

Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

The Impact of a Respiratory Fluoroquinolone Restriction Program on *C. difficile* Infection Rates within a Healthcare System

Author Block:

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Abstract Body:

Background: Fluoroquinolones are one of the most frequently prescribed antibiotic classes in the United States; however, up to 80% of use has been deemed inappropriate. Additionally, fluoroquinolones have been categorized as high risk agents for causing *C. difficile* infection (CDI). Interventions to limit fluoroquinolone utilization have been associated with a reduction in CDI rates. Investigators sought to assess the impact of a health system respiratory fluoroquinolone restriction program on *C. difficile* infection rates. **Methods:** This is a multi-center, retrospective study comparing hospital-acquired *C. difficile* infection rates at five hospitals within a healthcare system after implementation of a respiratory fluoroquinolone restriction program. The program included education as well as development and implementation of criteria for utilization. The Student's t-test was used to assess the impact of the restriction program on CDI rates and moxifloxacin utilization. **Results:** The hospital system experienced a 94% overall reduction in respiratory fluoroquinolone days of therapy per 1000 patient days (DOT/1000 patient days) and a 35% reduction in hospital-acquired CDI cases per 1000 patient days (CDI rate). The mean DOT/1000 patient days decreased post-education [24.5 (SEM 1.1) vs. 12.8 (SEM 1.5); abs reduction 11.7, p<0.001] and was further reduced post-restriction [12.8 (SEM 1.5) vs. 4.0 (SEM 0.6); abs reduction 8.8, p<0.001]. A significant reduction in CDI rate was experienced between the pre-intervention and post-restriction phases (absolute mean reduction of 0.1, p=0.036) as well as the pre-restriction and post-restriction phases (absolute mean reduction of 0.1, p=0.046). **Conclusions:** Implementation of a respiratory fluoroquinolone restriction program can reduce utilization and hospital-acquired *C. difficile* infection.

Author Disclosure Block:

K. Shea: None. **A. Hobbs:** None. **T. Jaso:** None. **J. Bissett:** None. **E. Douglass:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Rates of Surgical Site Infections and *Clostridium difficile* Infections before and after the Elimination of Post-Operative Antibiotic Prophylaxis at Wesley Medical Center

Author Block:

R. Louis¹, S. Kuhn², V. Creswell²; ¹Via Christi Hosp. Wichita, Inc, Wichita, KS, ²Wesley Med. Ctr., Wichita, KS

Abstract Body:

Background: Surgical site infections (SSI) are the most common cause of healthcare associated infections, and can lead to complications including increased length of hospital stay and mortality. Recent literature has concluded that the use of post-operative antibiotic prophylaxis does not affect SSI rates, and guideline updates from IDSA/SHEA and HICPAC recommend against their use. Additionally, it has been shown that patients are at a higher risk for *Clostridium difficile* infections (CDI). Based on current literature and guideline recommendations, Wesley Medical Center eliminated the routine use of post-operative antibiotics. **Methods:** This retrospective quality improvement project compared rates of SSI in patients receiving 24-48 hours of post-operative antibiotic prophylaxis versus patients receiving no post-operative antibiotics. In addition, a comparison of number of hospital acquired CDI was conducted. Patients who underwent surgery during August 2013-January 2014 and August 2014-January 2015 were eligible for inclusion. Data collected included SSI infection rates for each six month period reviewed, overall number of patients who developed hospital-acquired CDI, and number of surgical patients who developed CDI. **Results:** In the pre- and post- process change groups, there were 905 and 697 surgeries performed, respectively. SSI rates between the pre-process change and post-process change group by surgery type were as follows: Overall 1.1% vs 1.1%, total knee 0.5% vs 0.7%, total hip 0% in both groups, fusion 1.5% vs 0.4%, cardiac 0% in both groups, abdominal hysterectomy 2.4% vs 3.9%, and colorectal 2.5% vs 4.4%. The overall number of hospital-acquired CDI was 39 in both the pre and post process change groups, and the number of CDI in surgical patients was 15 in the pre-process change group and 11 in the post-process change group. **Conclusions:** There were similar rates of SSI in the patients receiving post-operative antibiotic

prophylaxis compared to patients who did not receive any additional post-operative antibiotics. The number of CDI was also similar between the two groups.

Author Disclosure Block:

R. Louis: None. **S. Kuhn:** None. **V. Creswell:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Impact of the National Antimicrobial Management Program of China on Patient Outcomes and Antimicrobial Resistance in a Large University Hospital

Author Block:

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Abstract Body:

Background: China has initiated a nationwide antimicrobial management program since May 2011. We retrospectively evaluated the impact of the program on outcomes of inpatients and antimicrobial resistance among clinical isolates in a 5,000-bed university hospital. **Methods:** The core components of the program were formulary restriction (≤ 50 agents for tertiary hospitals), preprescription authorization and postprescription review/feedback. The targets were that the proportion of inpatients received antimicrobials was $\leq 60\%$ and the number of defined daily dose (DDD) per 100 inpatients per day was ≤ 40 for tertiary hospitals. Numbers of patients discharged from West China Hospital, Sichuan University from 2009 to 2014, their length of hospital stay (LOS), all-cause mortality rates and susceptibility data of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. aureus*, *E. faecalis* and *E. faecium* were retrieved. The amount of antimicrobials used was converted into the number of DDD by divided the DDD value assigned by WHO. **Results:** The number of discharged patients was 155,609 in 2009 and 176,890 in 2014. The DDD number of antimicrobials per 100 discharged patients per day decreased from 70.08% in 2010 to 41.87% in 2014 and the proportion of discharged patients received antimicrobials during the hospital stay was dropped from 54.54% in 2011 to 42.40% in 2014. The average LOS was shorten from 9.99 d in 2009 to 9.60 in 2014 and the all-cause in-hospital mortality rate decreased from 1.36% in 2009 to 0.78% in 2014. Among Gram-negative bacteria, the non-susceptible rates of *A. baumannii* to all agents increased, while those of *E. coli* and *K. pneumoniae* to all but carbapenems decreased. Non-susceptible rates to carbapenems in *A. baumannii*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* were 66.0%, 28.9%, 1.7% and 2.7% in 2014, respectively. Among Gram-positive bacteria, from 2009 to 2014 MRSA decreased from 37.4% to 29.9%, while VRE increased from 0 to 2.7% in *E. faecalis* and from 3.9% to 12.9% in *E. faecium*, respectively. **Conclusions:** The management program significantly reduced

antimicrobial use and did not worsen patient outcomes in the hospital. The impact of the program on resistance varied depending on both the species and the agent. Carbapenem-non-susceptible Gram-negative bacilli and VRE have emerged.

Author Disclosure Block:

Z. Zhiyong: None. **J. Li:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Misuse of Antibiotics Reserved for the Hospital Settings in Outpatients: A Single-Center Prospective Study

Author Block:

M. Roche¹, C. Bornet², P. Monges², A. Stein², S. Gensollen², **P. Seng²**; ¹Assistance Publique - Hôpitaux de Marseille, Marseille, France, ²Univ. Hosp. La Conception, Marseille, France

Abstract Body:

Background: Many serious infections could be rapidly managed in the ambulatory and administration of antibiotics that are usually reserved for the exclusive use in hospitals in the outpatient setting is possible as long as the clinical situation allows. **Methods:** We performed a 30 days audit of outpatient antibiotic prescription in the four university hospital centers with 4,000-bed in Marseille, France. We evaluated the relevance of outpatient antibiotic prescribing. **Results:** Sixty prescriptions of antibiotics reserved for the hospital settings have been real-time analyzed during the study period. Seven prescriptions due to insufficient clinical data. In 64% of cases, the antibiotics reserved for the hospital settings was started in hospital cares; and 36% in ambulatory care. We observed that only 25% of cases were prescribed in infectious disease specialist and in 75%, antibiotics reserved for the hospital settings were prescribed by physicians of other specialties. The mean duration of antibiotic treatment prescribed was 37.5 days. The analysis of appropriateness of the prescription showed that only 40 % of cases were considered as appropriate prescription, and 60% were considered as an unnecessary or inappropriate prescription according to French Infectious Diseases Society and ANSM guideline. Among the 32 cases of unnecessary or inappropriate prescription, 3 cases were considered as unnecessary antibiotherapy with lack of microbial arguments, one case was not adequate to the infection type and the ecology of microorganisms, one case with incorrect in antibiotic dosage, one case with incorrect interval of dose administration, 3 cases with other therapeutic alternatives more adapted for ambulatory settings, and 23 cases with no recommendation mentioned in national guidelines. **Conclusions:** The result of our real-time analysis of outpatient's prescriptions of antibiotics reserved for the hospital settings showed that only 40% of prescription were appropriate. Implication of

infectious diseases expertise in this monitoring care has improved the quality of antibiotic use.

Author Disclosure Block:

M. Roche: None. **C. Bornet:** None. **P. Monges:** None. **A. Stein:** None. **S. Gensollen:** None. **P. Seng:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

The Integration of Pharmacokinetics-Pharmacodynamics (Pk-Pd) and the Practice of Antimicrobial Stewardship Through an Educational Mobile Application

Author Block:

C. C. Bulik, J. C. Bader, S. M. Bhavnani, D. R. George, C. M. Rubino, P. G. Ambrose, K. L. Sweeney; ICPD & ICPD Tech., Latham, NY

Abstract Body:

Introduction: Antimicrobial stewardship is essential to patient care. To improve patient care and preserve the activity of antimicrobials, state and federal governments have directed hospitals to implement antimicrobial stewardship practices. The PK-PD Compass is an educational mobile application developed to aid clinicians in evaluating competing therapeutic regimens from a PK-PD-patient-centric perspective. Initial user data are described herein. **Methods:** Data entered in the PK-PD Compass over a 3-month period (Oct - Dec, 2015) were evaluated. These data included: 1) user demographics (age, sex, medical/pharmacy specialty, years in practice); 2) healthcare institution type; 3) infection type, pathogen and antimicrobial(s) considered; 4) pathogen susceptibility; 5) patient demographics (age, sex, weight, height), serum creatinine and co-morbidities (diabetes, immune status); 6) user-selected antimicrobial and; 7) user-reported outcome. PK-PD Compass-calculated data included creatinine clearance and percent probability of attaining a PK-PD target associated with efficacy (%PTA) of selected and other antimicrobials. **Results:** Data for 134 patients from 71 users were available. Practitioner types included clinical pharmacists (52%), infectious disease and general attending physicians (22.5%), postdoctoral residents and fellows (8.5%) and others (17%). Infection types included bacteremia or infective endocarditis (46%), pneumonia (19%), urinary tract (13%), intra-abdominal (13%), and skin and skin structure (9%). Pathogen susceptibility was described using specific MIC value (45%), SENTRY distribution (41%) and susceptibility breakpoint (14%). The median (min, max) patient age and creatinine clearance were 49.5 (22, 89) years and 87.2 (2.5, 173.8) mL/min/1.73 m², respectively. 86% of users chose a regimen with %PTA \geq 80%; 78% chose a regimen with %PTA \geq 90%. Alternative dosing regimens provided by the PK-PD Compass were chosen in 16% of cases. **Conclusion:** Users were primarily clinical pharmacists or

attending physicians; complicated patients and infection types were more common. Data from the PK-PD Compass, which enables consideration of PK-PD optimized treatment plans, demonstrated selection of such regimens for the majority of patients.

Author Disclosure Block:

C.C. Bulik: C. Consultant; Self; ICPD Tech. D. Employee; Self; ICPD. **J.C. Bader:** C. Consultant; Self; ICPD Tech. D. Employee; Self; ICPD. **S.M. Bhavnani:** D. Employee; Self; ICPD. G. Member; Self; ICPD Tech. K. Shareholder (excluding diversified mutual funds); Self; ICPD. **D.R. George:** C. Consultant; Self; ICPD Tech.. D. Employee; Self; ICPD. **C.M. Rubino:** D. Employee; Self; ICPD. G. Member; Self; ICPD Tech. K. Shareholder (excluding diversified mutual funds); Self; ICPD. **P.G. Ambrose:** D. Employee; Self; ICPD. G. Member; Self; ICPD Tech. K. Shareholder (excluding diversified mutual funds); Self; ICPD. **K.L. Sweeney:** C. Consultant; Self; ICPD Tech.. D. Employee; Self; ICPD.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Reducing Time to Targeted Therapy: The Impact of Rapid Identification of *Staphylococcus aureus* via Polymerase Chain Reaction (PCR) in Combination with Pharmacist Intervention on Adults with Bacteremia

Author Block:

S. Kuhn, K. Beadle, V. Creswell; Wesley Med. Ctr., Wichita, KS

Abstract Body:

Background: Reducing the time to the initiation of appropriate antimicrobial has shown significant benefit in patient outcomes. Standard identification techniques can require at least 48-72 hours between specimen collection and organism identification. The use of rapid diagnostics enhances the time to optimal antibiotic use, which leads to decreased morbidity and mortality. In 2013 WMC acquired the Cepheid GeneXpert[®] (CG). The CG utilizes PCR to identify methicillin sensitive *Staphylococcus aureus* (MSSA) or methicillin resistant *Staphylococcus aureus* (MRSA) in blood. This allows physicians to tailor antibiotics based on presumptive sensitivities >24 hours earlier than standard methods of organism identification. **Methods:** This retrospective, observational study compared adult patients with bacteremia who had blood cultures (BC) positive for *S. aureus* before and after the use of PCR identification. The 6 month pre-intervention (pre-I) (July-December 2012) was compared to the post-intervention (post-I) (July-December 2014) to describe the differences in time to initiation of effective and targeted antibiotics. Differences in hospital stay (HS) and intensive care unit (ICU) length of stay were compared. Comparative continuous data was analyzed using a student's t-test. **Results:** Pre-I (n=43) vs. post-I (n=53): MRSA was isolated in 40% vs. 50% of patients. Effective therapy (ET) was started empirically on 72% vs. 87% of patients. Of those, 32% vs. 61% received empiric targeted therapy (TT). Vancomycin was the most common empiric therapy started (81% vs. 77%). Of the patients not started on empiric ET, the average time between first positive BC and ET was 4h18m vs. 2h17m (p>0.05). Of the patients who were not started on empiric TT, the median time between first positive BC and TT was 29h30m vs. 22h49m (p=0.01). Median time between ET and TT was 57h45m vs. 35h30m (p=0.007). The median ICU length of stay was 5.8 days vs. 4.5 days (p>0.05) with the same median HS of 9 days (p>0.05) for both groups. **Conclusions:** In patients

with *S. aureus* bacteremia, the use of rapid determination of MSSA vs. MRSA by PCR, in combination with pharmacists' interventions, significantly reduces the time until TT. This may lead to improved patient outcomes.

Author Disclosure Block:

S. Kuhn: None. **K. Beadle:** None. **V. Creswell:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Impact of Computerized Decision Support System on Antibiotic Prescribing in Singapore General Hospital

Author Block:

Y. P. ZHOU, S. S. L. TANG, W. LEE, Y. Y. CAI, T. P. LIM, M. P. CHLEBICKI, A. L. KWA; SINGAPORE GENERAL Hosp., Singapore, Singapore

Abstract Body:

Background: Computerized Decision Support System (CDSS) is proposed as an important tool in antimicrobial stewardship to promote appropriate antibiotic prescribing.¹ In Singapore General Hospital, CDSS was developed to incorporate hospital guidelines into our computerized physician order entry system. It was designed to be user friendly, not overly prescriptive with each antibiotic order completed within 6 mouse-clicks. Mandatory use of CDSS is only triggered when selected broad spectrum antibiotics are prescribed. It recommends empiric antibiotic and the corresponding dose based on the type, severity of infection and renal function of the patient. CDSS was launched in April 2015 and we seek to evaluate its impact on the appropriateness of selected broad-spectrum antibiotic use and to assess the impact of CDSS on patient safety. **Methods:** A 1 year pre- and 6 months post- implementation study was performed. All patients whose physicians intended to prescribe carbapenems, piperacillin-tazobactam and intravenous ciprofloxacin, including patients who subsequently received narrower spectrum antibiotics suggested by CDSS were analysed. Appropriateness of antibiotics use per month was determined by the hospital antimicrobial stewardship team based on choice, dose and route of antibiotics used upon prescribing. **Results:** An average of 1110 courses of selected broad spectrum antibiotics was prescribed per month. Appropriate empiric antibiotic use improved from a median of 90.3% to 93.8% ($p < 0.001$) after CDSS implementation. This was largely driven by improvement in antibiotics selection from 92.1% to 94.9% ($p < 0.001$). There were 133 (1.9%) patients whose prescribers chose alternative antibiotics based on CDSS recommendations. In comparison to patients who remained on inappropriate broad spectrum antibiotics ($n=421$), patients whose prescribers chose alternatives had a significantly lower 30-day mortality (5.3% vs 12.4%, $RR=0.426$, $p < 0.05$) and there was no significant difference in readmission rates. Appropriateness of

dosing also increased from 97.8% to 98.9% ($p=0.083$). **Conclusion:** CDSS promotes adherence to hospital antibiotic guidelines and has brought significant improvement in the appropriateness of empiric broad spectrum antibiotic prescribing without compromising patient safety.

Author Disclosure Block:

Y.P. Zhou: None. **S.S.L. Tang:** None. **W. Lee:** None. **Y.Y. Cai:** None. **T.P. Lim:** None. **M.P. Chlebicki:** None. **A.L. Kwa:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

Slovenian Undergraduate Training and Self-Assessments in the Infection Field - Student Prepare 2015

Author Block:

A. Saje¹, O. Dyar², A. Sustar³, U. Mrcic⁴, C. Pulcini⁵, **B. Beović**¹; ¹Clinic for Infectious Diseases and Febrile Illnesses, Ljubljana, Slovenia, ²Karolinska Inst., Ljubljana, Slovenia, ³Faculty of Med., Univ. of Ljubljana, Ljubljana, Slovenia, ⁴Faculty of Med., Univ. of Maribor, Maribor, Slovenia, ⁵Univ. de Lorraine, Nancy, France

Abstract Body:

Objectives: To evaluate self-assessed preparedness of Slovenian final year medical students on prudent antibiotic use. We compared the outcomes between the two Slovenian medical schools. **Methods:** The results presented here are part of a large European survey conducted in 2015 by ESGAP. Students in their final year of undergraduate medical studies at two medical schools in Slovenia were eligible to participate. Responses were obtained using an online questionnaire (19 questions), developed by a committee of international experts on antibiotic stewardship. **Results:** 154 Slovenian final year students completed the questionnaire (55 % response rate), including 109 of 196 eligible students at the University of Ljubljana and 45 of 84 eligible at the University of Maribor. The mean age was 24.6 years. The mean score of self-assessed preparedness on a scale from 1 (not at all prepared) to 7 (very well prepared) across all antibiotic use topics was 4.5 for both universities. The lowest preparedness was found for identifying indications for combination therapy, 3.3 for both universities. Insufficient preparedness (mean score of less than 4) was also found for identifying adequate duration of treatment; deciding when to switch from intravenous to oral therapy and in measuring the quality of care. The highest levels of preparedness were found for recognising clinical signs of infections (5.9 and 5.8, for Ljubljana and Maribor, respectively) and interpreting biochemical markers of inflammation (5.9, for both universities). Preparedness to use knowledge of antibiotic resistance was between 'sufficient' and 'very well' (5.0, for both universities). Small differences were observed between universities on individual topics, but none was statistically significant ($p > 0,05$). **Conclusions:** We identified topics that students assessed as being insufficiently

prepared for; the results could be used to improve the curriculum. There was no significant difference in self-assessed preparedness between the two Slovenian universities.

Author Disclosure Block:

A. Saje: None. **O. Dyar:** None. **A. Sustar:** None. **U. Mrsic:** None. **C. Pulcini:** None. **B. Beović:** None.

Session Number:

014

Session Title:

Antimicrobial Stewardship I

Publishing Title:

**Do Public Campaigns Decrease Antibiotic Prescription in the Community?
Evidence from a 12-Years Reimbursement Data Survey in Belgium**

Author Block:

P. M. Tulkens; Université catholique de Louvain, Bruxelles, Belgium

Abstract Body:

Background:Antibiotic consumption in the community varies widely amongst European countries (1), and its level has been correlated with the risk of emergence of resistance (2). Belgium was amongst the high consumers in 1997 (26.7 Defined Daily Doses [DDD; see ref. 3] per 10,000 inhabitants and per day; 3-fold larger than in the Netherlands). A first public campaign aiming at reducing patients' demand for antibiotics was therefore launched in 2000, yielding a statistically significant but transient reduction of antibiotic prescription (4). Similar campaigns were repeated each year up to now. Actions were also directed towards professionals for reducing unnecessary antibiotic prescriptions, especially quinolones. Our aim was to assess their long-term effects on antibiotic prescription.**Methods:**All antibiotics are under prescription and reimbursed in Belgium if delivered by pharmacists upon submission of the physician's prescription. We, therefore, retrieved the annual data of the National Institute for Health and Disability Insurance, which assembles yearly statistics of drug reimbursement (available at <http://tinyurl.com/hwu74sf>). Data were calculated as DDDs, which reflect drug most common usage and are not influenced by price or package size variations (3).**Results:**We analyzed data from 2000 to 2013 (see excerpt in the Table). The total consumption of systemic anti-infectives (group ATC J [> 95 % antibiotics]) increased 1.24-fold between 2001 and 2013, mostly due to β -lactams, while quinolones followed the same trend as all antibiotics taken globally.

Year	Systemic anti-infectives (ATC J) (DDD/10,000 inhabitants/day)	% β -lactams	% quinolones
2001	26.6	38.2	11.3

2005	27.4	49.9	10.1
2010	31.9	57.6	9.4
2013	33.2	57.0	8.9
Change 2001-13	1.24-fold	1.67-fold in DDD *	1.25-fold in DDD *
* DDDs in 2013 / DDSs in 2001			

Conclusions: During the period examined, there was no decrease but an increase in the prescription of systemic anti-infectives in the community (based on DDD data) with no specific effect (in absolute value) for quinolones. While other metrics (based on number of packages or prices) have suggested a decrease in total antibiotic prescription following public campaigns (5), the data show that overall antibiotic pressure in the community has not been reduced in spite of the actions undertaken.

Author Disclosure Block:

P.M. Tulkens: None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

A ‘Surprising’ Pair: The Synergistic Antimicrobial Activity of Polymyxin B in Combination with the Cystic Fibrosis Drug Ivacaftor against Polymyxin-Resistant *P. aeruginosa*

Author Block:

E. K. Schneider¹, M. Azad¹, Q. Zhou², J. Wang¹, J. Huang³, M. Cooper³, Y. Doi⁴, M. Muller⁵, J. LI¹, T. Velkov¹; ¹Monash Univ., Parkville, Australia, ²Purdue Univ., West Lafayette, IN, ³Univ. of queensland, St Lucia, Australia, ⁴Univ. of Pittsburgh, Pittsburg, PA, ⁵Burnett Sch. of BioMed. Sci., Orlando, FL

Abstract Body:

Background: Antibiotic resistance has evolved into a serious global health concern with the last line polymyxins now developing resistance due to the emergence of plasmid-mediated colistin-resistance-mechanisms MCR-1 in humans. Novel therapies are urgently required for combating lung infections caused by ‘superbugs’. This study investigated synergistic antibacterial activity of polymyxin B with the cystic fibrosis drug ivacaftor against Gram-negative pathogens that colonize the CF lung, particularly the problematic *Pseudomonas aeruginosa*. **Material/methods:** The *in-vitro* synergistic activity of polymyxin B with ivacaftor was evaluated against a panel of polymyxin-susceptible and polymyxin-resistant *P. aeruginosa* isolates including ciprofloxacin-resistant strains from the lungs of CF patients. **Results:** Polymyxin B and ivacaftor were ineffective when used individually against polymyxin-resistant isolates. However, the combination of polymyxin B and ivacaftor displayed synergistic killing activity against polymyxin-resistant isolates as a decrease. The combination displayed excellent antibacterial activity against *P. aeruginosa* under CF relevant conditions in a sputum medium assay. The potential antimicrobial mode of action against *P. aeruginosa* was investigated using different methods. The combination induced cytosolic GFP release, showed permeabilizing activity in the nitrocefin assay, indicating damage to both the outer and inner membranes. SEM/ TEM revealed that the combination produces outer membrane damage that is distinct from the effect of each compound *per se*. Ivacaftor was also shown to be a weak inhibitor of the bacterial DNA gyrase and topoisomerase IV. **Conclusions:** In summary, polymyxin B and ivacaftor exhibited synergistic activity

against highly polymyxin-resistant *P. aeruginosa* CF isolates including ciprofloxacin-resistant strains and offers a safe and potential highly effective treatment regimen against otherwise untreatable CF lung infections.

Author Disclosure Block:

E.K. Schneider: None. **M. Azad:** None. **Q. Zhou:** None. **J. Wang:** None. **J. Huang:** None. **M. Cooper:** None. **Y. Doi:** None. **M. Muller:** None. **J. Li:** None. **T. Velkov:** None.

Session Number:

015

Session Title:**Hot Topics in Antimicrobial Susceptibility Testing****Publishing Title:****Repurposing Pharmacologically Active Compounds to Enhance Antibiotic Killing of *Pseudomonas aeruginosa* Biofilms in Cystic Fibrosis****Author Block:**

M. Maiden, A. Hunt, C. Waters; Michigan State Univ., East Lansing, MI

Abstract Body:

Cystic fibrosis (CF) is a common genetic disease in Caucasians, affecting 30,000 people in the United States¹. Key to the pathogenesis of CF is a defective mucociliary transport system that results in chronic airway obstruction by dry mucopurulent secretions and chronic polymicrobial infections in the form of biofilms. Today, due to aggressive antibacterial treatments and better nutrition, median survival has increased from 15 to greater than 40 years old². However, the emergence of antimicrobial resistance and the inherent resistance of biofilms is of major clinical concern. The most common chronic lung infection in CF patients is *Pseudomonas aeruginosa*, which forms biofilms that are recalcitrant to conventional antibiotic therapies³. In this work, we aim to use pharmacologically active compounds and approved drugs to repurpose them for use in killing biofilms. The University of Michigan Center for Genomics performed a high throughput screen (HTS) on 6,090 bioactive small molecules to examine enhanced antibiotic killing of *P. aeruginosa* biofilms with tobramycin. From this drug repurposing screen, we have identified 26 hits that potentiate the action of tobramycin killing of *P. aeruginosa* biofilms but do not have activity on their own. These hits were further validated with MBEC (minimum biofilm eradication concentration) plates and their antimicrobial activity was quantified using BacTiter-Glo. One combination, tobramycin and imipenem, showed significant activity, eradicating greater than 97% of the standard lab strain PAO1 *in vitro*. These results indicate that our approach is a rapid and effective method for identifying compounds that can be repurposed to enhance antibiotic killing of biofilms. Future work will focus on studying biofilm killing activity in a CF mouse model using this combination and others from the HTS.

Author Disclosure Block:

M. Maiden: None. **A. Hunt:** None. **C. Waters:** None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Population Heterogeneity of *Pseudomonas aeruginosa* in Cystic Fibrosis (Cf) Patients on Cycled Inhaled Anti-*Pseudomonas* Antibiotics

Author Block:

A. Nguyen¹, A. Heirali¹, M. Workentine¹, R. Somayaji¹, D. Storey¹, W. Leung², B. Quon³, H. Rabin¹, M. Surette⁴, M. Parkins¹; ¹Univ. of Calgary, Calgary, AB, Canada, ²Univ. of Alberta, Edmonton, AB, Canada, ³Univ. of British Columbia, Vancouver, BC, Canada, ⁴McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Background: Inhaled antibacterials, such as aztreonam lysine for inhalation (AZLI), are critical therapies for CF patients with chronic *Pseudomonas aeruginosa* (PA) infection. Patient improvements on AZLI have been attributed to decreased sputum density of PA, however, no correlation between improvements in lung function and decline in PA density has been demonstrated. Studies have shown similar improvements in clinical response irrespective of in vitro predicted susceptibility. It is now apparent that chronic infecting strains in CF diversify over time, and that marked phenotypic heterogeneity evolves in factors such as virulence and antibiotic susceptibility, and that no single isolate is representative of the community. How these diverse PA communities change during therapeutic influence is hereto unknown. **Methods:** Patients enrolled (n=5) had chronic PA infections and were routinely administered AZLI for 28 days followed by a 28 day off period as licensed. PA was isolated from sputum collected at five time points (day 0, 14, 28, 42, and 56). High throughput techniques were used to screen approximately 190-isolates/time point/patient (n=4750). Antibiotic susceptibility testing was carried out with aztreonam, ciprofloxacin, and ceftazidime. Changes of population resistance between time points were measured by comparing the proportions of resistant PA isolates and visualized with stacked bar graphs. **Results:** Marked diversity in antibiotic susceptibility was observed amongst patients with chronic PA infection with respect to each antibiotic assessed. There were no significant effects of AZLI on population resistance to aztreonam (p=0.09), ciprofloxacin (p=0.55), or ceftazidime (p=0.22) between “on” vs “off” cycled therapy. Ciprofloxacin was the most effective antibiotic to inhibit growth followed by aztreonam then ceftazidime. **Conclusions:** Marked heterogeneity in PA

antibiotic susceptibility (aztreonam, ciprofloxacin, ceftazidime) was observed in individuals on chronic, cycled AZLI.

Author Disclosure Block:

A. Nguyen: None. **A. Heirali:** None. **M. Workentine:** None. **R. Somayaji:** None. **D. Storey:** None. **W. Leung:** E. Grant Investigator; Self; Gilead Sciences. **B. Quon:** E. Grant Investigator; Self; Gilead Sciences. **H. Rabin:** E. Grant Investigator; Self; Gilead Sciences. **M. Surette:** E. Grant Investigator; Self; Gilead Sciences. **M. Parkins:** E. Grant Investigator; Self; Gilead Sciences.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

***In Vitro* Potency of Amikacin and Comparators Against *E. coli* and *K. pneumoniae* Respiratory and Blood Isolates**

Author Block:

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Abstract Body:

Background: The management of nosocomial pneumonia has been made increasingly difficult due to the emergence of resistance and the potential for reduced antibiotic penetration in the intubated patient. Among the Enterobacteriaceae, *E. coli* (EC) and *K. pneumoniae* (KPN) are prominent etiologic agents in the setting of hospital-acquired lung infection. The delivery of antibiotics directly to the site of infection presents a unique clinical opportunity to enhance patient outcome. This approach is currently under study using amikacin (AMK) by inhalation (Amikacin Inhale, BAY41-6551) in combination with intravenous standard-of-care therapy for intubated and ventilated patients with Gram-negative pneumonia. To better understand the potential utility of Amikacin Inhale, the current study was undertaken to define the potency of AMK against a collection of US Gram-negative nosocomial isolates. **Methods:** 21 teaching and 3 community US hospital provided non-duplicate nosocomial blood and respiratory isolates of EC and KPN from adult inpatients. MICs were determined using CLSI defined broth microdilution methods for ceftolozane/tazobactam (C/T), cefepime (FEP), ceftriaxone (CRO), ciprofloxacin (CIP), piperacillin/tazobactam (TZP), meropenem (MEM), tobramycin (TOB) and AMK. CLSI and FDA breakpoints were used to define susceptibility. **Results:** 375 EC and 379 KPN were included in the analysis. Rank order % susceptibility (MIC₉₀, mg/L) was as follows: MEM 98% (0.06), AMK 97% (8), C/T 95% (1), TZP 90% (32), FEP 88% (16), TOB 86% (16), CRO 85% (128), and CIP 75% (32). For AMK, 97% of these organisms had MICs ≤ 16 mg/L, 2% = 32 mg/L, and 1% = ≥64 mg/L. Comparing AMK against FEP (n=92) and TZP resistant (n=76) only 1% of the organisms had MICs ≥128 mg/L. Furthermore, 45 EC and 41 KPN were confirmed to be ESBL positive. Of these ESBL producing EC 89% and KPN 90% were found to have MICs ≤ 16 mg/L to AMK. **Conclusions:** This study demonstrated a high level of activity for AMK to combat infections caused by *E. coli* and *K. pneumoniae* including ESBLs.

For AMK 97% of organisms had a MIC of ≤ 16 mg/L, moreover nearly all (99%) organisms have MICs ≤ 32 mg/L which is well below the achievable lung concentration of 5,000 mg/L with the administration of Amikacin Inhale. These data highlight the potential role of Amikacin Inhale in the management of nosocomial pneumonia due to *E. coli* and *K. pneumoniae*.

Author Disclosure Block:

C.A. Sutherland: None. **J.E. Verastegui:** None. **D.P. Nicolau:** L. Speaker's Bureau; Self; Bayer.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Antibiotics Resistance Pattern of *Klebsiella* Isolated from Clinical Samples and Their *In Vitro* Inhibition by the Secondary Metabolites of *Actinomyetes*

Author Block:

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Abstract Body:

A Total of 30 *Klebsiella* strains were isolated from clinical sample collected from a tertiary care hospital. The isolates were identified by morphological, physiological and biochemical and genetic characterization. Antibiotic susceptibility of the selected *Klebsiella* isolates was determined the standard Kirby Bauer disc diffusion assay. The results indicate that *Klebsiella* strains were mostly resistant to the antibiotics such as Ceftriaxone, Cephalexin. The genomic DNA extraction and gel purification of the isolated strains was done. The 16S rRNA gene sequencing of the selected isolates was performed using dye terminator chemistry on an automated sequencer. Further biological screening was done to check the antimicrobial activity of the actinomycetes against *Klebsiella* strains. In biological screening the well diffusion method showed good results and a maximum zone of inhibition about 12 “mm” was observed in case of strain A20. The actinomycetes strains which show the maximum activity on *Klebsiella* are: A19, A10, A20, A1, A2, A6, and A5. Strain A19 and A20 exhibited inhibitory effects on almost all the *Klebsiella* isolates. The chemical screening of the actinomycetes was performed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). HPLC/UV chromatogram show several peaks on the chromatogram at different retention times (t_R) which shows the presence of diverse compounds in the crude extracts. In comparative efficacy the antibiotics for which *Klebsiella* exhibited resistance were compared with the actinomycetes crude extracts which were sensitive to *Klebsiella*. The antibiotics Ceftriaxone, Cefepime, Cefpirome were ineffective or exhibited no inhibitory effect on *Klebsiella* while the crude extracts A19, A20, A10, A6, A1 were effective and showed maximum zones of inhibition up to 15-20 mm. Cytotoxicity of the crude extracts was determined against brine shrimps (*Artemia salina*) using a microwell cytotoxicity assay, most of the extracts were found to be mild cytotoxic. Biofilm forming ability of *Klebsiella* was detected on congo red agar,

after that the effect of the crude extracts of actinomycetes against the biofilm forming *Klebsiella pneumoniae* was tested on 96-well microtiter plates and significant inhibition of biofilm formation by the natural extracts was observed.

Author Disclosure Block:

N. noureen: None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Improved Turn-Around-Time for Identification and Susceptibilities for Blood Culture Isolates Utilizing Direct Identification and Susceptibility Testing from Positive Broth

Author Block:

K. Timm, **E. M. Richards**, M. Dodd, K. Culbreath; TriCore Reference Lab., Albuquerque, NM

Abstract Body:

Background: Rapid identification and susceptibility testing of blood culture isolates can lead to improved antimicrobial stewardship and outcomes. The purpose of this study was to evaluate the performance and impact on turn-around-time of performing rapid MALDI-TOF identification and automated susceptibility testing directly from positive blood culture bottles. **Methods:** 170 positive flagged blood culture bottles (54 retrospective spiked samples, 116 prospective clinical samples) were subcultured and identification and susceptibility testing was performed per routine methods. An aliquot of each sample was prepared for MALDI-TOF identification using the MALDI Sepsityper with the Bruker Biotyper RUO system (Bruker Daltonics, Billerica, MA) for identification from positive blood cultures. An aliquot was prepared for susceptibility testing for the BD Phoenix (BD, Sparks, MD). Results were compared for analytic correlation of identification by genus and species, categorical agreement and time to result. **Results:** Of the 143 identifications, species level identification was 95.8%. compared to routine MALDI-TOF identification. In total, the categorical agreement was (98.4%) for all of the drug-bug combinations. Additionally, for 66 samples, an evaluation was performed to determine the time gained in providing complete susceptibility compared to the routine method of performing ID/AST from the subculture plate. The average time to final result from positive blood culture bottle for routine and direct ID/AST were 41hr and 20hr, respectively. This demonstrated direct identification and susceptibility results available an average of 21hrs 10 min (± 9 hr) earlier than providing susceptibility from the routine culture plate. **Conclusions:** Our results demonstrate that direct testing of positive blood culture bottles for identification by MALDI-TOF identification and automated susceptibility testing provides accurate

identification and susceptibility testing. These results are available significantly earlier than results generated using the routine method of testing without significant additional cost.

Author Disclosure Block:

K. Timm: None. **E.M. Richards:** None. **M. Dodd:** None. **K. Culbreath:** None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Detection of Carbapenemase Activity in Gram Negative Rods by the Modified Carbapenem Inactivation Method (Cim)

Author Block:

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Abstract Body:

Introduction: Reliable detection of carbapenem resistant Gram negative rods (GNRs) is critical for effective infection control. Routine susceptibility tests can detect phenotypic resistance, but cannot confirm carbapenemase production or distinguish non-susceptible isolates due to other mechanisms including porin mutations, efflux pumps and other extended spectrum β lactamases. Therefore, we evaluated a low cost phenotypic method to reliably detect carbapenemase producing GNRs.**Methods:** The modified CIM test was derived by modifying the CIM assay ([PLoS One](#). 2015 Mar 23; 10(3):e0123690) using our in-house strain collection. For validation, 142 isolates (Enterobacteriaceae and non-fermenters) from CDC/FDA AR bank (132 isolates) and UCLA (10 isolates) were tested in triplicate. To perform the modified CIM, a suspension was made by using a full 1 μ l (Enterobacteriaceae) or 10 μ l (non-fermenter) inoculation loop of colony from a blood agar plate in 2ml Tryptic Soy Broth. A 10 μ g meropenem disk was added to the suspension. After brief vortexing, the broth was incubated for 4 hours at 35°C. Next, the meropenem disk was removed using an inoculation loop, placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* ATCC (25922) indicator strain and incubated at 35°C for 18-24 hours, when the zone of inhibition around the meropenem disk was measured.**Results:** All isolates tested had a known genetic mechanism for resistance and MIC results for carbapenems. Performance of the modified CIM test compared to the resistance mechanism is in Table 1. Sensitivity and specificity of the modified CIM test were 99% and 94%, respectively.

Table 1. Resistance Mechanism Compared with Modified CIM Test Results		
	CIM Test Results	Total Number tested

Resistance Mechanism	Positive	Negative	
NDM	31	0	31
VIM/IMP	17	0	17
KPC	31	1 [‡]	32
OXA	20	0	20
Other CRE (SME,SPM,IMI)	11	0	11
Non CRE	2*	29	31
‡ KPC by PCR but carbapenem MICs (0.25-1) in the susceptible range			
* No known resistance mechanism with high carbapenem MICs (8->64)			

Conclusions: This modification of the CIM test is a reliable, simple, and cost-effective method for detection of isolates producing carbapenemases.

Author Disclosure Block:

P. Patel: None. **R. Thomson:** None. **L. Peterson:** None. **K. Mangold:** None. **S. Das:** None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Comparison Of Cre Identification Based On Current (2015) Versus Previous (2012) Cdc Definitions

Author Block:

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Abstract Body:

Accurately identifying carbapenem resistant Enterobacteriaceae (CRE) is a challenge for clinical microbiology laboratories. In 2012, Centers for Disease Control (CDC) published a phenotypic definition for CRE that incorporated non-susceptibility to carbapenems (with the exception of ertapenem) as well as resistance to third generation cephalosporins. In 2015 the definition was revised: only carbapenems, including ertapenem, are evaluated for resistance. In order to evaluate the efficacy of these phenotypic definitions, and to assess the utility of the Verigene Gram Negative Blood Culture Nucleic Acid Test (BC-GN, Nanosphere, Northbrook, IL), we studied 26 clinical Enterobacteriaceae isolates. All of the isolates demonstrated non-susceptibility to at least one carbapenem. Susceptibility profiles were compared to both the 2012 and 2015 CDC definitions. Isolates were inoculated into previously tested negative blood cultures, and loaded onto Bactec FX (Becton Dickinson) instruments. When growth was detected, vials were removed and tested following manufacturer's protocols on the BC-GN, which detects KPC, NDM, IMP, OXA and VIM. The isolates were then blinded and sent to Maine's Health and Environmental Testing Laboratory for confirmation using an in-house laboratory designed PCR test that detects KPC, NDM, GES, OXA, and VIM. 17 of the 26 isolates were identified as CRE by the 2012 definition; 19 were identified as CRE by the 2015 definition. There was not good concordance between the two definitions; only 12 isolates were defined the same way by both definitions. Only 4 of the isolates demonstrated a molecular marker for carbapenemase production (all 4 for the KPC gene, by both molecular methods). 10 isolates demonstrated ESBL enzymes. In comparison to a molecular method, the sensitivity, specificity, positive predictive value, negative predictive value of the 2012 phenotypic CRE definition was 75%, 36.4%, 17.6%, and 88.9%. The values for the 2015 phenotypic definition were 100%, 31.8%, 21.1%, and

100%.By using the new definition with improved negative predictive value for screening, followed by Verigene BC-GN molecular method for confirmation, we obtained an accurate and efficient CRE identification algorithm that is easily incorporated into the workflow of a clinical diagnostic laboratory.

Author Disclosure Block:

C. Dragoni: None. **M. Ianosi-Irimie:** None. **N. Matluk:** None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

Imipenem Mic Shift Among Carbapenem-susceptible *Enterobacteriaceae*: A Single Center Metadata Analysis Between 2001 and 2014

Author Block:

M. J. Lee, J. A. Hindler, R. M. Humphries; David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract Body:

Background: The increase in carbapenem-resistant *Enterobacteriaceae* (CRE) in the US is a significant public health concern, driven predominantly by the spread of the *Klebsiella pneumoniae* carbapenemase (KPC). However, we have also anecdotally noted an upward shift in carbapenem MICs among susceptible isolates in recent years. We analyzed 14 years of imipenem (IPM) MIC data for 14,548 isolates of *Enterobacteriaceae*, to further evaluate this trend. **Methods:** Antimicrobial susceptibility testing was performed according to the CLSI reference broth microdilution method using panels prepared in-house. Analysis was performed using Prism (GraphPad Software, Inc.). The first isolate with MIC data per patient was included. *Proteus*, *Morganella*, and *Providencia* species were excluded due to natural elevated IPM MICs, as were all IPM non-susceptible isolates (MIC ≥ 2 $\mu\text{g/ml}$). IPM MICs were arbitrarily divided into 'low-MIC' (≤ 0.25 $\mu\text{g/ml}$) and 'elevated-MIC' (0.5-1.0 $\mu\text{g/ml}$) categories, for analysis. **Results:** Linear regression revealed a significant decrease in the proportion of low-MIC isolates over 14 years ($p=0.004$). In 2001-02, 77.4% of *Enterobacteriaceae* tested ($n=1,741$) had low-MIC and 21.1% of isolates had elevated-MIC values for IPM, whereas in 2013-14 ($n=2,320$), these numbers were 68.8% vs. 27.8%. The shift in IPM MICs was most apparent for isolates recovered from outpatients ($p=0.004$) and patients in the ICU ($p=0.006$). No change in IPM MICs was noted for *Escherichia coli* isolates, whereas a dramatic change was found for *K. pneumoniae*. In 2001-02, 95.8% of *K. pneumoniae* ($n=321$) had low-MIC values to IPM. In 2013-14, this number decreased to 77.7% ($p=0.0002$) ($N=365$). Isolates with elevated-MICs were associated with increased resistance to other cell wall-targeting agents, including ampicillin/sulbactam (28.2% vs. 18.7%), cefazolin (27.6% vs. 17.0%), and ceftazidime (14.5% vs. 10.4%). **Conclusion:** Collectively, our analysis suggests significant shift in IPM MICs over the past 14 years.

We hypothesized this may be due to the expansion of isolates that harbor extended spectrum β -lactamases that have weak activity against the carbapenems, including IPM. A larger, multi-center analysis is needed to clearly establish an IPM creep, and additional studies to further evaluate the mechanism of this shift in MIC.

Author Disclosure Block:

M.J. Lee: None. **J.A. Hindler:** None. **R.M. Humphries:** None.

Session Number:

015

Session Title:

Hot Topics in Antimicrobial Susceptibility Testing

Publishing Title:

**Regular Evaluation of Vancomycin for Automated Mic Problems (Revamp):
Trending of Vancomycin Minimum Inhibitory Concentrations Across Healthcare
Enterprises**

Author Block:

R. Clifford¹, M. Sparks¹, U. Chukwuma², C. Neumann², P. Waterman³, E. Milburn², J. Moran-Gilad⁴, M. Julius¹, M. Hinkle¹, **E. Lesho**¹; ¹Walter Reed Army Inst. of Res., Silver Spring, MD, ²Navy and Marine Corps Publ. Hlth.Ctr., Portsmouth, VA, ³Armed Forces Hlth.Surveillance Branch, Silver Spring, MD, ⁴Ben-Gurion Univ. of the Negev, Beersheba, Israel

Abstract Body:

Background: Vancomycin minimum inhibitory concentrations (MIC) of 1.5-2.0µg/mL portend poorer clinical outcomes of *Staphylococcus aureus* infections. Manual broth dilution (reference method) for MIC determination is not feasible for timely surveillance of thousands of isolates over entire health systems. We sought to develop a tool to trend and predict incidences of ‘problematic vancomycin-susceptible *S. aureus*’ (PVSSA) isolates. **Methods:** Electronic records of all patients in a geographically dispersed 288-facility managed care system during 2010 -2015 were reviewed for all cultures that grew *S. aureus* and for prescriptions of anti-staphylococcal antimicrobials. Data were stratified by facility, testing platform and MIC, and trended using time-series modeling. **Results:** From >230 million patient encounters and 6.5 million cultures, 81,700 unique VSSAs were isolated. The fractions of isolates tested on the Vitek, Phoenix and Microscan automated platforms were 58%, 14%, and 20%, respectively. Due to inherent characteristics of the platform, the Microscan reported significantly higher proportions of PVSSA than Phoenix or Vitek. Trends in the Vitek and Phoenix derived data were concordant. In facilities using the Vitek, the proportion of PVSSA decreased ($P < 0.0001$), while in facilities using Phoenix, the proportion of PVSSA remained flat with no significant trend ($P = 0.14$). Analysis of data from either platform predicts the proportion of PVSSA will remain steady at roughly 1.5%. Time trends were consistent regardless of hospital type (tertiary-referral or community), geographic location, or population served. Use of vancomycin and other anti-staphylococcal agents decreased. **Conclusion:** Trends

of PVSSA decreased or remained constant, and may be partly explained by antibiotic stewardship efforts. Current features in the Microscan system limit its ability to support PVSSA surveillance. Our approach for PVSSA can be used for trend analysis of other clinically relevant pathogens.

Author Disclosure Block:

R. Clifford: None. **M. Sparks:** None. **U. Chukwuma:** None. **C. Neumann:** None. **P. Waterman:** None. **E. Milburn:** None. **J. Moran-Gilad:** None. **M. Julius:** None. **M. Hinkle:** None. **E. Lesho:** None.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:**Optimization of Cd101 Formulation against *Candida albicans* in a Rat Model of Vulvovaginal Candidiasis (VVC)****Author Block:**

V. Ong¹, K. Bartizal¹, D. Hughes¹, L. Miesel², W. Lin³, J. Webb³, A. Chen³; ¹Cidara Therapeutics, San Diego, CA, ²Eurofins Panlabs, Taipei, Taiwan, ³Latitude Pharma, San Diego, CA

Abstract Body:

CD101 is a novel echinocandin with activity against *Candida* spp., including azole-resistant isolates known to cause VVC. Currently marketed echinocandins lack stability to be effectively formulated for topical use. As CD101 is stable in acidic/basic pH and at physiological temperatures, it is expected that CD101 will retain potent activity in the vaginal mucosa. The efficacy of topical CD101 administered vaginally in different formulations was compared with that of 2% miconazole cream in a rat model of VVC. Groups of 5 oophorohysterectomized Wistar rats were dosed with estradiol at 10 mg/kg subcutaneously 3 days before *C. albicans* (ATCC 44858) challenge and maintained with 4 mg/kg weekly injections throughout the study. Animals were immunosuppressed with dexamethasone in drinking water (2 mg/L) 3 days before challenge and throughout the study. To establish vaginal infection, anesthetized rats were inoculated intravaginally with *C. albicans* (10^7 CFU). Treatment began 48 h after challenge. CD101 from 1%-10%, or miconazole 2%, were administered intravaginally at 0.1 mL/rat daily for 3 days. Rats were sacrificed at time points (1, 3, 5, or 8 days) after treatment cessation followed by vaginal lavage for CFU enumeration. An unpaired t-test was performed to determine the significance of treatment effects relative to the vehicle control groups. CD101 appears more effective than miconazole in preventing recurrence of vaginal candidiasis, since vaginal CFU were below LOD and significantly lower than vehicle controls from 1 day after treatment and remained undetected thereafter. Efficacy at later timepoints post-treatment suggest correlation with viscosity/slower % drug release as vaginal CFU remained suppressed 8 days after treatment end with a slow release ointment, whereas faster release gels effectively lowered CFU 1 day after but did not suppress CFU for as long. Both gel and cream/ointment formulations of CD101 were effective at reducing

CFUs in the rat VVC model. Once daily application of 3% CD101 as a slower release formulation significantly reduced the fungal burden for at least one week after treatment cessation. Both topical formulations of CD101 may offer promising alternatives for the treatment of acute and recurrent VVC in humans.

Author Disclosure Block:

V. Ong: D. Employee; Self; Cidara Therapeutics. **K. Bartizal:** D. Employee; Self; Cidara Therapeutics. **D. Hughes:** D. Employee; Self; Cidara Therapeutics. **L. Miesel:** D. Employee; Self; Eurofins Panlabs. H. Research Contractor; Self; Cidara Therapeutics. **W. Lin:** D. Employee; Self; Latitude Pharma. H. Research Contractor; Self; Cidara Therapeutics. **J. Webb:** D. Employee; Self; Latitude Pharma. H. Research Contractor; Self; Cidara Therapeutics. **A. Chen:** D. Employee; Self; Latitude Pharma. H. Research Contractor; Self; Cidara Therapeutics.

Session Number:

016

Session Title:**New Antifungals****Publishing Title:****Prevention of *Pneumocystis* Pneumonia (PCP) by the Novel Echinocandin, Cd101****Author Block:**

M. T. Cushion¹, A. Ashbaugh¹, K. Lynch¹, M. J. Linke²; ¹Univ. of Cincinnati Coll. of Med., Cincinnati, OH, ²Univ. of Cincinnati, Cincinnati, OH

Abstract Body:

Pneumocystis spp. are obligate pathogenic fungi that cause PCP in mammalian hosts with weakened immune systems. PCP is not responsive to standard antifungal therapy and there are few treatment alternatives. A continuous *in vitro* culture system does not exist for *Pneumocystis* and preclinical drug development is conducted in immunosuppressed rodent models of PCP. Previously, we reported that currently approved echinocandins were not suitable candidates for monotherapy as large numbers of trophic forms remained after 3 wks of treatment, and when released from therapy, asci re-appeared and replication resumed. Asci formation may be essential for the *Pneumocystis* life cycle, so we explored the efficacy of CD101, a novel long-acting echinocandin, as a prophylactic agent to prevent PCP. C3H/HeN mice were infected with *P. murina* by intranasal inoculation of $2 \times 10^6/50 \mu\text{l}$. Mice were immunosuppressed by dexamethasone at 4 mg/L in acidified drinking water. Mice received vehicle (untreated control), trimethoprim/sulfamethoxazole (TMP/SMX 50/250 mg/kg/3X/wk) and CD101 at 20-, 2-, and 0.2- mg/kg individually at 1X and 3X/wk. All drugs were administered at the time mice were inoculated. After 6 wks, mice were sacrificed, lungs homogenized and slides prepared for quantification of trophic forms by rapid Wright-Giemsa stain and for asci by cresyl echt violet staining. Efficacy was based on the reduction of organism burden between treatment and untreated control groups. Counts were log transformed and analyzed by ANOVA and Student-Newman Keuls for multiple comparisons (GraphPadPrismv.6). Prophylaxis of *P. murina*-infected mice with CD101 showed a statistically significant reduction in nuclei levels at all doses except for the 0.2 mg/kg/1x/week group vs the untreated control group. Three of the CD101 treatment groups were as efficacious as TMP/SMX with no nuclei observed by microscopic evaluation. All CD101 treatment groups showed a statistically significant reduction in asci levels vs the untreated control group. There was no difference in efficacy between 5

of the CD101 treatment groups and TMP/SMX, with no asci observed by microscopic evaluation. CD101 inhibits the formation of asci which appears to be critical for replication of *Pneumocystis*. CD101 represents a viable candidate for prophylactic therapy of PCP.

Author Disclosure Block:

M.T. Cushion: None. **A. Ashbaugh:** None. **K. Lynch:** None. **M.J. Linke:** None.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

Efficacy of a Novel Echinocandin, CD101, in a Mouse Model of Azole-Resistant Disseminated Candidiasis

Author Block:

L. MIESEL¹, K. Y. Lin¹, J. C. Chien¹, M. L. Hsieh¹, V. Ong², K. Bartizal²; ¹Eurofins Panlabs, Ltd., Taipei, Taiwan, ²Cidara Therapeutics, San Diego, CA

Abstract Body:

Background: CD101 is a novel echinocandin with long-acting pharmacokinetics and exceptional stability that is being developed for treatment of serious fungal infections. This study objective was to evaluate the *in vivo* efficacy of CD101 in a neutropenic mouse model of azole-resistant candidiasis. **Methods:** An azole-resistant *C. albicans* human blood isolate, strain R357, was used for the mouse disseminated candidiasis model. R357 is resistant to fluconazole (Flu), voriconazole, and posaconazole but is susceptible to amphotericin B (AmB) and echinocandins. Mice were rendered neutropenic with cyclophosphamide then infected by tail vein injection with an inoculum of 10⁵ CFU/mouse. Test articles were administered once, 2 hrs after infection, to groups of five mice treated with AmB (3 mg/kg, intravenous (IV) administration), Flu (20 mg/kg, oral (PO) administration), and CD101 (3, 10 or 30 mg/kg intraperitoneal (IP) administration). Animals were humanely euthanized at 72 hr after infection and *C. albicans* counts in kidney tissue were measured (CFU/g-tissue). One-way ANOVA followed by Dunnett's test was performed to assess the significance of differences between vehicle and test article treatment groups. **Results:** A single 3-mg/kg dose of CD101 was sufficient to yield a significant reduction in *C. albicans* bioburden relative to the vehicle control group (>99.9% reduction in CFU; P<0.05; Table). AmB (3 mg/kg) was also efficacious in this model (>99% reduction in CFU; P<0.05). Flu (20 mg/kg) was less efficacious (83.9% reduction in CFU). **Conclusions:** CD101 by IP administration was effective in a mouse model of disseminated *C. albicans* infection with an azole-resistant *C. albicans* strain. The efficacy supports the potential advancement of CD101 for human use against azole-resistant *Candida* infections.

Azole resistant candidiasis model

Test article (Administration route)	Dose (mg/kg)	Kidney Counts, CFU/g, mean	Kidney Counts, CFU/g, Standard Error (SEM)	Reduction (%) relative to vehicle treatment
Vehicle (IP)	Not applicable	5.6×10^7	1.3×10^7	0
AmB (IV)	3	1.4×10^5	4.6×10^4	99.8%
Flu (PO)	20	9.1×10^6	4.5×10^6	83.9%
CD101 (IP)	3	1.9×10^4	7.4×10^3	100%
CD101 (IP)	10	1.6×10^2	5.7×10^1	100%
CD101 (IP)	30	2.8×10^2	1.2×10^2	100%

Author Disclosure Block:

L. Miesel: D. Employee; Self; Eurofins Panlabs, Ltd.. H. Research Contractor; Self; Cidara Therapeutics, Inc. **K.Y. Lin:** D. Employee; Self; Eurofins Panlabs, Ltd.. H. Research Contractor; Self; Cidara Therapeutics, Inc. **J.C. Chien:** D. Employee; Self; Eurofins Panlabs, Ltd.. H. Research Contractor; Self; Cidara Therapeutics, Inc. **M.L. Hsieh:** D. Employee; Self; Eurofins Panlabs, Ltd.. H. Research Contractor; Self; Cidara Therapeutics, Inc. **V. Ong:** D. Employee; Self; Cidara Therapeutics, Inc. **K. Bartizal:** D. Employee; Self; Cidara Therapeutics, Inc..

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

Absolute Oral Bioavailability of the Novel Antifungal Glucan Synthesis Inhibitor Scy-078 in Healthy Human Volunteers

Author Block:

S. Wring¹, R. Blum², M. Hyman³, W. Kraft⁴, M. Murphy⁵, K. O'Hayer⁴, R. Outcalt¹, M. Willett⁶, D. Angulo¹; ¹SCYNEXIS, Inc, Jersey City, NJ, ²Buffalo Clinical Res. Ctr., Buffalo, NY, ³BCH Res. Solutions, LLC, West Chester, PA, ⁴Thomas Jefferson Univ., Philadelphia, PA, ⁵Riverside Consulting, LLC, Wilmington, DE, ⁶Ready Clinical, LLC, Princeton, NJ

Abstract Body:

Background: SCY-078 is a first-in-class, triterpene-based, β -1,3-D-glucan synthesis inhibitor (GSI), currently in clinical development for treatment of invasive fungal infections. Here we report an estimate of oral bioavailability for SCY-078 determined during an interim sub-analysis of pharmacokinetic (PK) data from 2 Phase 1 clinical studies following either oral (PO) or intravenous (IV) administration of SCY-078 to healthy male volunteers. **Methods:** Twenty-three healthy fasted male subjects received a single 500-mg PO dose of SCY-078 in an open-label Phase I study. SCY-078 was delivered as a novel salt form formulated as a compressed tablet. Sixteen healthy fasted male subjects received the SCY-078 salt as an IV infusion in a double-blind, randomized, placebo-controlled, alternating-panel, single-rising-dose Phase I study, with 6, 6 and 9 subjects receiving 50-, 100- and 200mg IV doses, respectively. Blood samples were collected for PK assays. Descriptive PK parameters were calculated by non-compartmental analysis. **Results:** Mean \pm SD plasma clearance rate was 0.35 \pm 0.025 L/kg/hr for the IV doses. Half-lives for the 50-, 100- and 200mg IV and 500mg PO doses of SCY-078 were 29.3 \pm 4.87 h, 26.2 \pm 3.6 h, 28.2 \pm 8.11 h and 22.3 \pm 4.56 h, respectively. In each instance, half-life was consistent with once per day dosing. Corresponding values for exposure, expressed as area under the curve from 0 h to infinity (AUC_{0- ∞}), were 4.23 \pm 0.73 μ M \cdot h, 6.95 \pm 1.05 μ M \cdot h, 15.2 \pm 3.52 μ M \cdot h and 13.8 \pm 6.37 μ M \cdot h, respectively. Exposure increased in a generally proportional manner across these IV doses. A daily IV dose of \sim 200mg SCY-078 is expected to achieve the \sim 15 μ M \cdot h exposure target associated with efficacy in murine models of disseminated candidiasis. Absolute oral

bioavailability, defined as the ratio of the dose-normalized $AUC_{0-\infty}$ values for the oral to IV doses, was 36.2 ± 3.5 %. **Conclusion:** SCY-078 is orally bioavailable at a clinically practicable level in healthy male volunteers and merits continued development as an oral alternative to azole therapy in the treatment or prophylaxis of fungal infections.

Author Disclosure Block:

S. Wring: D. Employee; Self; Scynexis. **R. Blum:** C. Consultant; Self; Scynexis. **M. Hyman:** C. Consultant; Self; Scynexis. **W. Kraft:** F. Investigator; Self; Scynexis. **M. Murphy:** C. Consultant; Self; Scynexis. **K. O'Hayer:** F. Investigator; Self; Scynexis. **R. Outcalt:** D. Employee; Self; Scynexis. **M. Willett:** C. Consultant; Self; Scynexis. **D. Angulo:** D. Employee; Self; Scynexis.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

Scy-078 is Fungicidal in Time-kill Studies Against *Candida* Species

Author Block:

B. Scorneaux¹, D. Angulo¹, K. Borroto-Esoda¹, M. Ghannoum², M. Peel¹, **S. Wring¹**;
¹SCYNEXIS, Inc, Jersey City, NJ, ²Case Western Reserve Univ., Cleveland, OH

Abstract Body:

Background: SCY-078 is an orally bioavailable β -1,3-D-glucan synthesis inhibitor (GSI) and the first-in-class of structurally novel triterpene antifungals in clinical development for treatment of candidemia and invasive candidiasis. Here we report the time-kill (TK) dynamics of SCY-078 against multiple *Candida* spp. **Methods:** *In vitro* susceptibility by broth micro-dilution (CLSI M27-A3, RPMI pH 7.0 with MOPS, 24 hr incubation) and TK dynamics were determined for 3 reference (Ref) strains (*C. albicans* 90028, *C. parapsilosis* 90018, and *C. tropicalis* 750), a Quality Control (QC) strain (*C. krusei* 6258) and 4 others (*C. albicans* MYA-2732, 64124, 76485 and *C. glabrata* 90030). Amphotericin B (AMB), caspofungin (CSP), fluconazole (FLC) and voriconazole (VRC) were included as comparators. For TK, SCY-078 and CSP were evaluated at 0.25, 1, 2, 4, 8 and 16x MIC₈₀. FLC and VRC were evaluated at 4x MIC₈₀. TK data were fitted to a 3-parameter exponential decay model (SigmaPlot) to determine time to reach 50, 90 and 99.9% reductions in growth from the starting inoculum. Values for net change in CFUs/mL were fitted to 3-parameter sigmoidal Hill model to determine EC₅₀, EC₉₀ and E_{max}. **Results:** *In vitro* MIC values for SCY-078, AMB, CSP, FLC, and VRC against all strains tested were determined by visual inspection and spectrophotometry; agreement between methods was within 2 dilutions. MICs for Ref or QC strains were within acceptable limits (CLSI M27-A3 and M27-S45) for AMB, FLC or VRC. Across all strains MICs were within the range 0.0625 - 1 μ g/mL for SCY-078, CSP and AMB. MICs were generally higher for FLC and VRC (0.0625 - 16 μ g/mL). SCY-078 was fungicidal with a ≥ 3 log₁₀ reduction in CFUs/mL against 7 of the 8 isolates at ≥ 1 x MIC following 24 h incubation. The exception, *C. albicans* 90028, appeared highly robust *in vitro* and fungal reduction was 1.59 and 1.45 log₁₀ for SCY-078 and CSP, respectively. EC₅₀ and EC₉₀ values for SCY-078 were reached between 1.2 - 2.8 h and 3.4 -15.5 h, respectively across strains. Mean values for E_{max} determined at 8, 12 and 24 h were -2.4, -

2.9 and -3.3 log₁₀ CFU/mL for SCY-078 and -2.2, -2.9 and -3.1 log₁₀ CFUs/mL for CSP. FLC and VRC were fungistatic (24 h CFU/mL ≥ 0h) against all isolates. **Conclusion:** SCY-078 is rapidly fungicidal against clinically relevant *Candida* spp.

Author Disclosure Block:

B. Scorneaux: D. Employee; Self; Scynexis. **D. Angulo:** D. Employee; Self; Scynexis. **K. Borroto-Esoda:** D. Employee; Self; Scynexis. **M. Ghannoum:** C. Consultant; Self; Scynexis. **M. Peel:** D. Employee; Self; Scynexis. **S. Wring:** D. Employee; Self; Scynexis.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

Alginate Oligosaccharides Modify Phospholipase Activity and Inhibit Hyphal Infiltration by *Candida albicans* in an *In Vitro* Model of Human Candidosis

Author Block:

M. F. Pritchard¹, A. A. Jack¹, L. C. Powell¹, H. Sadh¹, E. Onsøyen², P. D. Rye², **K. E. Hill¹**, D. W. Thomas¹; ¹Cardiff Univ. Sch. of Dentistry, Cardiff, United Kingdom, ²Algipharma AS, Sandvika, Norway

Abstract Body:

Background: *Candida* spp. are responsible for over 50% of systemic fungal infections and as their incidence increases annually they represent a significant global challenge. A novel alginate oligomer, OligoG CF-5/20, has been shown to interact with a range of fungi inducing anti-fungal activity and inhibiting candidal hyphae formation *in vitro*. This interaction was investigated at the nanoscale to determine if OligoG modified candidal virulence factor expression and invasion of mucosal surfaces *in vitro*. **Methods:** OligoG interactions with the carbohydrate cell-surface of *C. albicans* ATCC 90028 and *C. tropicalis* 519468 were studied using atomic force microscopy and electrophoretic light scattering. Plate substrate assays and 3-dimensional, organotypic, skin-equivalent (reconstituted human epithelium; RHE) models (n=28) were employed to study invasion with *C. albicans* (ATCC 90028). *Candida* invasion in the RHE model was analyzed via histochemistry (Periodic Acid-Schiff staining), immunocytochemistry and confocal laser-scanning microscopy. *Candida* virulence was further assessed utilizing secreted aspartyl proteinase (SAP), phospholipase (PL), coagulase, hemolysis and protease assays. Quantification of changes in relative expression of hydrolytic enzymes was confirmed using quantitative PCR (qPCR). **Results:** Direct interaction between OligoG CF-5/20 and the fungal cell-wall failed to induce significant alterations in surface-charge, but marked reduction in hyphal formation in plate substrate assays (n=3) was evident. This was reflected in reduced invasion by OligoG CF-5/20-treated *C. albicans* in the RHE (0.2%; p<0.001). Significant dose-dependent inhibition in PL assays occurred following OligoG CF-5/20 treatment (p<0.05; n=3). Substrate assays revealed no difference in SAP activity in treated samples. qPCR demonstrated the changes in activity reflected significant, OligoG-CF5/20 -induced, decreases in expression of PLB2 and SAP4 (p<0.05) but

interestingly, not SAP5 and SAP6. **Conclusions:** The inhibition of fungal growth and inhibition of hydrolytic PL enzyme systems of *C. albicans* by OligoG-CF-5/20, represent a novel potential therapeutic approach to fungal disease.

Author Disclosure Block:

M.F. Pritchard: I. Research Relationship; Self; Algipharma AS. **A.A. Jack:** I. Research Relationship; Self; Algipharma AS. **L.C. Powell:** I. Research Relationship; Self; Algipharma AS. **H. Sath:** I. Research Relationship; Self; Algipharma AS. **E. Onsøyen:** D. Employee; Self; Algipharma AS. **P.D. Rye:** D. Employee; Self; Algipharma AS. **K.E. Hill:** I. Research Relationship; Self; Algipharma AS. **D.W. Thomas:** I. Research Relationship; Self; Algipharma AS.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

Antifungal Potential of Antimicrobial Peptide Mimetics in a Mouse Model of Disseminated Candidiasis

Author Block:

M. H. Chowdhury¹, L. K. Ryan², K. B. Freeman³, R. W. Scott³, G. Diamond¹; ¹Univ. of Florida Coll. of Dentistry, Gainesville, FL, ²Univ. of Florida Coll. of Med., Gainesville, FL, ³Fox Chase Chemical Diversity Ctr., Doylestown, PA

Abstract Body:

Disseminated candidiasis is a life threatening fungal infection, associated with a high morbidity and mortality in immunocompromised patients; especially those caused by *Candida albicans* and other non-albicans *Candida* (NAC) species. Due to limited therapeutic options, unintended toxicity as well as emergence of resistant strains emphasizes the urgent need to develop novel antifungal drugs. Antimicrobial peptides (AMPs) are small cationic peptides, are effective as broad-spectrum antibiotics and antifungals, and exhibit limited resistance development. However, high production cost and protease degradation are the main drawback of AMPs. Here we studied *in vitro* activity of eight nonpeptidic molecules that mimic the structure and function of AMPs, and demonstrated their efficacy in a mouse model of disseminated candidiasis. The aim of the study was to establish these AMP mimetics as potential agents to treat systemic candidiasis. We studied *in vitro* activity of these mimetics against *C. albicans* and five other NAC, in the presence and absence of human and mouse serum using an MIC assay. We then quantified their activity in 8 week old CD-1 Swiss-Webster male mice injected (i.p.) with 150 mg/kg cyclophosphamide prior to injecting (i.v.) 3.6×10^4 cfu, a defined mouse model of systemic candidiasis. All mimetics exhibited MIC values below 10 $\mu\text{g/ml}$ against the *Candida* species, and low cytotoxicity as quantified by MTT assay in three human cell lines. All mimetics tested caused rapid fungal membrane permeability as measured by propidium iodide uptake using flow cytometry. A competitive inhibition of the mimetics by divalent cations was also observed. In the mouse model of systemic candidiasis, injection (s.q.) of several of the mimetics in 20% kleptose, 2 hr post-infection, resulted in a reduction of kidney burden at 24 hr post-infection with an efficacy that was comparable to fluconazole. Our data demonstrate that antimicrobial peptide

mimetics are a potential source of antifungal agents that could be developed as novel therapies for systemic candidiasis.

Author Disclosure Block:

M.H. Chowdhury: None. **L.K. Ryan:** None. **K.B. Freeman:** D. Employee; Self; Employee of Fox Chase Chemical Diversity Center. **R.W. Scott:** D. Employee; Self; Employee of Fox Chase Chemical Diversity Center. **G. Diamond:** None.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

A Novel Porous Antimicrobial Space Maintainer Eluting Econazole Has Activity against *Candida* and *Aspergillus*

Author Block:

A. M. Tatar¹, A. J. Salter¹, P. D. Kontoyiannis¹, E. Watson¹, N. D. Albert², G. R. Bennett¹, A. G. Mikos¹; ¹Rice Univ., Houston, TX, ²The Univ. of Texas MD Anderson Cancer, Houston, TX

Abstract Body:

Background: While relatively rare, fungal periprosthetic joint infections (FPJIs), most commonly caused by *Candida* and *Aspergillus* species, result in significant patient morbidity and mortality. Current therapy involves replacing the infected prosthesis with a polymethylmethacrylate (PMMA) spacer impregnated with antifungals for local release and infection clearance. However, these solid spacers have limited drug elution concentration and release duration. Therefore, we have designed a new type of porous spacer capable of extending the local release of therapeutics. **Methods:** Porous spacers made of PMMA and a non-toxic aqueous gel composed of carboxymethylcellulose (CMC) were fabricated as described previously[1]. These porous spacers were loaded with either 2.5% or 5% wt/wt econazole and compared to traditional non-porous econazole-loaded spacers. Cylinders of 6mm in diameter and 12mm in height (n=4 per group) were subject to mechanical analysis per established standards (ISO5833). Spacer discs of 6mm in diameter and 1mm in height were tested in a modified Kirby-Bauer disc diffusion assay (n=3 per group per organism) against clinical isolates of *C. albicans* and *A. fumigatus*. **Results:** Introduction of econazole at either loading dosage did not significantly reduce spacer mechanical properties in either porous or solid spacers. Introducing porosity significantly reduced spacer mechanical properties (offset yield strength and compressive modulus)- however, the properties of the porous spacers still remained sufficient for the demands of space maintenance of the joint[2]. Compared to solid econazole-loaded spacers, porous econazole-loaded spacers in the 5 wt/wt% loading dosage group created a significantly larger zone of inhibition against *C. albicans* and *A. fumigatus*. Unloaded spacers of either type produced no zone of inhibition. **Conclusions:** This new type of spacer can mitigate the growth of the most common fungal pathogens

responsible for FPJIs while preserving sufficient mechanical properties for maintaining space in the joint. By incorporating porosity into the design of the spacer, we have demonstrated significantly increased inhibition of fungal species compared to the solid spacers currently used in clinical practice.

Author Disclosure Block:

A.M. Tatara: None. **A.J. Salter:** None. **P.D. Kontoyiannis:** None. **E. Watson:** None. **N.D. Albert:** None. **G.R. Bennett:** None. **A.G. Mikos:** None.

Session Number:

016

Session Title:

New Antifungals

Publishing Title:

The Use of a Combined Therapy of Itraconazole Plus a Monoclonal Antibody Specific to Neutrophils in Experimental Paracoccidioidomycosis Improves the Control of Infection and Attenuates the Lung Fibrosis

Author Block:

J. D. Puerta-Arias¹, P. A. Pino¹, J. Arango¹, **A. Gonzalez**²; ¹CIB-UdeA-UPB, Medellin, Colombia, ²Univ. de Antioquia, Medellin, Colombia

Abstract Body:

Background: We have previously demonstrated that depletion of neutrophils during the chronic stages of experimental paracoccidioidomycosis (PCM) is beneficial for controlling fungal infection, effect that is accompanied by a decrease of the inflammatory response and fibrotic process, the later considered the most important sequelae in those PCM patients. In the present study, we evaluated the efficacy of a combined therapy using a specific monoclonal antibody (mAb) anti-neutrophils plus itraconazole (ITZ) in experimental pulmonary PCM. **Methods:** Isogenic BALB/c male mice were inoculated i.n. with 1.5×10^6 *P. brasiliensis* yeasts. Groups of mice were treated with a mAb specific to neutrophils, an isotype control and/or ITZ. The treatment started at week 4 post-infection (PI), the mAb was administered i.p. every 48h during two weeks and ITZ was administered (via oral) daily during 8 weeks. Animals were sacrificed at week 12 PI in order to determine fungal burden, collagen levels, neutrophil populations, as well as histopathological aspects. **Results:** We observed that the use of a combined therapy (mAb anti-neutrophils plus ITZ) in experimental PCM was associated with a better control of infection (decreased fungal burden in lungs, liver and spleen) and reduced collagen levels. Surprisingly, in those groups of mice treated with ITZ, it was observed an increase of neutrophils mainly neutrophils type II (Ly6G+/CD11b+), even in those animals depleted of these phagocytic cells. **Conclusions:** Combination of a mAb anti-neutrophils plus ITZ not only improve the control of *P. brasiliensis* infection, but also attenuate the lung fibrosis caused by this fungal pathogen. In addition, these results support the hypothesis that ITZ could modulate the immune response, in this case acting as a chemoattractant for neutrophils. Study supported by Colciencias (project No.183-2010).

Author Disclosure Block:

J.D. Puerta-Arias: None. **P.A. Pino:** None. **J. Arango:** None. **A. Gonzalez:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Comparison of Primary Streaking Results of Two Full Laboratory Automation Systems

Author Block:

M. Jetter, M. Marchesi, **M. Hombach**, P. M. Keller; Univ. of Zurich, Zurich, Switzerland

Abstract Body:

Background: Automation increasingly enters clinical labs. WASP™/WASPLab™ automation system (Copan) uses a loop for sample streaking, while InoquIA™ (BD Kiestra™) uses a ball. Studies comparing the two systems analysed single strains and urine samples. To evaluate the systems for other specimen types, various clinical specimens were streaked with WASP™ and InoquIA™ benchtop (BT) in parallel. Objectives of this study were to: i) Compare WASP™ and benchtop InoquIA™ to inoculate sputum, wound, vaginal, and stool specimens and positive blood culture broth (PBC); ii) Compare both systems ability to generate single colonies; iii) Assess the feasibility of streaking results for a complete diagnostic workup (MALDI-TOF identification, susceptibility testing [AST]). **Methods:** Consecutive clinical specimens and PBC were included in this study. Sputum samples pre-treated with SLSolution™ (1:1 ratio), wound and vaginal specimens in ESwab™, and stool samples in FecalSwab™ were loaded on WASP™ and automatically streaked using a 10 µl loop (WASP™) and 4-quadrant streaking pattern (4Q5 SP); PCB were transferred into BC+™ tubes (Copan) and streaked using both 1 µl and 10 µl loops and a 4Q3 SP. For InoquIA™ 10ul of each sample were manually dispensed on the appropriate agar plates using a manually calibrated pipettor and streaked using SP similar to the WASP™, or SP as suggested by the manufacturer. Plates were incubated in WASPLab™ incubators. Images were taken by WASPLab after various periods of incubation. Growth, streaking pattern, CFU counts, and feasibility for further processing were evaluated by experienced personnel on-screen. **Results:** Overall, comparable numbers of single colonies were present in all WASP™ “loop based” and “ball-based” benchtop InoquIA™ processed specimens. Single colonies in appropriate numbers were obtained in all specimen types allowing MALDI-TOF based identification, AST and additional testing, in particular comparing the PBC with the 1 µl loop versus the 10 µl loop or pipettor. **Conclusions:** Comparison of

streaking results for complex clinical specimens demonstrated that WASP™ and Inoqula BT™ produced sufficient single colonies for MALDI-TOF identification and AST. WASP™ was superior to the Inoqula BT™ in particular for PBC. In addition, WASP™ produced SP were more user-friendly and allowed easier adaptation from manual to automated reading.

Author Disclosure Block:

M. Jetter: None. **M. Marchesi:** None. **M. Hombach:** None. **P.M. Keller:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Left Out: Analysis Of Time Primary Culture Medium Spends Outside The Incubator During Routine Workup

Author Block:

N. Anderson¹, A. McMullen¹, R. Jennemann²; ¹Washington Univ. in Saint Louis Sch. of Med., Saint Louis, MO, ²Barnes Jewish Hosp., Saint Louis, MO

Abstract Body:

Background: The workflow of a microbiology laboratory requires cultures spend time outside of incubation to perform necessary workup. Bacterial recovery from clinical specimens relies on adequate incubation of culture media. We evaluated the amount of time primary culture medium is not in an optimal incubation environment during routine workup in the microbiology laboratory of a 1250 bed tertiary care hospital. **Methods:** We recorded time spent outside of the incubator by aerobic primary media for routine bacterial culture from wounds, tissues, and sterile fluids. Specimens were plated immediately following receipt, 24 h a day. Primary media were incubated in 5% CO₂ at 35°C. Plates were removed in the morning and returned to the incubator in batches as workup was finished. Plates were not returned if they required additional analysis, such as MALDI-TOF, susceptibility testing, or consultation. All plates were removed for a second reading in the afternoon, and plates that required extra testing were returned to the incubator. On days 1-4, culture age and time outside of the incubator was recorded for each plate when examined. **Results:** The age of each plate and the cumulative time outside of the incubator are shown in Table 1. While time spent outside of incubation is small for plates read on day 1, this dramatically increases over days 2-4. When examined on day 2, plates are 26-50h old and have spent an average 2h9m outside the incubator, possibly affecting the recovery of organisms taking 24-48h to grow. Extra testing, including MALDI-TOF, susceptibility testing, or consultation, added an average 3h53m to the total time spent outside of the incubator. **Conclusion:** Time spent outside of the incubator can be significant and may affect growth of certain organisms. The introduction of process improvements, including total lab automation, may address this problem.

	Day 1 n=232	Day 2 n=232	Day 3 n=147	Day 4 n=35
Plate age (range)	1h51m- 25h37m	26h29m- 50h2m	51h5m- 75h17m	78h22m- 96h50m
Plate age (median, IQR)	17h58m, 6h46m	42h1m, 6h32m	65h3m, 6h10m	86h59m, 9h24m
Cumulative time outside incubator (average)	26m	2h9m	5h48m	9h58m
Cumulative time outside incubator (range)	2m-2h1m	52m-7h20m	3h3m- 11h57m	6h22m- 18h27m
Cumulative time outside incubator (median, IQR)	22m, 27m	1h56m, 50m	5h23m, 2h52m	8h45m, 5h3m

Author Disclosure Block:

N. Anderson: None. **A. McMullen:** None. **R. Jennemann:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Changing from Transystem to Eswab™, a Liquid Based Microbiology (LBM) Device, for Processing Clinical Specimens with WASP™/WASPlab™ in a Microbiology Laboratory

Author Block:

L. Navarria, A. Giambra, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Background: Transystem (TS) has a rayon swab with a plastic tube with Amies' agar-gel or a sponge with liquid Amies and has been used for specimen's collection for bacteria culture. Copan developed the ESwab™ (ES), associating a FLOQswab™ to a 1 ml tube of Liquid Amies medium, introducing the LBM concept to enable automated bacteriology specimens processing. ES is device for the collection of clinical specimens for bacterial culture and can be used with manual and automated specimen's inoculation methods. An adequate validation is necessary to change from TS to ES with WASP™ (WA) automation, since different inoculation methods are required: TS by direct swabbing, ES by volumetric system. The objectives of this study were to: 1) Quantitate the volume of ES that corresponds to direct TS swabbing for bacteria culture inoculation. 2) Compare culture growth in plates and Gram-smears (GS) generated from ES spiked clinical specimens processed by WA versus manual direct TS swabbing. **Methods:** 1) ES and TS were spiked with 100uL of a countable suspension of *E.coli* (EC), *S.aureus* (SA) *C.albicans* (CA) and *P.aeruginosa* strains. TS were seeded with manual swabbing on the first quadrant (FQ) in blood agar plates (BAP) while ES were loaded on WA and seeded on BAP with 30ul loop and 5QT1 SP. 2) ATCC strains, 10uL each (10^7 or 10^9 CFU/ml) were used to spike ES samples collected from volunteers, Vaginal with both *EC* (10^7) and *CA* (10^9), Nasal and throat with SA and *S.pyogenes* (10^7). All TS were seeded manually, swabbing the FQ in agar plate and preparing the GS, while all ES were loaded on WA using 30ul loop for smear preparation and for seeding appropriate agar plates with a 5QT1 SP. All plates were transferred to WL and incubated at appropriate conditions. After 24h, all plates were recorded and colonies counted and interpreted. **Results:** The CFUs obtained from ES streaking by WA were comparable to manual TS. WA plated ES samples had the same results as manual TS. ES WA streaked plates had more isolated

colonies than manual TS. The ES WA prepared GS were well distributed and stained with more elements than the TS. **Conclusion:** It was demonstrated that 30ul ES sample is the optimal volume to use with WA in comparison to manual TS. Better results in terms of colonies quality and distribution were obtained from ES plates and GS processed by WA automation than TS.

Author Disclosure Block:

L. Navarra: None. **A. Giambra:** None. **S. Castriciano:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Evaluation of Eswabtm transport Medium with the Latest Clsi M40-A2 Standard

Author Block:

R. Botrugno, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Objectives: The latest revision of the CLSI standards for Quality Control of Microbiological Transport System (M40-A2 published June 2014) introduced new acceptance criteria for the evaluation of the transport systems. Copan ESwabTM (a flocculated swab and a tube with 1 ml of modified Liquid Amies medium), has been already confirmed to be in full compliance with the previous standard. The objective of this study is to verify the conformity of the ESwabTM with the latest CLSI M40-A2 standard acceptance criteria using both Swab Elution Method and Roll-plate Methods suggested by the guideline. **Methods:** As per guideline instructions, ATCC strains of *P.aeruginosa* ATCC BAA-427 (only for overgrowth study), *H. influenzae* ATCC 10211, *S. pneumoniae* ATCC 6305, *S. pyogenes* ATCC 19615, *P. melaninogenica* ATCC 25845, *B. fragilis* ATCC 25285, *P. anaerobius* ATCC 27337, *P. acnes* ATCC 6919, *F.nucleatum* ATCC 25586, *N.gonorrhoeae* ATCC 43069 were used for this study. Bacterial suspensions for each strains were prepared from fresh subculture to obtain a 0.5 McF, and then diluted tenfold in physiological saline to provide working suspensions of approximately 1.5×10^7 CFU/mL for the Swab Elution Method and 1.5×10^3 CFU/mL for the Roll-Plate Method. For the Swab Elution Method the procedure applied is described in the CLSI M40-A2 document par. 8.11. For the Roll-Plate Method the procedure applied is described in the CLSI M40-A2 document par. 8.12. All testing was performed in triplicate, three devices for zero-time and three devices for each subsequent incubation/storage time (24/48 hours Room Temperature, 24/48 hours Refrigerated Temperature, with the exception for *N. gonorrhoeae* only 24 hours). At each interval, the inoculated devices were plated and incubated as per M40-A2. **Results:** For all ATCC bacteria strains, for both methods at each storage condition and time, results obtained were all well within the acceptance criteria defined in the guideline. **Conclusions:** ESwabTM transport medium is fully compliant to the new CLSI M40-A2 standard for all the bacteria strains tested. These results confirm the high performance and quality of the

ESwab™ as transport medium for aerobes, anaerobes and fastidious bacteria in the clinical diagnosis.

Author Disclosure Block:

R. Botrugno: None. **S. Castriciano:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Comparison of the Original SLSolution (SLS1) to the New SLSolution (SLS2) to Pre-Treat Mucus-Rich Specimens for the Detection of Bacteria Using WASP™ Automation

Author Block:

L. Conter, B. Massetti, R. Paroni, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Background: Copan developed many LBM devices to facilitate processing of bacteriology clinical specimens' with WASP™ automation. Originally, the SLSolution (SLS1), a ready to use mucus dissolving solution, was developed for the pre-treatment of sputum samples to process on the WASP™ and its performance was validated against other mucus dissolving solutions. During 2015, a PET tube was used for the SLSolution (SLS2) to prevent oxidation and eliminate the five tubes vacuum package. The objective of this study was to compare the original (SLS1) to the new (SLS2) ability to dissolve mucus samples to process with for WASP™ automation for bacteria detection. **Methods:** Sputum samples (N=80) (30 reported negative, containing only normal flora and 50 unknown), were used for this comparison. The 30 negative samples were spiked with ATCC bacteria stains (*S. pneumoniae* 6305; *H. influenzae* 10211; *M. catarrhalis* 25238; *K. pneumoniae* 700603; *L.pneumophila* 33152; *B.pertussis* 8467; *S.pyogenes* 19615; *P. aeruginosa* 27853; *S. aureus* 6538; *C. albicans* 10231) and the 50 unknown samples treated in duplicate, one with a 1:1 ratio (SP/SLS1), and the other with a 1:1 ratio (SP/SLS2). Samples were loaded on the WASP™ and processed using the predefined protocol (Vortex at 2500 RPM for 30 seconds, prepare a Gram smear, and plated with 10ul loop and 4Q T5 streaking pattern on appropriate agar). All smears were Gram stained and results recorded. The 30 spiked samples were cultured after 2, 4, and 6 hrs storage at RT. All plates were incubated at 24/48 hrs at 35⁰C under appropriated conditions and culture results were recorded. **Results:** The 30 spiked sputum samples treated with SLS1 and SLS2 showed 100% concordance with both Gram and culture results. Good culture stability results compared to zero time was detected after 2, 4 and 6 hrs at RT. In the 50 unknown samples, treated with SLS1 and SLS2, were detected 36 positive and 14 negative. **Conclusions:** All results obtained in this study, demonstrated

that Copan original SLS1 and the new SLS2 showed no difference in recovering bacteria by culture and Gram stain with WASP™ automated processing. Good culture performance and stability was found in sputum samples treated with new SLS2. The new SLS2 in PET tubes can be used to pre-treat samples, and enables rapid and uniform fluidization without pre-incubation.

Author Disclosure Block:

L. Conter: None. **B. Massetti:** None. **R. Paroni:** None. **S. Castriciano:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Fecalswab™, Selenite Broth and WASP™ Improve the Detection of Enteric Pathogens from Human and Veterinary Stool Samples

Author Block:

S. Castriciano¹, J. Steenbergern²; ¹Copan Italia, Brescia, Italy, ²AML, Antwerpen, Belgium

Abstract Body:

Background: Enteric pathogens are causing infections in human and veterinary population. Detection and prevention of enteric infections require screening for *Salmonella* (SA), *Shigella* (SH), *Campylobacter* (CA) and *Yersinia* (YA). For enhanced detection of SA and SH selective broths are widely used. Stool specimens from human and veterinary origins (~1500/month) are received in the laboratory. Copan is producing the FecalSwab™ (FS), a FLOQSwab™ and a 2mL tube of Cary Blair medium for stool collection and the Selenite (SE) broth for selective enrichment of SA and SH. Prior implementing these devices in our laboratory we validate its performance. The objectives of this study were to: 1) Validate the performance for the FS and SE for the detection of enteric pathogens from stool samples processed on the WASP™. 2) Implement FS and SE broth in our laboratory for processing stool specimens on the WASP™. **Methods:** For the validation of the FS 100 stools, received in dry containers, were tested in duplicate, one using a swab to inoculate the agar plates and SE broth and Gram-smear. All agar plates were manually using a 4Q streaking pattern. For the FS protocol, the FLOQSwab™ was used to transfer the stool to the Cary Blair medium and loaded on the WASP™ and processed using a defined protocol (10ul loop for Gram-smear, SE broth inoculation and agar seeding with 4Q streaking pattern). After incubation all media plates for both protocols were read as per SOPs; SE broth were loaded on the WASP™ and streaked onto selective media plate for SA and SH. **Results:** In comparison to the manual method, standardized and easy to read Gram-smears, and more single colonies were obtained with the WASP™ processed FS samples. One more CA species was isolated using WASP™ automation. Positive (N=16) samples were detected (12 CA, 2 SH *sonnei* and 2 SA). **Conclusions:** More positive samples were detected from stool collected with the Copan FS. The SE broth detected all SA and SH and was easily processed on the

WASP™. FS and SE broth demonstrated to be better devices for transporting, storing and selective detection of gastrointestinal pathogens. To date, 30,000 stools samples have been tested. Stool protocol of FecalSwab™ collection, selective enrichment in Selenite broth and WASP™ processing, improved laboratory workflow, productivity and positivity rate.

Author Disclosure Block:

S. Castriciano: None. **J. Steenbergern:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Wasp™ and Wasplab™ Automation Allows Faster Turn Around Time to Identification and Susceptibility Testing of Positive Blood Cultures

Author Block:

M. Gaskin, D. Yamamura, J. Korver; Hamilton Regional Lab. Med. Program, Hamilton, ON, Canada

Abstract Body:

Background: Rapid identification and susceptibility testing (IDS) of positive blood cultures is crucial for establishing effective antibiotic therapy in patients with sepsis. With the introduction of WASP™WASPLab™ automation in our laboratory, we decided to establish a workflow to process all the positive Blood cultures (BC) using WASP™WASPLab™ automation in order to improve turn-around time for reporting IDS. **Methods:** 203 blood culture bottles flagged positive by the BacTAlert system (Biomerieux) at the HRLMP microbiology laboratory were included. BC samples were transferred to a BC+ vacuum tube (Copan) and loaded onto the WASP™ (Copan) and tested with the established protocols (1ul loop for the gram smear preparation and streaked on appropriate agar plates) and incubated in a CO₂ WASPLab™ smart incubator. Automated digital imaging of the plates was done at 4h, 6h, 12h and 18h. Images were analyzed digitally. Plates with growth detected at 6h were called out of the smart incubator and cultures were tested for identification by MALDI-ToF MS using Vitek MS (Biomerieux). Sensitivities were performed using the Vitek 2 (Biomerieux). Plates were then loaded back into the smart incubator for further image analysis. Gram stain results, identification and sensitivity were compared to the routine laboratory work up. **Results:** Use of WASP™WASPLab™ automation optimized incubation times and workflow. In the 203 blood cultures analyzed, 43% had growth at 4h, 93% at 6h and 99% at both 12 h and 18h. Of those with growth at 6h, 94% were identified by MS and matched the routine laboratory identification 100% of the time. Gram stain results matched 100% as well. Nine of the 203 blood had polymicrobial cultures. Susceptibility testing with 6 h growth, showed 100% essential and categorical agreement (EA, CA) for gram positive organisms except for clindamycin and coag negative staph (CA 57%). For gram negative bacilli, EA and CA were 99.1 and 99%. **Conclusion:** The data obtained

from this validation demonstrated that WASP™WASPLab™ automation effectively facilitated processing BCs samples. Early identification and susceptibility testing at 6 hours using the WASP WASPLab automation with VitekMS and Vitek2 is an accurate and efficient method to support antimicrobial stewardship and facilitate patient care.

Author Disclosure Block:

M. Gaskin: None. **D. Yamamura:** None. **J. Korver:** None.

Session Number:

090

Session Title:

Front-end Automation in the Clinical Microbiology Laboratory

Publishing Title:

Short Time to Diagnosis by Fully Automated Streaking of Positive Blood Cultures

Author Block:

M. Jetter, M. Marchesi, P. M. Keller, **M. Hombach**; Univ. of Zurich, Zurich, Switzerland

Abstract Body:

Introduction: Time-to-diagnosis is critical in sepsis patients as an early targeted treatment significantly decreases morbidity and mortality. We aimed to speed-up pathogen identification and antimicrobial susceptibility testing (AST) from positive blood culture samples using fully automated WASP/WASPLabTM (Copan) sample processing and MALDI-TOF-MS identification. **Methods:** 63 individual positive blood culture broths reported positive by BacT/ALERT[®] (BioMérieux) were immediately transferred into vacuum tubes (Copan, Italy) and processed in the WASP/WASPLabTM automation system including automated streaking by WASPTM (Copan), incubation in WASPLabTM incubators, and automated imaging by WASPLabTM after 3h, 6h, 18h, 24h, and 40h of incubation. Images were checked for growth on-screen. If growth was detected, MALDI-TOF identification (ID) using a Bruker Biotyper (Bruker Daltonics) and AST were done. If MALDI-TOF and/or AST were not possible, plates were re-incubated until the next imaging time. Results were compared a classical manual workup including overnight incubation. **Results:** After 3h of incubation growth rate was 45%. MALDI-TOF and AST were possible for 37% and 45% of samples, respectively. After 6h of incubation MALDI ID and AST rates were at 93%. At 18h and 24h of incubation MALDI-TOF ID and AST-rates of 97% and 100% were yielded. Discrepancies in successful MALDI-TOF ID and AST rates were observed mainly for coagulase-negative staphylococci and streptococci at early time-points. **Conclusion:** Automated periodic plate-screening accelerates time-to-diagnosis providing same-day results in contrast to the classical manual work-up. The accelerated work-up with Copan WASPLabTM facilitated rapid MALDI-TOF ID superseding additional extraction procedures and enabled AST at the same time with potential impact on patient morbidity and mortality.

Author Disclosure Block:

M. Jetter: None. **M. Marchesi:** None. **P.M. Keller:** None. **M. Hombach:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Seroepidemiology of Japanese Encephalitis Virus Infection in Taiwanese Adults

Author Block:

J-T. Lee¹, C-L. Su², S-F. Chang², P-Y. Shu², L-M. Huang³; ¹Natl. Taiwan Univ. Hosp., Yun-Lin Branch, Yunlin, Taiwan, ²Ctr.s for Disease Control, Ministry of Hlth.and Welfare, Taipei, Taiwan, ³Natl. Taiwan Univ. Children's Hosp. and Coll. of Med., Natl. Taiwan Univ., Taipei, Taiwan

Abstract Body:

Background: Japanese encephalitis (JE) has become a disease mainly in adults in the past decade. A survey conducted by Taiwan Centers for Disease Control in 2002 showed that those born between 1963 and 1975 had the lowest seroprevalence of JE-neutralizing antibodies. We aimed to investigate the trend of JE seroepidemiology and its implication of vaccination strategy.**Methods:** We collected serum samples from swine farmers and general population older than 20 years old in 2012 and 2013. The sera were subjected to plaque reduction neutralization test (PRNT). Seropositivity was defined as the PRNT₅₀ titer $\geq 1:10$. Immunoglobulin G (IgG) antibodies against JE virus non-structural protein 1 (NS1) was assayed using an in-house enzyme-linked immunosorbent assay. A positive anti-NS1 IgG implied natural infection by JE virus.**Results:** A total of 444 serum samples (swine farmers: 149; general population: 295) were evaluated. The seropositive rate of PRNT₅₀ was the lowest (~57%) for birth cohort 1970-1980 who received the last dose of JE vaccine ~30 years ago and the highest (86.3%) for those born before 1952 who did not receive the vaccine. Compared with the seroepidemiological surveillance done in 2002, the seropositivity of PRNT₅₀ in 2012 tended to be higher in the population born before 1970-1975, especially for swine farmers in birth cohorts 1963-1969 (76.9% vs. 54.3%, $p=0.022$) and 1953-1962 (82.5% vs. 68.3%, $p=0.056$). Swine farmers (21/149, 14.1%) were more likely ($p=0.005$) to be positive for anti-NS1 IgG than the general population (18/295, 6.1%).**Conclusions:** Taiwanese adults, who received the last dose of JE vaccine more than 30 years ago and especially for swine farmers, were potential target groups for JE virus vaccine booster.

Author Disclosure Block:

J. Lee: None. **C. Su:** None. **S. Chang:** None. **P. Shu:** None. **L. Huang:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Concurrent Infections of Chikungunya Virus with Malaria and Typhoid in Children

Author Block:

A. W. Mwangula¹, L. A. Mwamburi¹, M. Mwau², D. N. Siamba³, F. W. Wanyama⁴;
¹Univ. of Eldoret, Eldoret, Kenya, ²Ctr. for Infectious & Parasitic Diseases Control Res. (CIPDCR), Kenya Med. Res. Inst., Busia, Kenya, ³Masinde Muliro Univ. of Sci. and Technology, Kakamega, Kenya, ⁴Univ. of Kabianga, Kericho, Kenya

Abstract Body:

Background: Fever is common medical sign and may result from many different conditions ranging from benign to potentially serious. Children typically get higher and quicker fevers, reflecting the effects of the pyrogens upon an inexperienced immune system. Symptoms and signs of chikungunya virus (CHIKV) infections are quite similar to those of malaria and typhoid fever. Malaria and typhoid investigations are routinely carried out to establish the cause of pyrexia of unknown origin (PUO) and treatment follows with complete neglect of CHIKV infections. Thus, CHIKV fever cases can sometimes be misdiagnosed or occur simultaneously with malaria, typhoid fever or both. **Methods:** This study was conducted to determine the concurrent infections of malaria and/or typhoid fever with CHIKV, among febrile children aged 1- 12 years seeking treatment in Alupe District Hospital, Busia Kenya. Blood smears were prepared for detection of malarial parasites and serum sample for widal test. Serum (1 ml) was stored in cryogenic vials and transported in dry ice to Kenya Medical Research Institute (KEMRI), Centre for Infectious and Parasitic Disease Control Research (CIPDCR) for laboratory testing. Enzyme-linked Immunosorbent Assay (ELISA) and Plaque Reduction Neutralisation Test tests were performed to detect the CHIKV antibodies. **Results:** The median (IQR) age for the febrile children was 54 months and 55.5% were female. Concurrent infection of CHIKV with malaria or typhoid was 9.6% and 7% respectively using the ELISA technique and 10.5% and 9.9% using PRNT technique, respectively. **Conclusions:** CHIKV should be tested for in cases of patients presenting with fever.

Author Disclosure Block:

A.W. Mwongula: None. **L.A. Mwamburi:** None. **M. Mwau:** None. **D.N. Siamba:** None. **F.W. Wanyama:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Reemergence of Novel Reassortant Avian Influenza H14N3 from Wet Market in Pakistan

Author Block:

N. Siddique¹, M. A. Abbas¹, S. Rafique¹, R. Farooq², K. Naeem¹; ¹Natl. Agricultural Res. Ctr., Islamabad, Pakistan, ²Directorate of Poultry Production and Res., Karachi, Pakistan

Abstract Body:

Background: Since the isolation of original four isolates of H14 AIV in central Asia during 1982, these rare viruses had never been reported in any Asian country. In 2013, during routine AIV surveillance three H14N3 isolates recovered from live bird market in Pakistan. These AIV isolates were subjected to sequencing, phylogentic and serological evaluation. **Methods:** The specimens of cloacal swabs were subjected to virological evaluation through embryonated SPF chicken egg inoculation. Subtype identification was determined by HA, HI techniques along with RT-PCR and QRT-PCR procedures using sequence specific primers and probes. The purified PCR products were directly used for cycle sequencing reactions and then sequenced in a genetic analyzer. Phylogenetic analysis was conducted using *MEGA -4*. **Results:** The whole genome sequencing revealed introduction of a unique reassortant Eurasian- American avian strain into Pakistan. Phylogenetically HA gene shares 92% nucleotide sequence identity with H14 isolates from Wisconsin and California. The NA genes showed 97.7% sequence homology with European H11N3 isolates. The M, PB1, PB2 and PA genes were Asian in origin whereas NS and NP genes showed maximum sequence identity with the Pakistani H3N1 and H4N6 AIVs recovered during 2010-2011 respectively. Sequence analysis revealed a LP amino acid motif (PDKQAK) at HA cleavage site, avian-like receptor specificity (Q243 and G241) and 7 N-linked glycosylation sites while 2 glycosylation sites were lost due to S23I and N278G mutations. Moreover, the glycosylation sites in NA gene were conserved. The amino acids known to be associated with sensitivities to antiviral drugs (oseltamivir, zanamivir, amantadine) were found conserved. The NS gene C-terminal possess ESEV PDZ domain motif. Seroprevalence of H14N3 was negligible in various birds species. **Conclusions:** These observations suggested that these AIVs may persist in

environmental stasis for long periods of time, undetected by surveillance, until a time when agent, host, or environmental factors allow for reemergence in susceptible hosts within a region. The various point mutations in these novel reassortant H14N3 and close relationship with American, European and Asian AIVs also reflect the genetic diversity and intercontinental spread.

Author Disclosure Block:

N. Siddique: None. **M.A. Abbas:** None. **S. Rafique:** None. **R. Farooq:** None. **K. Naeem:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Georgian Public Health Response to Newly Diagnosed Cases of Crimean-Congo Hemorrhagic Fever

Author Block:

N. Mamuchishvili¹, K. Zakhashvili¹, D. Echeverria¹, N. Heyer², P. Imnadze¹; ¹Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, ²Battelle Georgia Branch, Tbilisi, Georgia

Abstract Body:

Background: The first laboratory confirmed case of Crimean-Congo Hemorrhagic Fever (CCHF) was diagnosed in 2009 in Tbilisi. Since then, 44 cases have been reported with a case-fatality of 11.3%. The majority of cases came in contact with infected ticks from the genus *Hyalomma* (genus *Nairovirus*, family *Bunyaviridae*). **Objective:** In 2014 the NCDC conducted a risk communication campaign (RCC) in 24 impacted villages to inform the population in areas where cases of CCHF occurred and to encourage early detection and treatment. **Methods:** The RCC that was conducted in 24 villages located in 4 foci in Eastern Georgia determined by the location of CCHF cases. Teams included members of NCDC, Tbilisi ID Hospital, regional Public Health Centers, and local doctors. A short, structured interview and data collection form was developed. Descriptive statistics were used to characterize the villages with respect to location, size and history of CCHF cases as well as the demographics and personal risk factors associated with initial and post-education knowledge of CCHF. Logistic regression was used to evaluate potential associations between village and informant characteristics with initial and post-education CCHF knowledge. **Results:** The RCC teams were well received. An average of 26% of houses was contacted in each village. A total of 1512 household informants were interviewed and 73.1% were female. The majority were informal farmers (77.9%). Average initial CCHF knowledge was 12.8% which increased to 68.3% post-RCC. The size of village (<=1000 inhabitants) and history of cases in villages, and owning a pig were associated with greater initial knowledge. Village size and owning a pig remained strongly associated with post-RCC improvement in knowledge, along with holding a non-farm job, and being interviewed in the street as opposed to inside the informant's house. **Discussion:** The RCC had an impact on overall

knowledge of CCHF. Scores were higher when residents were more engaged in groups on the street by outreach teams. The results provide a baseline against which proposed future RCCs can determine retention over time.

Author Disclosure Block:

N. Mamuchishvili: None. **K. Zakhashvili:** None. **D. Echeverria:** None. **N. Heyer:** None. **P. Innadze:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Epidemiological Analysis of Dengue Fever and Estimate the Potentially Beneficiaries of Newly Developed Vaccine

Author Block:

M. Modi, K. Madhavani, N. Patel, S. Nanda, T. Javadekar; Med. Coll. Baroda, Maharaja Sayajirao Univ., Vadodara, India

Abstract Body:

Background: In recent years, Dengue fever is re-emerged as mosquito borne viral disease across the Globe, which is endemic in Tropics & expanding to the new regions of the world without any hurdle. This study was conducted to estimate the burden of the disease in Vadodara, in western part of India, & to estimate the number of cases where newly developed dengue vaccine might help. Epidemiological analysis was done to characterize the age & gender related prevalence & seasonal variation in infection. **Methods:** Retrospective analysis was done spanning over the period of 5 years and 9 months, from March, 2010 to December, 2015. Samples were received in cold chain from within the hospital and from peripheral centers in rural areas depending on the clinical signs, symptoms, laboratory investigations and condition of the patient. Testing was done by Enzyme Linked Immuno-Sorbent Assay (ELISA) for presence of NS1 antigen and IgM antibody depending on the days of illness. Both, internal and external, positive and negative controls were tested in each run for the validation and quality control. **Results:** Out of total 6,716 patients, 1,749 (26.04%) patients had confirmed dengue infection. 1,151 (65.81%) were males and 598 (34.19%) were females.

		Total	Positive	%	Male	%	Female	%	
	2010	483	146	30.23	92	63.01	54	36.99	
	2011	718	58	8.08	38	65.52	20	34.48	
	2012	827	160	19.35	100	62.50	60	37.50	
	2013	1763	497	28.19	315	63.38	182	36.62	

	2014	1222	342	27.99	234	68.42	108	31.58	
	2015	1703	546	32.06	372	68.13	174	31.87	
	TOTAL	6716	1749	26.04	1151	65.81	598	34.19	
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	TOTAL
2010	41	45	32	12	10	2	4	0	146.00
2011	9	19	13	9	4	2	2	0	58.00
2012	38	51	44	9	9	6	2	1	160.00
2013	79	169	141	58	26	19	4	1	497.00
2014	37	122	92	47	23	11	7	3	342.00
2015	63	176	188	63	27	15	13	1	546.00
TOTAL	267	582	510	198	99	55	32	6	1749.00
%	15.27	33.28	29.16	11.32	5.66	3.14	1.83	0.34	100.00

Highest numbers of the infection was noted during September and October in each year, with intermediate number was recorded during November and December. Adolescent (11-20) & young age (21-30) were highly affected as compared to children, middle and older age. Nearly 75% of patients fall in the age group of 9-45 years, which is the recommended age group for the newly developed vaccine. **Conclusion:** Study result shows dengue fever is affecting males more than females. Adolescent as well as young people's are more affected as compared to extremes of ages. Effective preventive measures should be implemented in pre-monsoon season as considerable increase in infection is noted in post-monsoon season. Newly developed vaccine for dengue can able to help tremendously.

Author Disclosure Block:

M. Modi: None. **K. Madhavani:** None. **N. Patel:** None. **S. Nanda:** None. **T. Javadekar:** None.

Session Number:

091

Session Title:

Global Health and Emerging Infections

Publishing Title:

Public Awareness About Dengue Fever, Its Perceived Severity and Preventive Practices in Dengue Endemic City: Karachi, Pakistan

Author Block:

T. R. Siddiqui, S. Ghazal, S. Bibi, W. Ahmed; Pakistan Med. Res. Council, Karachi, Pakistan

Abstract Body:

Background: Dengue prevention is the most effective way to reduce the risk of dengue infection especially in endemic countries like Pakistan. Review of public awareness about dengue fever (DF) & its perceived severity and susceptibility plays an important role to make strategies for disease control. This study was conducted to assess the level of knowledge, perceived severity and susceptibility and preventive practices against DF in Karachi. **Methods:** This was a community based cross sectional study. Randomly selected 6 towns were visited, 2 persons (male+ female) from each household were interviewed through structured questionnaire and household preventive practices were observed. Latter the information regarding DF was shared among participants through printed leaflet written in native language. **Results:** Total 608 resident mean age 33.2 ± 13.35 of Karachi were interviewed. Mean knowledge score about DF was 10.83 ± 3.22 out of 23. Perceived severity and susceptibility against DF was 4.4 ± 1.61 out of 9. Mean score of preventive practices was 9 ± 1.8 out of 17. Higher knowledge and high perceived severity and susceptibility is positively correlated with preventive practices ($r = 0.370, 0.318$) (p-value 0.000 for both) respectively. Literacy and socioeconomic status was significantly associated with knowledge and perceived severity and susceptibility of DF (p-value 0.000 each). **Conclusions:** Public knowledge regarding DF is little in Karachi. Positive correlation between preventive practices and high knowledge and severity perception indicate that dengue preventive strategies should focus on growing awareness in the population regarding risk of acquiring diseases, its severity and preventive measures.

Author Disclosure Block:

T.R. Siddiqui: None. **S. Ghazal:** None. **S. Bibi:** None. **W. Ahmed:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Nanomicrobiology: An Alternative Approach to Control Infections

Author Block:

R. Amin; Natl. Inst. of Laser Enhanced Sci. (NILES), Cairo Univ., Giza, Egypt

Abstract Body:

The threat of resistance to antimicrobial drugs has reached a global emergency stage, therefore, there is a need for developing a new approach to tackling antimicrobial drug resistance. Nanomicrobiology research is gaining a great importance in controlling infectious disease due to the unique properties of nanomaterials. Antimicrobial activity of some newly biosynthesized nanomaterials like Curcumin, Chitosan and their metal conjugates will be presented. A microplate assay for monitoring the antimicrobial activity of the nanomaterials was evaluated and established. Results showed that microplate assay could be used as a rapid and sensitive method to assess the antimicrobial activity of the prepared nanomaterials. Biosynthesis method produced nanomaterials in a clean, non-toxic and ecologically sound manner. Silver nanoconjugates of both Curcumin and Chitosan increased the antimicrobial effect on multi-drug resistant bacteria. In conclusion, nanomicrobiology research should gain considerable attention to understanding the properties of nanomaterials and their effect on microbes for considerable applications in controlling infectious disease.

Author Disclosure Block:

R. Amin: None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Gold Nanoparticle-assisted Laser Therapy for the Disruption of Methicillin-resistant *Staphylococcus aureus* Biofilms

Author Block:

D. Kirui, G. Weber, T. Burton, N. Millenbaugh; Naval Med. Res. Unit San Antonio, Joint Base San Antonio(JBSA), San Antonio, TX

Abstract Body:

Multi-drug resistant (MDR) pathogens are becoming the most common cause of infectious disease-related deaths around the world, killing more Americans every year than colon and breast cancer combined. Furthermore, infections caused by MDR bacterial strains, which comprise 46-51% of all isolates, complicate the treatment and recovery of combat-injured US military personnel and adversely impact battlefield readiness. MDR pathogens commonly exhibit increased tolerance to antibiotics due to formation of biofilms that serve as a protective barrier against the immune system and antibiotic treatment. There is an urgent need to discover alternative strategies that are less prone to drug resistance and can improve the efficacy of mainstay antibiotic regimens. Here, we evaluated the use of laser therapy as a potential alternative strategy to destroy and disperse bacterial biofilms and cells using a methicillin-resistant *Staphylococcus aureus* (MRSA) infection model. Overnight cultures of MRSA were suspended at 10^7 CFU/mL in tryptic soy broth supplemented with 10% human plasma and grown in fibrinogen-coated glass bottom 96-well microplates at 37°C under static conditions. Biofilms were pre-treated with 0, 125, 250 or 500 µg/mL of gold nanoparticles (GNPs) for 5 h and then treated with pulsed laser irradiation (532 nm, 8 ns, 1 Hz). Confocal microscopy was used to assess the extent of biofilm damage, while colony forming unit assays were used to evaluate cell viability. Treatment of MRSA biofilms with GNPs at 500 µg/mL plus followed by laser therapy led to the most significant bacterial cell killing, with a $63 \pm 8\%$ reduction in viability relative to controls. The treatment also led to $79 \pm 10\%$ dispersion of biofilms as evidenced by confocal microscopy. In contrast, biofilms and cells in the untreated control remained robust and viable. Likewise, biofilms treated with laser or GNPs alone remained unaffected and comparable to the untreated controls. These results demonstrate the use of gold nanoparticle-assisted laser therapy as a potential strategy to

physically destroy and debride biofilms and cells from a wound site, and thus eradicate a protective and obstructive barrier. This strategy would improve delivery of antibiotics to the remaining bacterial cells and enhance indices of mainstay antimicrobial regimens.

Author Disclosure Block:

D. Kirui: None. **G. Weber:** None. **T. Burton:** None. **N. Millenbaugh:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Impacts Of Titanium Nanoparticles On Multidrug Efflux Pump Of *enterobacter Cloacae*

Author Block:

G. He¹, X. Li¹, J. Jin², D. Lam¹, H. Chen², E. Nou¹; ¹Univ. of Massachusetts Lowell, Lowell, MA, ²Natl. Ctr. for Toxicological Res., Jefferson, AR

Abstract Body:

Background: Nanoscale materials (NMs) have shown antibacterial function, however, their impacts on drug efflux pump are not clear. Thus, assessing the impacts of NMs on bacterial multidrug efflux pumps (MDEP) is very important. Here, we report the impacts of titanium NPs on MDEP of *Enterobacter cloacae*. Antibacterial tests were performed with CLSI broth dilution method. **Methods:** *E. cloacae* strains with or without efflux pump, EmmdR were grown in the L-broth with different type TiO₂ NPs. Ciprofloxacin, benzalkonium, norfloxacin, ethidium bromide, and trimethoprim were utilized for susceptibility tests. The drug accumulation/efflux alteration of EmmdR after exposure to TiO₂ NPs was determined through measuring ethidium bromide accumulation. The expression of *emmdR* with and without exposure to TiO₂ NPs were measured by using a real-time PCR method. **Results:** Antibacterial tests showed a TiO₂ NPs (10-30nm) with the specific surface area in the range of 80-100m²/g decreased the susceptibilities of antibiotics such as ciprofloxacin and (1/8) benzalkonium (1/4) on the growth of *E. cloacae*. The accumulation/efflux of ethidium bromide was decreased after exposure to the TiO₂ NPs. The ciprofloxacin-H⁺ antiport activity was decreased after exposure to the TiO₂ NPs also. The expression of *emmdR* showed about 37.3% increase after exposure to the TiO₂ NPs. **Conclusions:** In conclusion, this study discovered a novel antibacterial TiO₂ NPs. This NPs is able to improve the antimicrobial effectiveness of ciprofloxacin, benzalkonium, ethidium bromide, and norfloxacin in *E. cloacae*. The antibiotic-H⁺ antiport alteration and expression of *emmdR* indicated that the TiO₂ NPs affect efflux pump, EmmdR in *E. cloacae*.

Author Disclosure Block:

G. He: None. **X. Li:** None. **J. Jin:** None. **D. Lam:** None. **H. Chen:** None. **E. Nou:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Synergistic Actions of Carbon Monoxide-Releasing Molecules and Antibiotics on an Antibiotic-Resistant Uropathogenic *Escherichia coli* (EC958)

Author Block:

S. Ali, R. K. Poole; The Univ. of Sheffield, Sheffield, United Kingdom

Abstract Body:

Background: Antibiotic-resistant pathogenic bacteria pose a major, growing public health risk. Antibiotics affect specific targets so that bacteria may develop cognate resistance to the target site. It is therefore necessary to investigate other antimicrobial agents. Carbon Monoxide-Releasing Molecules (CO-RMs) have been shown to exert antimicrobial actions on several bacterial species both *in vitro* and *in vivo*. To cope with antibiotic resistance, combinatorial therapies involving two (or more) antimicrobial agents may be useful; here we report that combination of two CO-RMs with antibiotics.**Methods:** Minimal Inhibitory Concentrations (MIC) and Fractional Inhibitory Concentrations (FIC) were calculated as before [1,2].**Results:** Table 1. FICs of CO-RMs in combination with antibiotics on *E. coli*.**Conclusions:** CORM-2 and CORM-3 have synergistic antibacterial activities when combined with gentamicin, chloramphenicol, spectinomycin or doxycycline.1-Albertson, et al., 1998. *J Clin Microbiol*, 36,1232-5. 2-Verma P. 2007. Pages 275-298 in Antimicrobial Susceptibility testing protocol. R. Schwable, L. Steele-Moore, and A. Goodwin, ed. Taylor and Francis Group, New York, NY.

Table 1 FICs of CORMs in combination with antibiotics on *E. coli*

Antibiotic	FIC= MIC of antibiotic in combination with CORM-2/ MIC of antibiotic alone	FIC= MIC of CORM-2 in combination / MIC of CORM alone	FIC= antibiotic + FIC CORM-2	Interpretation
Gentamicin	0.12	0.25	0.37	Synergy
Spectinomycin	0.16	0.25	0.41	Synergy
Doxycycline	0.16	0.25	0.41	Synergy

	FIC= MIC of antibiotic in combination with CORM-3/ MIC of antibiotic alone	FIC= MIC of CORM-3 in combination / MIC of CORM alone	FIC= antibiotic + FIC CORM-3	
Gentamicin	0.25	0.25	0.50	Synergy
Spectinomycin	0.16	0.25	0.41	Synergy
Doxycycline	0.18	0.25	0.43	Synergy

Author Disclosure Block:

S. Ali: None. **R.K. Poole:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

inhibition Of Aac(6')-Ib-Mediated Acetylation Of Aminoglycosides By Metal Ions: Reversion Of Amikacin-Resistance In A Multiresistant *klebsiella Pneumoniae* Clinical Isolate

Author Block:

K. Chiem, B. A. Fuentes, D. L. Lin, T. Tran, A. Jackson, M. S. Ramirez, M. E. Tolmasky; California State Univ. Fullerton, Fullerton, CA

Abstract Body:

Background: Aminoglycosides are used to treat infections caused by gram-negatives, or in combination with other antibiotics, gram-positives. However, the success of treatments has become more limited due to the dissemination of genes coding for aminoglycoside modifying enzymes (AMEs). One of the most common resistance enzymes found in gram-negatives is the aminoglycoside 6'-N-acetyltransferase type Ib [AAC(6')-Ib]. Although new aminoglycosides refractory to this and other AMEs are being designed, an alternative way to overcome the action of AMEs is the discovery of inhibitors of the modification reaction. We found that metal ions interfere with acetylation of aminoglycosides *in vitro* and can also interfere with growth of resistant bacterial cells when combined with aminoglycosides. **Methods:** Monovalent and divalent metal ions were added to acetylation reactions and monitored by the increase in OD₄₁₂ when Ellman's reagent reacts with the CoA-SH released from acetyl CoA after acetylation of the substrate. Growth inhibition assays were carried out in Mueller-Hinton broth in microtiter plates determining the OD₆₀₀. **Results:** Cu⁺², Zn⁺², and Ag⁺¹ inhibited the acetylation reaction *in vitro* with IC₅₀ values of 2, 15, and 2.5 μM, respectively. The concentration of each ion needed to inhibit growth of an amikacin resistant clinical *Klebsiella pneumoniae* strain in the presence of 16 μg/ml amikacin showed that while more than 500 μM was needed for CuCl₂ and ZnCl₂, 5 μM of silver acetate was sufficient. When zinc or copper were assayed in complex with the ionophore pyrithione, the concentrations required for inhibition in the same conditions of growth were 5 and 10 μM, respectively. **Conclusions:** Our results show that metal ions, alone, complexed to ionophores, or in combination with other inhibitors such as the recently described small molecule 1-[3-(2-aminoethyl)benzyl]-3-(piperidin-1-ylmethyl)pyrrolidin-3-ol could be a

viable strategy to extend the life of existing aminoglycosides that are threatened by the dissemination of AAC(6')-Ib.

Author Disclosure Block:

K. Chiem: None. **B.A. Fuentes:** None. **D.L. Lin:** None. **T. Tran:** None. **A. Jackson:** None. **M.S. Ramirez:** None. **M.E. Tolmasky:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Lipoteichoic Acid Synthesis Inhibition in Multi-Drug Resistant *Enterococcus faecium* as Potential Novel Combination Therapy with Antibiotics

Author Block:

F. L. Paganelli, T. van de Kamer, R. J. L. Willems, H. L. Leavis, M. J. M. Bonten, **A. P. Hendrickx**; Univ. Med. Ctr. Utrecht, UTRECHT, Netherlands

Abstract Body:

Background: Antibiotic resistant *Enterococcus faecium* (*Efm*) is a nosocomial pathogen with human morbidity and mortality. New antimicrobials are needed, since antibiotic treatment options are decreasing. *Efm* incorporates lipoteichoic acid (LTA; 1,3-polyglycerol-phosphate linked to glycolipid) in its cell wall. The small molecule inhibitor 1771 (2-oxo-2-(5-phenyl-1,3,4-oxadiazol-2-ylamino)ethyl 2-naphtho[2,1-b]furan-1-ylacetate) blocks the *Staphylococcus aureus* LtaS synthase, the enzyme that polymerizes glycerol-phosphate into LTA polymers. The objective was to characterize LTA expression and growth inhibition by 1771 in 32 *Efm* strains in combination with either gentamicin, vancomycin, daptomycin, ampicillin or linezolid antibiotics in cultures or biofilms. **Methods:** Effects of inhibitor 1771 on growth inhibition of 32 *Efm* strains was studied using bioscreen, flow cytometry (FACS) or biofilm assays by growing *Efm* in the presence or absence of varying concentrations of 1771, and in combination with 5 different antibiotics. Effects of LTA inhibition on cell integrity was analyzed by SDS-PAGE, confocal (IF) and scanning electron microscopy (SEM). **Results:** Incubation of *Efm* with 0 to 100 μ M of 1771 showed a 1771-dependent inhibitory effect on growth and IF and FACS using an anti-LTA mAb confirmed a reduction of LTA at the surface. Bioscreens and FACS showed a variable 20-60% growth inhibition and LTA reduction at the surface of the cell of all 32 *Efm* strains incubated with 20 μ M of 1771. SEM showed damaged *Efm* cell walls, and SDS-PAGE indicated protein release. A combination of 20 μ M 1771 with 10 μ g/ml daptomycin or 15 μ g/ml gentamicin lead to a 95-100% growth reduction of 2/3 of the dapto/genta-resistant strains, but not in combination with either 5 μ g/ml linezolid, vancomycin or 15 μ g/ml ampicillin. Biofilms could not be reduced by 1771 or in combinations with antibiotics. **Conclusions:** The LTA synthesis inhibitor 1771: (1) inhibits growth of 32 tested *Efm* strains, (2) reduces surface-exposed LTA, (3)

leads to alterations in cell wall morphology, (4) cannot reduce biofilms and (5) has together with daptomycin and gentamicin a detrimental growth inhibitory effect, suggesting that it may provide a novel combination therapy to treat *Efm* infections.

Author Disclosure Block:

F.L. Paganelli: None. **T. van de Kamer:** None. **R.J.L. Willems:** None. **H.L. Leavis:** None. **M.J.M. Bonten:** None. **A.P. Hendrickx:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Clopidogrel, an Antiplatelet Drug, Potentiates Antibiotic Effect of Beta-lactams Against *Staphylococcus aureus*

Author Block:

D. Ono, T. Yamaguchi, M. Hamada, Y. Ishii, K. Tateda; Toho Univ., Omori, Ota-ku, Tokyo, Japan

Abstract Body:

Background: *Staphylococcus aureus* is a major cause of nosocomial and community-acquired infections. Because therapeutic agents against methicillin-resistant *S. aureus* (MRSA) are limited and anti-MRSA drug resistance is expanding, a novel agent against *S. aureus* infection is needed. Previously, ticlopidine, an antiplatelet drug, was suggested to potentiate the antibiotic effect of beta-lactams against *S. aureus*. This study aimed to clarify the effect of clopidogrel, a ticlopidine analog, in potentiating effects of beta-lactams against *S. aureus*. **Methods:** Minimum inhibitory concentrations (MICs) of 15 beta-lactams against 3 MRSA clinical strains were tested using the broth microdilution method with 5 different clopidogrel concentrations (0, 1, 10, 100, and 1000 µg/ml). Similarly, MICs of 3 beta-lactams (oxacillin, cefazolin and ceftazidime) that showed high synergy with clopidogrel against 16 MRSA strains (including N315 and USA300) and 5 methicillin-susceptible *S. aureus* (MSSA) strains (including ATCC 25923 and ATCC 29213) and those of 4 anti-MRSA agents (vancomycin, daptomycin, linezolid, and arbekacin) against 7 MRSA strains (including N315 and USA300) were determined. **Results:** For 100 and 1000 µg/ml clopidogrel, MICs of all 15 beta-lactams against 3 clinical MRSA strains were decreased by 1-6 fold. At similar clopidogrel concentrations, MICs of 3 beta-lactams (oxacillin, cefazolin and ceftazidime) against 14 out of 16 MRSA strains were decreased by 1-6 fold (MICs of cefazolin against 1 MRSA strain and of oxacillin against another strain were unchanged), and those against 5 MSSA strains were decreased by 1-4 fold. Clopidogrel itself did not show an antibacterial effect at any concentration. Furthermore, no change was observed in MICs of the anti-MRSA agents against 7 MRSA strains. **Conclusions:** We confirmed that clopidogrel potentiates the antibiotic effect of beta-lactams against *S. aureus* including MRSA. Because clopidogrel did not potentiate effects of anti-MRSA agents, clopidogrel may affect penicillin-binding

proteins, which are beta-lactam targets, including PBP2a. Compared with developing novel agents, using existing agents like clopidogrel and beta-lactams saves time and reduces costs. Clopidogrel could be used as a novel treatment against *S. aureus* infections.

Author Disclosure Block:

D. Ono: None. **T. Yamaguchi:** None. **M. Hamada:** None. **Y. Ishii:** None. **K. Tateda:** None.

Session Number:

092

Session Title:

Novel Strategies to Help Antibiotics to Play their Role

Publishing Title:

Green Tea Polyphenols as Synergistic Agents to Enhance Antibacterial Activity of Erythromycin

Author Block:

S-A. B. Mohamed-Yussof¹, A. L. V. Melok¹, T-C. Chu², L. H. Lee¹; ¹Montclair State Univ., Montclair, NJ, ²Seton Hall Univ., South Orange, NJ

Abstract Body:

Background: The rise in antibiotic resistant cases has caused a global concern and researchers around the world are trying to find a novel alternative to combat this issue. Green tea with its many health benefits including antibacterial and antiviral has shown to be one of the most promising candidates to be used as an agent to solve this problem. In this study, modified crude lipophilic tea polyphenols (cLTP) and pure lipophilic tea polyphenols (pLTP) were used to determine their potential synergistic antibacterial effect on erythromycin. **Methods:** Three bacteria, Gram negative *Pseudomonas aeruginosa* (*P. aeruginosa*); Gram positive *Bacillus megaterium* (*B. megaterium*) and acid-fast *Mycobacterium smegmatis* (*M. smegmatis*), were tested with different concentrations of Erythromycin (E) (2.5, 5, 10, 15, 25, 60 and 100 µg/ml) and cLTP and pLTP (25, 50, 75 and 100 µg/ml) either alone or in combination. Kirby-Bauer disk diffusion test was carried out to determine the susceptibility of the bacteria to E and the colony-forming unit (CFU) was recorded to quantitatively determine the best combination of E with cLTP/pLTP. LIVE/DEAD® BacLight assay provided a qualitative data to confirm viability while Scanning Electron Microscopy (SEM) showed changes in bacterial cell surface. **Results:** Kirby-Bauer tests showed that *P. aeruginosa* and *M. smegmatis* were intermediately sensitive to E15 while *B. megaterium* is sensitive to E15 treatment. The CFU results indicated that the LD₅₀ for *P. aeruginosa* and *M. smegmatis* was 10 µg/ml and for *B. megaterium* was 2.5 µg/ml. The best combination with E15 for all three bacteria is pLTP25. The results from viability assay showed that the combination of E15 and pLTP25 resulted in more dead cells than E15 or pLTP25 individually. The bacterial synergism increased 67.6% for *P. aeruginosa*, 67.0% for *B. megaterium* and 77.8% for *M. smegmatis* respectively. pLTP25 converted E15 from intermediate to susceptible category in Gram negative and acid-fast tested bacteria. SEM images showed that the cell

membrane of the bacteria was more impaired when exposed to the combination of E15 and pLTP25 than individual compound. **Conclusion:** This study showed that the combination of Erythromycin with modified green tea polyphenols may be used as a potential therapeutic agent for antibiotic resistant bacteria.

Author Disclosure Block:

S.B. Mohamed-Yussof: None. **A.L.V. Melok:** None. **T. Chu:** None. **L.H. Lee:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Siderophore Vaccine Conjugates Stimulate Adaptive Immunity To Protect Against *Escherichia Coli* urinary Tract Infections

Author Block:

L. A. Mike, S. N. Smith, C. Sumner, H. L. T. Mobley; Univ. of Michigan - Ann Arbor, Ann Arbor, MI

Abstract Body:

Uropathogenic *Escherichia coli* (UPEC), the primary cause of uncomplicated urinary tract infection (UTI), gains access to the bladder upon periurethral colonization and ascension via the urethra. If infection is not restricted by the host or antibiotics, bacteria may ascend to the kidneys causing pyelonephritis, sepsis, and even death. Over half of all women in the United States will suffer from a UTI in their lifetime and 1 in 40 women will endure recurrent infections. UTIs are typically resolved using standard antibiotic regimens. Managing recurrent infections by means of frequent or continuous antibiotic courses, however, inevitably leads to increased resistance to those antibiotics typically used to treat UTIs. Currently, no UTI vaccines are approved for use in the United States and the development of a highly effective vaccine remains elusive. Here, we have developed a vaccine that targets bacterial-secreted natural products called siderophores. Siderophores, which are small molecules produced by bacteria, capture iron from the environment to deliver the nutrient metal to the bacterial cell during infection. Pathogen-associated siderophores evade host immune defenses and enhance bacterial virulence. Previous studies have shown that immunization with siderophore receptor proteins is protective in a murine model of UTI. These integral membrane proteins, however, have poor solubility in aqueous solutions, thus limiting their practical utility. Conversely, their cognate siderophore ligands are water soluble. Therefore, we hypothesized that pathogen-specific siderophores are prime vaccine candidates. To test this hypothesis, we vaccinated mice with two UPEC siderophores conjugated to immunogenic carrier proteins both individually and in combination. Vaccination with individual siderophores reduced bacterial burdens by an order of magnitude in the urine and kidneys. Also, co-immunization decreased bacterial burdens in the urine 14-fold and, most dramatically, reduced bacterial burden in the kidneys 126-fold. The vaccine courses elicited a B cell-

mediated immune response that targeted bacterial siderophores and protected against UTI. Our study has identified two new antigens for use in a UTI vaccine and highlights the untapped resource of bacteria-specific small molecules as potential vaccine antigens.

Author Disclosure Block:

L.A. Mike: None. **S.N. Smith:** None. **C. Sumner:** None. **H.L.T. Mobley:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Characterization of New Live, Attenuated *Shigella flexneri* Vaccine Candidates

Author Block:

B. DeLaine, C. Grassel, T. Wu, E. M. Barry; Univ. of Maryland, Baltimore, Baltimore, MD

Abstract Body:

Shigella flexneri, an enteroinvasive bacterium, is one of the leading causes of diarrheal disease in children under five in developing countries. The development of an efficacious vaccine has been elusive, in part due to the requirement for serotype specific protection. We assessed two new vaccine candidates utilizing the well-studied live, attenuated *Shigella flexneri* 2a vaccine CVD 1208S as a standard. The new vaccine strains, CVD 1213 and CVD 1215 (derived from *S. flexneri* serotypes 3a and 6), represent two prominent *S. flexneri* serotypes. Combined with CVD 1208S, they could provide broad spectrum immunity against shigellosis. Each vaccine strain was attenuated with a deletion in the *guaBA* operon encoding critical enzymes of the de novo guanine nucleotide biosynthesis pathway. Host responses to the vaccines were measured using: macrophage cytotoxicity assays, gentamicin protection assays to assess invasion and replication rates, and enzyme-linked immunosorbent assays to quantify cytokine secretion from infected cells. Additionally, guinea pigs studies were performed to assess *Shigella* LPS-specific antibody responses and protection against wild type (WT) challenge following immunization with each vaccine individually and as a trivalent mixed vaccine. Epithelial cell invasion by the vaccine strains was not significantly decreased compared to WT; however, the attenuated vaccines were unable to replicate intracellularly. Vaccine candidates demonstrated varied cytotoxic effects in human macrophages but remained comparable to their parental WT strains. Cytokine secretion from macrophages and epithelial cells infected with the vaccine strains was consistent with secretion from WT infected cells and significantly higher than uninfected cells. Guinea pig immunization studies revealed a robust induction of *Shigella* serotype specific LPS- specific IgA and IgG antibodies (100% seroconversion) following immunization with each individual vaccine strain and a mixture of all three. Challenge using the guinea pig Sereny test demonstrated protection against WT *S. flexneri* serotypes 2a, 3a and 6 following

immunization with a mixed immunization including 1208S, 1213 and 1215. These data indicate that CVD 1213 and CVD 1215 are viable candidates for the creation of a multivalent vaccine that could confer broad spectrum protection against *Shigella flexneri* infections.

Author Disclosure Block:

B. DeLaine: None. **C. Grassel:** None. **T. Wu:** None. **E.M. Barry:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Development of a Whole Cell Cholera Vaccine Using a Biofilm Matrix Protein as an Antigen Presentation Platform

Author Block:

J. Liao¹, D. R. Smith², P. I. Watnick¹; ¹Boston Children's Hosp., Boston, MA, ²KnipBio, Lowell, MA

Abstract Body:

Vibrio cholerae causes cholera, an acute diarrheal disease that afflicts millions of people around the world annually. While oral rehydration therapy and antibiotics can effectively reduce the duration and severity of disease, cholera remains a significant contributor to fatal enteric disease in children under the age of 5. This burden is especially high in developing countries where many communities have limited access to medical care. Thus, there is an urgent need to develop inexpensive, effective interventions that can be implemented on a large scale to lower the incidence of disease in these nations. We previously identified RbmA as a biofilm matrix protein that is secreted and associates with the *V. cholerae* cell surface, and reasoned that RbmA can be used as an antigen presentation platform to create a whole cell *V. cholerae* vaccine. Dukoral® is a commercially available vaccine against cholera that consists of killed, whole *V. cholerae* cells administered with purified cholera toxin subunit B (CtxB). Inclusion of purified CtxB confers protection against *V. cholerae* as well as enterotoxigenic *E. coli*, the third most common cause of childhood diarrhea. But as a result, Dukoral® is relatively more expensive and less easy to administer than a simple whole cell vaccine. By expressing an RbmA-CtxB fusion protein and relying on its spontaneous association to the cell surface, we developed a simple, whole cell cholera vaccine prototype that contains CtxB and administered it in mice. We have shown that the recombinant RbmA-CtxB remains robustly cell-associated in our prototype vaccine. Quantitative Western blot indicated that levels of cell-associated RbmA-CtxB is comparable to the amount of purified CtxB included in an equivalent dose of Dukoral®. Vaccine immunogenicity was determined by measuring cholera-specific serum IgA, IgG, and IgM titers as well as secretory IgA levels in stool with ELISA. Each condition tested included at least 10 mice and antibody titers were compared to that of mice receiving a control vaccine strain (*V. cholerae* that does

not express RbmA-CtxB). We are now developing a live attenuated whole cell vaccine with the hope that it will provide proof of concept for using RbmA as an antigen presenting platform, and support future studies to improve immunogenicity or adapt the platform for the delivery of other antigens.

Author Disclosure Block:

J. Liao: None. **D.R. Smith:** None. **P.I. Watnick:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Gen-004, a Recombinant Protein-based Vaccine, Protects Against Colonization with *Streptococcus pneumoniae* Strains 6b and 23f in an IL-17a-Dependent Manner

Author Block:

L. C. Gavrilescu, N. Siddall, L. Sobezenski, B. Le, D. Yu, M. Skoberne, J. Flechtner, **E. Flaño**; Genocea BioSci.s, Inc, Cambridge, MA

Abstract Body:

Introduction: The first and necessary step to establishment of pneumococcal disease is bacterial colonization of the nasopharynx. There is growing evidence that T_H17 cells play a key role in preventing pneumococcal colonization and subsequent disease. The GEN-004 vaccine was designed to be a universal pneumococcal vaccine protecting via a T_H17 mechanism. GEN-004 consists of three recombinant proteins conserved across all sequenced variants of pneumococcus, adsorbed to aluminum hydroxide adjuvant. The antigens were selected using the ATLAS™ platform, which identified the pneumococcal proteins to which humans naturally made T_H17 responses. The objective of this study was to look at GEN-004 protection against colonization by several pneumococcal serotypes, and to confirm that protection was dependent on IL-17A secretion. **Methods:** C57BL/6 mice were immunized subcutaneously three times, two weeks apart, with the GEN-004 vaccine, or control. Mice were challenged intranasally with *S. pneumoniae* strains 6B or 23F three weeks after the last vaccine dose. In IL-17A blocking studies, mice were treated 1 day before and 2 days after challenge with 100 µg of an anti-mouse IL-17A monoclonal antibody. The number of *S. pneumoniae* colony forming units (CFU) isolated from nasopharyngeal washes was assessed 10 days post challenge for each mouse.

Results: Mice immunized with GEN-004 developed antigen-specific IgG and T_H17 immune responses. After challenge with live pneumococcus, the geometric mean bacterial CFUs/mouse in GEN-004 vaccinated mice were reduced to 4% (p=0.0021) or 8% (p=0.0028) of that observed in the control mice, for the 6B and 23F strain, respectively. Furthermore, *in vivo* treatment of GEN-004-vaccinated animals with IL-17A neutralizing antibody eliminated all protection against 6B colonization.

Conclusions: Immunization with GEN-004 protected against colonization by at least two distinct *S. pneumoniae* serotypes in the murine model of nasopharyngeal colonization.

Blocking of IL-17A with a neutralizing antibody completely abrogated vaccine efficacy, supporting the dependence on IL-17A cytokine secretion for protection.

Author Disclosure Block:

L.C. Gavrilesu: D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **N. Siddall:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **L. Sobzenski:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **B. Le:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **D. Yu:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **M. Skoberne:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **J. Flechtner:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences. **E. Flaño:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversified mutual funds); Self; Genocea Biosciences.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Novel PVL Toxoid Combination, LukVax Induces Broad Neutralizing Immune Responses to *Staphylococcus aureus* Leukotoxins

Author Block:

A. Venkatasubramaniam, T. Kort, N. Williston, S. Patel, G. Liao, R. Campos, R. Adhikari, S. Krishnan, J. Aman; Integrated Biotherapeutics, Gaithersburg, MD

Abstract Body:

Background: Bi-component leukotoxins play a crucial role in *Staphylococcus aureus* (*S.aureus*) pathogenesis. We have previously reported novel PVL toxoid components that are able to confer protection in a mouse model of *S. aureus* sepsis. In the current study, we sought to expand our understanding of immunogenicity and efficacy of these novel PVL toxoids and determine their role as part of a multivalent *S. aureus* vaccine.

Methods: Immunogenicity studies in Balb/c mice were initiated to evaluate (1) the functional immune response to individual PVL toxoid components LukS-mut9 (harboring mutations LukS-PV_T28F/K97A/S209A) and LukF-mut1 (harboring a single point mutation LukF-PV_K102A), (2) whether combination of LukS-mut9 and LukF-mut1, termed as LukVax, provides superior and broader neutralizing response and (3) whether the addition of HlgB to LukVax can be beneficial. Total binding antibodies by ELISA, neutralizing antibody titers by HL-60 cell based cytotoxicity assay and neutralizing antibody titers by rabbit RBC assay were determined to canonical and non-canonical leukotoxin pairs. **Results:** LukS-mut9 immunized mice generated robust neutralizing titers against PVL and LukED toxins. LukF-mut1 immunized mice on the other hand generated robust neutralizing titers against PVL, HlgAB and HlgCB. Mice immunized with LukVax generated higher neutralizing titers against PVL (*NT50* values two fold or higher) and provided increased breadth of binding and neutralizing activity against the leukotoxins (PVL, HlgAB, HlgCB and LukED). More so, LukVax generated neutralizing titers to a lethal non-canonical leukotoxin pair of HlgA and LukD. Addition of HlgB to LukVax, however did not appear to significantly increase total binding or the breadth of neutralizing activity against leukotoxins as compared to LukVax alone. **Conclusions:** We demonstrate that this novel LukS-mut9 and LukF-mut1 combination, LukVax induced broad neutralizing antibody response against canonical and non-canonical *S. aureus*

leukotoxin pairs. The superiority of LukVax over the individual components and the fact that, in contrast to wild type PVL, LukVax has no cytotoxic activity, provides merit for LukVax to be part of a multivalent *S. aureus* vaccine.

Author Disclosure Block:

A. Venkatasubramaniam: None. **T. Kort:** None. **N. Williston:** None. **S. Patel:** None. **G. Liao:** None. **R. Campos:** None. **R. Adhikari:** None. **S. Krishnan:** None. **J. Aman:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

A Phase 2, Dose-Confirmation Immunogenicity and Safety Study of VLA84, A *Clostridium difficile* Vaccine Candidate, in Adults Aged 50 Years and Older

Author Block:

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Abstract Body:

Background: *Clostridium difficile* infection (CDI) is the leading cause of nosocomial diarrhoea. *C. difficile* Toxin A and Toxin B are considered primary mediators of disease. There are no licensed vaccines against CDI. A monoclonal antibody against Toxin B has recently demonstrated preventive efficacy against recurrence of CDI, while earlier studies associated higher Toxin A-specific antibody levels with protection from recurrence. We aimed to confirm the dose/formulation of VLA84, a recombinant protein vaccine candidate featuring relevant portions of the cell-binding domains of Toxin A and B, in a Phase 2 study. **Methods:** Randomized, observer-blind, placebo-controlled, multi-centric study. 500 subjects, age range 50-90 years, of good general health including subjects with controlled chronic conditions, received either 75µg VLA84 without Alum (N=148), 200 µg without Alum (N=152), 200 µg with Alum (N=152), or placebo (PBS, N=48); as intramuscular injections on Days 0, 7 and 28. Subjects were followed for 6 months after the last immunization. The primary endpoint, Seroconversion Rate (SCR, rate of subjects with ≥4-fold IgG antibody titer increase over baseline) against both Toxin A and B on Day 56, was assessed in a pre-planned interim analysis. **Results:** On Day 56, SCRs against both Toxin A and B were 72%, 83% and 60% for groups VLA84 75 µg w/o Alum, 200 µg w/o Alum, and 200 µg w/ Alum; and 0% for placebo (p<0.05 for all comparisons between groups, per protocol set). SCR against Toxin A was higher, 92% to 97% in the VLA84 groups, Toxin B SCRs were similar to SCR against both Toxins. For the VLA84 groups, GMTs for Toxin A specific IgG ranged from 2925 to 4756 EU/mL, and for Toxin B from 1451 to 3322 EU/mL. Toxin-neutralizing antibodies were induced in all dose groups. VLA84 was generally safe and well tolerated in all treatment groups, no severe local reactions were noted. Injection site pain and tenderness were the most

common adverse events. **Conclusions:** VLA84 was well tolerated. The 200 µg without Alum dose showed superior immune response with the highest SCR, hence should be considered for further development.

Author Disclosure Block:

K. Dubischar: D. Employee; Self; Valneva SE. **N. Bézy:** D. Employee; Self; Valneva SE. **R. Hochreiter:** D. Employee; Self; Valneva. **T. Jelinek:** C. Consultant; Self; Baxter, Boehringer Ingelheim, Novartis Vaccines, Pfizer, r-biopharm, Sigma Tau. **F. Investigator:** Self; Abbott, Baxter, GSK, Novartis Vaccines, Sanofi, Sigma Tau, Valneva. **J. Scientific Advisor (Review Panel or Advisory Committee):** Self; Boehringer Ingelheim, Sigma Tau, Valneva. **L. Speaker's Bureau:** Self; Baxter, Boehringer Ingelheim, Crucell, GSK, Hoffmann LaRoche, Novartis Vaccines, Pfizer, r-biopharm, Sanofi, MSD Sharp & Dohme, Sekizui-Virotech, Sigma Tau, Valneva. **V. Kadlec:** D. Employee; Self; Valneva. **S. Kiermayr:** D. Employee; Self; Valneva. **K. Westritschnig:** D. Employee; Self; Valneva.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Formulation of a Subunit Vaccine in Mucus-penetrating Nanoparticles Protects Against Tuberculosis Infection and Enhances Chemotherapy Responses in Mice

Author Block:

H. N. Soni, H. Patel, A. Ormond, L. Ensign, J. Hanes, E. Nuermberger; Johns Hopkins Univ, Baltimore, Baltimore, MD

Abstract Body:

Background: Mucosal immune responses in the lung may be important in restricting initial infection and progression of tuberculosis (TB). However, the mucus layer overlying the respiratory epithelium is an obstacle to antigen delivery. We hypothesized that formulating vaccines in mucus penetrating nanoparticles (MPPs) will improve the efficacy of intranasal vaccination with *Mycobacterium tuberculosis* (Mtb) antigens in murine models of TB. **Experimental methods:** MPPs were prepared from maleimide-PEG-PLGA polymer by the single emulsion method, incorporating monophosphoryl lipid A and dioctyldecylammonium bromide as adjuvants before conjugating antigen-85B and ESAT-6 to the surface. Conventional PEG-PLGA nanoparticles (CPs) were prepared using the same adjuvants. Antigen-conjugated MPPs and CPs were tested for particle size, morphology, zeta potential and ex-vivo mucus diffusion. Two mouse strains, C57BL/6 and C3HeB/FeJ, were used to evaluate protective efficacy of MPP and CP vaccines administered (1) alone and (2) as boosters following BCG priming. Following vaccination, mice were challenged with ~100 CFU of virulent *Mycobacterium tuberculosis* (Mtb). Controls included PBS (sham) and antigens alone. MPPs were also evaluated for their ability to accelerate clearance of Mtb from the lungs of mice when administered together with standard combination chemotherapy with rifampin, isoniazid and pyrazinamide (RHZ). **Results:** MPPs were mucus-diffusive while CPs were mucus-adhesive. Primary vaccination with BCG resulted in ~0.5 and 1.0 log₁₀ fewer CFU compared to sham vaccine in both mouse strains. The MPP vaccine performed similarly to BCG and superior to CP vaccine. As a booster, MPP vaccine performed significantly better than sham and CP vaccine. Finally, addition of MPP vaccine significantly reduced the number of mice relapsing after abbreviated RHZ treatment. **Conclusion:** Formulation

of *Mtb* antigens in MPPs provided superior efficacy over sham and CPs as both preventive and therapeutic vaccines in mice.

Author Disclosure Block:

H.N. Soni: None. **H. Patel:** None. **A. Ormond:** None. **L. Ensign:** None. **J. Hanes:** A. Board Member; Self; Kala Pharmaceuticals Inc, 100 Beaver Street #201, Waltham, MA 02453. **B.** Collaborator; Self; Kala Pharmaceuticals Inc, 100 Beaver Street #201, Waltham, MA 02453. **C.** Consultant; Self; Kala Pharmaceuticals Inc, 100 Beaver Street #201, Waltham, MA 02453. **K.** Shareholder (excluding diversified mutual funds); Self; Kala Pharmaceuticals Inc, 100 Beaver Street #201, Waltham, MA 02453. **E. Nuernberger:** None.

Session Number:

093

Session Title:

Novel Vaccines: From Basic Research Through Clinical Development

Publishing Title:

Antibody Targeting of Matrix Protein M1 of Influenza A Virus Identifies an Epitope as a Potential Immunogen for a Universal Flu Vaccine

Author Block:

R. Seedarnee¹, M. Baer², D. Bucher¹; ¹New York Med. Coll., Valhalla, NY, ²EnGen Bio Inc, Redwood, CA

Abstract Body:

Background: Influenza is a highly contagious respiratory illness that causes an acute and severe disease led by influenza viruses type A (IAV). According to CDC and WHO, it is estimated that IAV causes 25-30 million cases of Influenza per year in the USA, resulting in 150,000 hospitalizations and up to 30,000 to 40,000 deaths annually. Globally, there are approximately 1 billion cases of influenza with 3-5 million cases of severe illness and 300,000 to 500,000 deaths annually. This morbidity and mortality can be decreased by the use of seasonal influenza vaccines or by antivirals such as the neuraminidase inhibitors or M2 ion channel inhibitors. Because the IAVs undergo antigenic drift, antibodies generated against the currently circulating IAVs (H1 and H3 subtypes) may not grant protection against future strains of IAVs. Therefore, vaccine formulation must change and match the circulating subtype strains annually. Therefore development of a Universal Influenza Vaccine would provide our best opportunity to reduce the burden of IAV. **Methods:** Western Blot; ELISA; PRNT; *In Ovo* assay; Immuno-EM ; Linear B-Cell Epitope mapping; Immunization Study. **Results:** Previous work done in our laboratory generated a monoclonal antibody (mAb) that recognizes the M1 protein component of heterosubtypic IAVs. This mAb is from a hybridoma cell line created from mice immunized and boosted with purified M1 protein from A/Puerto Rico/8/1934 H1N1virus. ELISA studies have demonstrated this antibody to be broadly reactive against IAVs H1 and H3 including the IAVs currently in the 2015-2016 vaccine formulation. *In vitro* Plaque Reduction Neutralization Test (PRNT) and *in ovo* testing with this mAb has demonstrated that it can neutralize the parental virus from which it was generated, A/Puerto Rico/8/1934 H1N1 virus, as well as other IAVs. Immuno-EM revealed that the epitope is surface exposed, and was subsequently mapped by Linear B-Cell Epitope mapping to the C-terminal region of the M1 protein. An animal model

demonstrated that a vaccine composed of an M1 peptide conjugate from the C-terminus generated an immune response against the peptide as well as an immune response against purified M1 protein. **Conclusion:** Thus our mAb has elucidated a response against a novel epitope on the surface of the IAV which may lead to development of a Universal Flu Vaccine.

Author Disclosure Block:

R. Seedarnee: None. **M. Baer:** None. **D. Bucher:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Assessing the Risk of Hand-to-hand Transmission of Bacteria

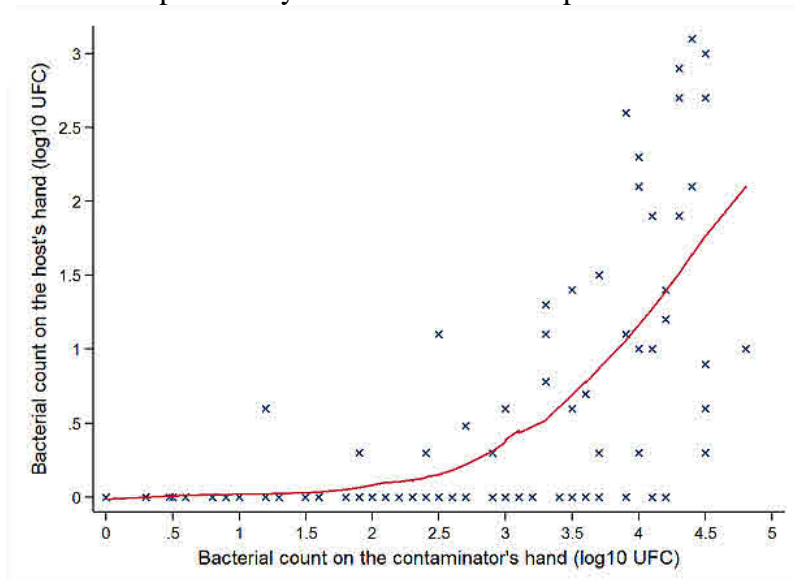
Author Block:

F. Bellissimo-Rodrigues, H. Soule, D. Pires, A. Gayet-Ageron, D. Pittet; Univ. of Geneva Hosp., Geneva, Switzerland

Abstract Body:

Background: The minimal bacterial load on hands associated with cross-transmission remains unknown. We aimed to evaluate the relationship between the bacterial load on hands and the risk of cross-transmission after hand-to-hand contact. **Methods:** Experimental study conducted with 6 HCWs with different hand sizes. In each experimental series, one HCW played the role of “contaminator” and the other of “host”, in a crossover design. Contaminators had their hands contaminated in suspensions of increasing inoculum (from 10^3 to 10^6 cfu/mL) of *E. coli* ATCC 10536. Following the contamination procedure, one hand of the contaminator was cultured using the “fingertips method”, while the other hold the host’s hand for 1 min with fingers interlocked. Fingertips cultures of the host’s hand were subsequently performed to assess the degree of cross-transmission (primary outcome of the study). We used a mixed logistic regression model with a random effect on the intercept to analyze the results. **Results:** Cross-transmission was significantly ($P < 0.001$) associated with bacterial counts on contaminators’ hands (Figure). The likelihood of transmission, adjusted for gender and hand size, increased by 4.4-fold when the inoculum ranged 3 to $3.5 \log_{10}$ compared to $< 3 \log_{10}$, by 16.4-fold when it ranged 3.5 to $4 \log_{10}$, and by 46.8-fold when it was superior to $4 \log_{10}$. When the inoculum was inferior to $1 \log_{10}$, cross-transmission was not detected. **Conclusion:** There is a direct relationship between the bacterial load on HCWs hands and the risk of cross-transmission following hand-to-hand contact. Under the described experimental conditions, at least $1 \log_{10}$ of *E. coli* must be present on HCWs

hands to be potentially transmitted to other persons.



Author Disclosure Block:

F. Bellissimo-Rodrigues: None. **H. Soule:** None. **D. Pires:** None. **A. Gayet-Ageron:** None. **D. Pittet:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Alcohol-Based Hand Gel Use and Incidence of Healthcare Associated Infections in Mbale Regional Referral Hospital, Rural Eastern Uganda

Author Block:

H. Saito¹, K. Inoue², J. Ditai³, B. Wanume⁴, J. Abeso⁴, A. Weeks⁵; ¹Univ. of California, Irvine, Orange, CA, ²Nagasaki Univ., Nagasaki, Japan, ³Sanyu Africa Res. Inst., Mbale, Uganda, ⁴Mbale Regional Referral Hosp., Mbale, Uganda, ⁵Univ. of Liverpool, Liverpool, United Kingdom

Abstract Body:

Background: Use of alcohol-based hand gel (ABHG) at health facilities is limited in Uganda. Data on the practice of hand hygiene (HH) and the incidence of healthcare associated infections (HAIs) is sparse in resource-limited settings. We conducted a study to evaluate HH practices of health care providers (HCPs) utilizing locally made ABHG and the incidence of HAIs. **Methods:** HH compliance and the incidence of HAIs were assessed at a referral hospital in rural Uganda. Inpatients on the obstetrics/gynecology (OB/GYN), pediatric and surgical wards were followed during their hospital stay. A 12-week baseline phase was followed by a 12-week intervention phase where training for HH was provided and ABHG was supplied on the wards. The incidence rate ratio (IRR) of HAIs and or systemic inflammatory response syndrome (SIRS) was calculated to compare the baseline and intervention phases. **Results:** A total of 3626 patients were enrolled over a 24-week period from October 2014 to April 2015. HH compliance rate was significantly improved from 10.3% during the baseline phase to 55.7% during the intervention phase. The incidence rate of HAIs/SIRS was 18.8 cases per 1000 patient-days during the whole study period and the incidence was not significantly changed between the baseline and intervention phases (IRR 1.03, 95% CI: 0.76 - 1.39). However, subgroup analyses showed significant reduction in HAIs/SIRS on the pediatric and surgical wards (IRR 0.17 (95% CI: 0.07 - 0.44) and IRR 0.37 (95% CI: 0.15 - 0.92), respectively) while a significant increase in HAIs/SIRS was found on the OB/GYN ward (IRR 2.90 (95% CI: 1.85 - 4.54)). **Conclusions:** Little is known about HAIs in the resource limited countries. To our knowledge, our study is one of the largest studies that address HAIs in Africa. Significant improvement in HH compliance was observed by

providing training and ABHG. The intervention was associated with a significant reduction in HAIs and or SIRS on the pediatric and surgical wards. Given the fact that SIRS cases were more commonly identified than HAIs, further research is warranted to accurately survey HAIs in resource limited settings.

Author Disclosure Block:

H. Saito: None. **K. Inoue:** None. **J. Ditai:** None. **B. Wanume:** None. **J. Abeso:** None. **A. Weeks:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

The Global Hand Sanitizing Relay: Impact on Hand Hygiene Compliance

Author Block:

E. Tartari¹, F. Bellissimo-Rodrigues¹, D. Pires¹, M. De Kraker¹, C. Kilpatrick², B. Allegranzi², D. Pittet¹; ¹Univ. of Geneva Hosp., Geneva, Switzerland, ²World Hlth.Organization, Geneva, Switzerland

Abstract Body:

Background: Although hand hygiene (HH) is the core element of patient safety and great strides have been made, sustained HH compliance amongst health-care workers (HCW) remains sub-optimal. To further encourage hospitals to promote HH, the World Health Organization (WHO) Collaborating Centre (WCC) in Geneva launched the initiative “Global Hand Sanitizing Relay 2015” (HSRelay). To assess its impact in participating hospitals, we evaluated HH compliance data before and after the initiative. **Methods:** The HSRelay consisted of performing a sequential chain of HH actions according to the WHO “How to Handrub” technique by as many HCWs as possible during a single event. Important aspects included a hospital-wide engagement and pre-intervention training of the HH technique. Participating facilities were encouraged to measure HH compliance rates before and after the HSRelay according to the WHO standardized direct HH observation method. HH compliance rates were compared using Chi-square test, applying the Bonferoni correction for multiple comparisons. **Results:** A total of 133 hospitals in 43 countries across all WHO regions completed the HSRelay. All hospitals completed the HSRelay between May and September 2015. Fourteen hospitals submitted pre- and post- HH compliance data, collected in April-May and June-September 2015, respectively. The number of HCWs participating in the event ranged from 100-500. Most hospitals were tertiary care (n=9, 64%) and non-university-affiliated (n=12, 86%), had a median of 294 beds (IQR 135-471), and had handrub available at the bedside (n=11, 85%). Eight out of 14 facilities showed a statistically significant (p< 0.01) improvement of HH compliance, ranging from +3.2 to +32.1%. **Conclusion:** The Global HSRelay engaged health facilities in action and monitoring of compliance to understand the impact of this activity. This novel strategy appeared effective in promoting a HH approach to supplement other campaign activities. HH compliance improvement was

observed in the majority of hospitals evaluated. It seemed effective in overcoming campaign fatigue and strengthened HCW's awareness and commitment towards improving practices.

Author Disclosure Block:

E. Tartari: None. **F. Bellissimo-Rodrigues:** None. **D. Pires:** None. **M. De Kraker:** None. **C. Kilpatrick:** None. **B. Allegranzi:** None. **D. Pittet:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Hand Hygiene with Alcohol-Based Handrub: How Long Is Long Enough?

Author Block:

D. Pires, H. Soule, F. Bellissimo-Rodrigues, A. Gayet-Ageron, D. Pittet; Univ. of Geneva Hosp., Geneva, Switzerland

Abstract Body:

Background: Hand hygiene (HH) is the most important preventive measure against healthcare-associated infections. Great efforts are made to improve hand hygiene compliance, but less attention has been given to HH action's quality. We analyzed the influence of hand friction duration in the reduction of bacterial load on healthcare workers' (HCW) hands. **Methods:** In an experimental study at the University of Geneva Hospitals, 23 HCWs rubbed their hands with 3 ml of isopropanol 60% according to World Health Organization's "how to handrub" technique, varying the duration of friction: 10, 15, 20, 30, 45 and 60 seconds (sec). According to European Norm 1500 standards, hands were contaminated with *E. coli* ATCC 10536 and bacterial counts were retrieved using the fingertips method after no HH (baseline) and after each HH action. We used a generalized linear mixed model with a random effect on the subject assessing the log₁₀ bacteria count recovered from HCWs' hands from baseline and following each duration of friction. **Results:** Among the 23 participants, 15 were women; 110 measures were obtained. After adjustment for gender and hand size, bacterial count was significantly associated with duration of friction (P<0.001): it was reduced by a mean -2.27 log₁₀ (95% CI -2.99; -1.54) after 10 sec (reference baseline); by a mean -2.52 log₁₀ (CI -2.93; -2.10) after 15 sec; by a mean -2.69 log₁₀ (CI -3.16; -2.23) after 20 sec; by a mean -2.70 log₁₀ (CI -3.11; -2.28) after 30 sec; by a mean -2.17 log₁₀ (CI -2.71; -1.62) after 45 sec and by a mean -2.26 log₁₀ (CI -2.80; -1.71) after 60 sec. The difference in bacterial count reduction, adjusted for gender and hand size, was significantly smaller for 45 sec compared to 15, 20 or 30 sec and for 60 sec compared to 20 or 30 sec. There was no statistically significant difference between 15, 20 and 30 sec or between 45 and 60 sec. **Conclusions:** Bacterial count was significantly reduced from baseline whatever the duration of HH friction, but we did not show a linear increase in the log₁₀ reduction across the 6 durations of friction. Our results suggest that, under the current experimental

conditions, there is no gain in performing HH for longer than 30 sec. Further studies need to assess the clinical significance of performing HH for 15, 20 or 30 sec.

Author Disclosure Block:

D. Pires: None. **H. Soule:** None. **F. Bellissimo-Rodrigues:** None. **A. Gayet-Ageron:** None. **D. Pittet:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Improving Hand Hygiene Behavior by Scenario Based Simulation Education

Author Block:

I. Nakamura, T. Matsumoto; Tokyo Med. Univ. Hosp., Tokyo, Japan

Abstract Body:

Background: Proper hand hygiene by health care workers is important in preventing nosocomial infection, but compliance is usually poor. Simulation education technology is widely used in medical education and has great potential. However, scenario-based simulation education, similar to that with cardiac life support, has not been described to prevent nosocomial infections. This study aimed to determine the effectiveness of a scenario-based education program to prevent infections and improve hospital care—especially with hand hygiene. **Methods:** We conducted a single-center, prospective, cohort study among nursing staff and doctors attending an infection-control training course (ICTC) from April 2011 to March 2015 at Tokyo Medical University Hospital (1015 beds), an acute-care teaching hospital. Each ICTC was held every month and lasted 2 hours. Trainees put on and removed personal protective equipment under scenarios of standard precaution (2 scenarios) and contact precaution with MRSA (1 scenario). Using a questionnaire about standard precautions and transmission-based precautions before and after the course, we evaluated trainee reactions. We determined the correlation between the participation rate in the simulation education and use of alcohol-based hand disinfection. In our estimates, we employed Pearson's product-moment correlation coefficient. **Results:** There were 1078 trainees; total participation rate was 76% by the end of the study. All questionnaire items dealt with observed improvements. The correlation between course participation rate and use of hand hygiene agents was significant for 19 of 24 hospital wards (correlation coefficient, 0.8-0.99). Three of 24 wards showed an intermediate correlation coefficient (0.45-0.67). The overall correlation between use of hand hygiene agents in the hospital and course participation rate was also significant (0.99). **Conclusions:** Our ICTC had a positive impact on hand hygiene. This study is the first effective scenario-based simulation education for infection prevention and control.

Author Disclosure Block:

I. Nakamura: None. **T. Matsumoto:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Identifying the Source of Healthcare-associated *Mycobacterium chimaera* Infections Using Environmental Sampling and Cardiac Surgery Simulation

Author Block:

M. S. Keckler¹, H. Moulton-Meissner¹, M. M. Lyman¹, C. T. Grigg¹, C. Bicking Kinsey², K. A. Perry¹, N. C. Toney¹, B. Jensen¹, J. Miller², J. Perz¹, K. M. Perkins¹, M. J. Arduino¹, J. Noble-Wang¹; ¹CDC, Atlanta, GA, ²CDC/Pennsylvania Dept. of Hlth., Harrisburg, PA

Abstract Body:

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment and can cause severe opportunistic disease. A case-control study of *M. chimaera* healthcare-associated infections (HAI) showed that patients with NTM infection had greater odds of having undergone cardiopulmonary bypass (CPB), and this effect was further increased if CPB lasted greater than 2 hours. Based on these findings and relevant literature, we designed an environmental sampling strategy to better understand the potential source and routes of NTM exposure. As part of our environmental sampling, we simulated a five-hour cardiac surgery which included surgical staff, operating room environmental controls and relevant medical devices operating as they would during a surgery. Air and settle plate samples were taken hourly from multiple locations to better understand the dissemination of NTM within this environment. All samples were cultured and stained for acid-fast bacilli (AFB). AFB+ samples and clinical isolates were identified using matrix-assisted laser desorption/ionization using a time-of-flight mass spectrometer (MALDI-TOF). Species and strain were confirmed by 16S rRNA gene and *rpoB* sequencing and pulsed-field gel electrophoresis (PFGE). Available clinical isolates were confirmed as *M. chimaera* and were PFGE-indistinguishable. *M. chimaera* was found in both water and biofilm samples from heater-cooler units and in air samples taken 2 or more hours into the simulation. The NTM bioburden in water samples was measurable after 15 minutes of operation and was highest in units sampled after 5 hours of use (1.5×10^5 CFU/ml). Characterization of the NTM biofilm is ongoing. These results indicate that operation of contaminated heater-cooler units can aerosolize and disperse NTM within the operating room, placing patients at risk for NTM infection. The results also shed light on the

dynamic nature of NTM dispersion and exposure during surgery. These findings can contribute to the design of data-driven interventions regarding heater-cooler unit design, placement, operation, maintenance and monitoring to prevent device-associated NTM infections.

Author Disclosure Block:

M.S. Keckler: None. **H. Moulton-Meissner:** None. **M.M. Lyman:** None. **C.T. Grigg:** None. **C. Bicking Kinsey:** None. **K.A. Perry:** None. **N.C. Toney:** None. **B. Jensen:** None. **J. Miller:** None. **J. Perz:** None. **K.M. Perkins:** None. **M.J. Arduino:** None. **J. Noble-Wang:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Comparison of Two Methods for Culturing Reprocessed Duodenoscopes

Author Block:

B. Dylla, P. Kohner, S. Ihde, J. Berry, K. Monson Jobe, D. J. Hata, B. Petersen, A. English, R. Yates, P. Sampathkumar, R. Thompson, T. Grys, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Background: Improperly cleaned and reprocessed duodenoscopes have been implicated in transmission of resistant enteric bacteria. We compared 2 potential methods for culturing reprocessed duodenoscopes, including a novel approach using blood culture bottles. **Methods:** A prospective side by side comparison of two culture methods was performed on 51 samples collected between 5/27/2015 to 10/26/2015 from duodenoscopes that had been reprocessed using high level disinfection and sterilization. Distal tip brushings and channel samples were collected using a modified CDC protocol. For 19 scopes, channel flushes and distal tip brushings were separately cultured; the remainder were combined and assayed together. The original sample (including brush) was vortexed, placed in a 50 mL tube and centrifuged at 4000 rpm for 5 min. The supernatant was removed and the remaining 1 mL vortexed for 15 seconds. 0.1 mL was inoculated onto sheep blood and EMB agars, and into T-soy broth; media were incubated for 5 days at 35-37° C in CO₂ with daily examination. Cloudy broths or plate growth were worked-up. The remaining concentrated sample was inoculated into a BD BACTEC Peds Plus/F culture bottle (BD Peds). For the final 30 duodenoscopes, 3 mL of the vortexed un-concentrated sample was additionally inoculated into a BD Peds bottle. Bottles were incubated for 5 days on a BACTEC FX platform. Gram stain and subculture of bottles were performed if they flagged positive. **Results:** 9 duodenoscopes (18%) were culture-positive for skin contaminants (table). No enteric bacteria were recovered. No bacteria were recovered using the 30 BD Peds bottles inoculated with un-concentrated fluid.

Organism Detected	BACTEC Peds Plus/F culture	Sheep	Trypticase	EMB
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	bottle		Blood Agar	Soy Broth	Agar
	Un-concentrated	Concentrated			
<i>Bacillus</i> species	0	0	1	0	0
Coagulase negative <i>Staphylococcus</i> species	0	2	1	1	0
<i>Micrococcus</i> species	0	4	1	0	0
<i>Streptococcus salivarius</i> group	0	1	0	0	0
<i>Streptococcus mitis</i> group	0	1	0	0	0

Conclusion: This study confirms that proper cleaning followed by ethylene oxide sterilization is effective in reprocessing scopes. Use of blood culture bottles is promising for partially automating the process of duodenoscope culture.

Author Disclosure Block:

B. Dylla: None. **P. Kohner:** None. **S. Ihde:** None. **J. Berry:** None. **K. Monson Jobe:** None. **D.J. Hata:** None. **B. Petersen:** None. **A. English:** None. **R. Yates:** None. **P. Sampathkumar:** None. **R. Thompson:** None. **T. Gryns:** None. **R. Patel:** E. Grant Investigator; Self; nanoMR, BioFire, Check-Points, Curetis, 3M, Merck, Actavis, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Allergan, The Medicines Company. N. Other; Self; Dr. Patel has a patent Bordetella pertussis/parapertussis PCR with royalties paid to TIB, a patent Device/method for sonication with royalties paid to Samsung, and a patent anti-biofilm substance issu, non-financial support from bioMérieux, Bruker, Abbott, Nanosphere, Siemens, BD, other from Curetis.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

Early Results of a Duodenoscope Microbiological Surveillance Program

Author Block:

D. Sampat, A. Wanger, G. Holzmann-Pazgal, J. Butler, N. Thosani, L. Ostrosky-Zeichner; Mem. Hermann- TMC, Houston, TX

Abstract Body:

BACKGROUND: Following recent outbreaks of CRE, the FDA has provided guidance recommending periodic culturing of duodenoscopes. There is limited data on the logistics and results of such surveillance. **METHODS:** Duodenoscopes were cultured monthly from JUN to NOV 2015 at a tertiary care center. Two operators obtained cultures while wearing PPE. After processing, the scopes were tested on Fridays on a rotational basis. They were not used until results were negative and the scopes were cleared by infection prevention the following Monday. Three samples were obtained from each scope; one from the elevator-closed position, one with the elevator-open, and one from the channel. The outer surface of the scope was sanitized with an alcohol swab, without wiping the elevator or the lens. Sterile brushes, pre-moistened with sterile 0.01M PBS with 0.02% Tween-80 solution, were used to obtain samples from the elevator. 50mL of sterile water was then flushed through the biopsy valve and collected into a sterile container. If any sample yielded growth, the scope was reprocessed, re-cultured, and kept out of circulation. If a scope came back positive for any microorganism, a chart review was completed for patients in whom the duodenoscope was used between the dates of the previous negative result and the current positive result; assessing for post-procedure bacteremia or cholangitis. If a scope came back positive 3x, it was sent to the manufacturer for analysis. **RESULTS:** As seen in the table, 3/32 (9.3%) cultures were positive. One scope had persistent contamination despite repeated reprocessing. No post-procedure cases of bacteremia or cholangitis were identified. **CONCLUSION:** Our process provides a logistically feasible way to comply with current FDA recommendations. We found contamination in 9% of cultures; however, none of these were associated with post-procedure infections. We found one scope persistently contaminated with *Pseudomonas* and sent it back to the manufacturer. The cost and benefits of duodenoscope culture surveillance programs need to be further characterized.

Month	# scopes cultured	Results
JUN	6	1 + for <i>P. aeruginosa</i> , flush, 3x
JUL	5	-
AUG	6	1 + for Alpha hemolytic <i>Streptococcus</i> , open position
SEP	6	-
OCT	3	-
NOV	6	1 + for <i>P. aeruginosa</i> , flush

Author Disclosure Block:

D. Sampat: None. **A. Wanger:** None. **G. Holzmann-Pazgal:** None. **J. Butler:** None. **N. Thosani:** None. **L. Ostrosky-Zeichner:** None.

Session Number:

127

Session Title:

Hand Hygiene and Environmental Issues in the Healthcare Setting

Publishing Title:

How Clean Is Cleaned? Cleaning Thoroughness, Effectiveness, And Briefly Intervening To Affect Cleaning Outcomes

Author Block:

R. Clifford¹, M. Sparks¹, E. Hosford¹, A. Ong¹, D. Richesson¹, S. Fraser², Y. Kwak¹, S. Miller², M. Julius¹, P. McGann¹, **E. Lesho¹**; ¹Walter Reed Army Inst. of Res., Silver Spring, MD, ²Ft. Belvoir Community Hosp., Ft. Belvoir, VA

Abstract Body:

Objectives: The most efficient approach to monitoring and improving cleaning outcomes remains unresolved. We sought to determine whether cleaning thoroughness (dye removal) correlates with cleaning efficacy (absence of molecular or cultivable biomaterial) and whether one brief educational intervention improves cleaning outcomes. **Design:** Before-after trial **Setting:** Newly built community hospital **Intervention:** 90 minute training refresher with surface specific performance results **Methods:** Dye removal, measured by fluorescence, and biomaterial removal and acquisition, measured with culture and culture-independent PCR-based assays were clandestinely assessed for eight consecutive months. At this midpoint, results were presented to the cleaning staff (intervention) and assessments continued for another eight consecutive months. **Results:** 1273 surfaces were sampled before and after terminal room cleaning. In the short-term, dye removal increased from 40.3% to 50.0% (not significant). For the entire study period, dye removal also improved but not significantly. After the intervention, the number of rooms testing positive for specific pathogenic species by culturing decreased from 55.6% to 36.6% (not significant), and those testing positive by PCR fell from 80.6% to 53.7% (P=0.016). For nonspecific biomaterial on surfaces: a) removal of cultivable Gram-negatives (GN) trended toward improvement (P=0.056), b) removal of any cultivable growth was unchanged but acquisition (detection of biomaterial on post-cleaned surfaces that were contaminant-free before cleaning) worsened (P=0.017); c) removal of PCR-based detection of bacterial DNA improved (P=0.046, but acquisition worsened (P=0.003); d) cleaning thoroughness and efficacy were not correlated. **Conclusion:** A minor intervention or minimally more aggressive

cleaning may reduce pathogen-specific contamination, but not without unintended consequences.

Author Disclosure Block:

R. Clifford: None. **M. Sparks:** None. **E. Hosford:** None. **A. Ong:** None. **D. Richesson:** None. **S. Fraser:** None. **Y. Kwak:** None. **S. Miller:** None. **M. Julius:** None. **P. McGann:** None. **E. Lesho:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Occurrence Of Aminoglycoside (Amg) Modifying Enzymes (Ame) And 16s Rna Methylases (RNamet) Among Enterobacteriaceae (Ent) And Activity Of Plazomicin (Plz) Against Common Resistance (R) Mechanisms

Author Block:

M. Castanheira, A. P. Davis, T. B. Doyle, R. E. Mendes, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: AMEs are the primary mechanisms of AMG R in ENT and confer variable R levels to AMG, but not PLZ. RNAmets confer high level R against all AMG. We screened ENT isolates for common AME and RNAmets genes, and analyzed the PLZ activity against R groups/genes. **Methods:** 929 ENT isolates collected worldwide during 2014 and displaying R to gentamicin (GEN), amikacin (AMK) or tobramycin (TOB) were screened for *aac(6′)-Ib*, *aac(3)-IIa*, *ant(2′′)-Ia*, *aph(3′)-VIa* and *aac(3)-Ia*, *-Ib*, *-Ic*, *-Id*, *-Ie* and *aac(3)-IVa*. Isolates displaying PLZ MIC results at ≥ 128 $\mu\text{g/mL}$ were tested for RNAmets. **Results:** *aac(6′)-Ib* was the most common AME detected (625 isolates; 67.3%) and was found in 424 *K. pneumoniae* (KPN; 84.0%) and 171 *E. coli* (EC; 45.0%). 581 (62.5%) isolates yielded positive results for *aac(3)-IIa*: including 287 EC (75.5%) and 273 KPN (54.0%). These two genes were noted on all continents and were detected together in 367 isolates (239 KPN, 110 EC and 18 other spp.). Among other genes detected, *ant(2′′)-Ia* and *aac(3)-IVa* were observed in 38 and 29 isolates and *aac(3)-Ia* and *aac(3)-Id/e* were detected in 3 and 1 isolates from Europe, respectively. Only twenty-two (2.4%) isolates were negative for AME genes tested. Forty (4.3% of total) isolates displayed PLZ MIC values ≥ 128 $\mu\text{g/mL}$ and were positive for RNAmets. PLZ was very active against AMG non-S isolates (Table). AMK non-S isolates had higher PLZ MICs due to the high proportion of RNAmets producers in this group (40/121). PLZ inhibited all isolates harbouring the four most common AME genes at ≤ 16 $\mu\text{g/mL}$. PLZ was more active against AME carrying isolates than AMK, GEN or TOB. PLZ MIC values at 8-64 $\mu\text{g/mL}$ were noted among only 5 (0.5%) isolates. **Conclusions:** PLZ displayed potent activity against ENT carrying common AMEs and only 5 (0.6%) AME-producers displayed PLZ MICs >4 $\mu\text{g/mL}$. All 40 RNAmets-producers were R to all AMGs. These

data warrant further investigation of PLZ as a new antibiotic for treatment of serious infections due to AMG-R ENT.

Organism Groups(no. of isolates)	MIC _{50/90}			
	PLZ	AMK	GEN	TOB
Overall (929) ^a	0.5/1	4/32	>32/>32	>8/>8
AMK non-S (121) ^a	0.5/>128	32/>32	16/>32	>8/>8
GEN non-S (693) ^a	0.5/2	2/16	>32/>32	>8/>8
TOB non-S (820) ^a	0.5/1	4/32	>32/>32	>8/>8
aac(6')-Ib (625)	0.5/1	4/32	32/>32	>8/>8
aac(3)-IIa (581)	0.5/1	2/8	>32/>32	>8/>8
aac(3)-IVa (29)	0.5/1	4/32	32/>32	>8/>8
ant(2'')-Ia (38)	0.5/1	2/8	16/>32	>8/>8
RNAmet (40)	>128/>128	>32/>32	>32/>32	>8/>8
a. Include 40 RNAmet-producing isolates.				

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Achaogen, Inc. **A.P. Davis:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Achaogen, Inc. **T.B. Doyle:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Achaogen, Inc. **R.E. Mendes:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Achaogen, Inc. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Achaogen, Inc..

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Emergence of Plasmid-Mediated Mcr-1 Protein Leading to Colistin Resistance in South Africa

Author Block:

L. Poirel¹, N. Kieffer¹, A. Brink², J. Coetze², P. Nordmann¹; ¹Univ. of Fribourg, Fribourg, Switzerland, ²Univ. of Johannesburg, Johannesburg, South Africa

Abstract Body:

Background: Increasing antibiotic resistance in gram-negative bacteria recently led to an increasing use of colistin. Due to this selective pressure, emergence of colistin resistant Enterobacteriaceae was expected. Chromosomal gene modifications involved in the PmrAB and PhoPQ two-component regulatory system, as well as inactivation of the *mgrB* gene, are known to be sources of colistin resistance in *Klebsiella pneumoniae*. In addition, a novel plasmid-mediated mechanism has been described, corresponding to the production of the phosphoethanolamine transferase MCR-1 protein. **Material/methods:** Colistin susceptibility testing was determined by using broth microdilution according to CLSI, and by using the Rapid Polymyxin NP test that is based on a rapid culture in presence of a defined concentration of colistin and a defined culture medium. Search of the *mcr-1* gene was performed by PCR. Results were interpreted according to EUCAST and were compared to results obtained with the BMD method (gold standard).

Escherichia coli ATCC 25922 and a *M. morgannii* strain naturally resistant to colistin were included in all experiments as control strains. **Results:** This study was carried out on a total of seven non-duplicate clinical *Escherichia coli* isolates recovered in different South African hospitals. They were all showing an MIC of colistin at 8 or 16 µg/ml. Six out of the seven isolates were found to produce the MCR-1 protein. The corresponding gene was located onto a ca. 70-kb IncI2 plasmid in five out of six isolates, and onto a ca. 150-kb IncHI2 plasmid in a single isolate. No other resistance marker were identified on those plasmids. Four out of the seven isolates were clonally-related, although three isolates corresponded to single non clonally-related clones. **Conclusions:** This study revealed the emergence of the MCR-1 protein responsible for colistin resistance in a series of colistin-resistant *E. coli* isolates in South Africa.

Author Disclosure Block:

L. Poirel: None. **N. Kieffer:** None. **A. Brink:** None. **J. Coetze:** None. **P. Nordmann:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Travelers Can Import *E. coli* Possessing the Plasmid-Mediated Colistin Resistance *Mcr-1* Gene from the Indian Subcontinent

Author Block:

O. Bernasconi¹, E. Kuenzli², J. Pires¹, S. Kasraian¹, A. Carattoli³, C. Hatz², V. Perreten¹, **A. Endimiani¹**; ¹Univ. of Bern, Bern, Switzerland, ²Swiss TPH, Basel, Switzerland, ³ISS, Rome, Italy

Abstract Body:

Background: Plasmid-mediated colistin (COL) resistance encoded by the *mcr-1* gene has been recently described in *Enterobacteriaceae* (*Ent*) from food animals, food, and humans in several countries. However, data regarding the prevalence of these life-threatening pathogens in humans is still needed. We explored the prevalence of travelers colonized at intestinal level with COL-resistant (COL-R) *Ent*. **Methods:** Stools obtained before and after traveling (at return and after 3 and 6 months) were enriched in LB broth and plated on MacConkey with 4 mg/L COL. BLSE, ChromID ESBL, and Supercarba plates were also used to detect ESBL/carbapenemase producers. Species ID was achieved with the MALDI-TOF MS. MICs were obtained with microdilution panels and Etest. Microarray CT103XL was used to identify *bla* genes, whereas PCR/DNA sequencing was implemented to detect *mcr-1*. Plasmids were characterized with PBRT and transformation. Clonality was determined by MLST and phylogenetic group. DNA was submitted for WGS using Illumina. Travelers filled in a questionnaire. **Results:** So far, we screened 36 people living in Switzerland and traveling to the Indian subcontinent. At return, a 57-year old woman who visited India for 15 days had a *mcr-1*-carrying *E. coli* with a COL MIC of 8 mg/L and another *E. coli* producing CTX-M-15 (MIC for COL ≤0.25 mg/L). The COL-R strain was of ST10, group A1, and positive for IncHI2, X1, and P plasmids; it was resistant to quinolones, tetracycline, and cotrimoxazole but not to cephalosporins and carbapenems. The person was not colonized before traveling. Notably, the *mcr-1*-carrying *E. coli* was not found in the follow up stools collected at 3 and 6 months after the trip. **Conclusions:** This is the first study investigating Swiss travelers for colonization with COL-R *Ent*. Our results indicate that traveling to the Indian subcontinent can be a risk factor to acquire the plasmid-mediated colistin

resistance *mcr-1* gene. This phenomenon should be carefully monitored because it may rapidly jeopardize COL, our last efficient anti-Gram-negative antibiotic. The importance to use selective plates containing only COL is also emphasized.

Author Disclosure Block:

O. Bernasconi: None. **E. Kuenzli:** None. **J. Pires:** None. **S. Kasraian:** None. **A. Carattoli:** None. **C. Hatz:** None. **V. Perreten:** None. **A. Endimiani:** None.

Session Number:

128

Session Title:**New insights on Emerging Resistance Mechanisms in Gram-negatives****Publishing Title:****Co-Carriage of Plasmid-Mediated Fosfomycin Resistance Determinants (*fos*) by Carbapenem Resistant Enterobacteriaceae in the United Kingdom****Author Block:****S. Zainal**, L. Phee, H. Abdul Momin, D. Wareham; Queen Mary Univ. London, London, United Kingdom**Abstract Body:**

Carbapenem resistant Enterobacteriaceae (CRE) are recognised as a major threat to human health. Strains producing serine (KPC) or metallo- β -lactamases (NDM, IMP, VIM) are often extensively drug resistant (XDR) and carry multiple resistance determinants on large conjugative plasmids. Fosfomycin (FOS), an old drug with proven efficacy in the treatment of uncomplicated urinary tract infections, has been proposed as a potential therapy for CRE infections. Resistance to FOS can occur either through mutation of intrinsic genes involved in drug uptake (*uhpT*, *glpT*), or by acquisition of fosfomycin glutathione S-transferase enzymes (FR-GST) able to modify or hydrolyse fosfomycin (*fosA-I, X*). In this study we investigated the prevalence of plasmid encoded FOS resistance in XDR Enterobacteriaceae recovered in the UK. A total of 110 unique CRE (ertapenem MIC > 1mg/L) isolates collected from 2011-15 were analysed. β -lactamase and carbapenemase genes were detected and identified by multiplex PCR. Susceptibility to FOS was assessed by disc diffusion using 50 μ g and 200 μ g discs on MH II agar supplemented with 25 μ g/ml of Glucose-6-Phosphate (G6P). Growth of FOS resistant isolates on M9 minimal media supplemented with G6P as a sole carbon source was used to confirm the integrity of FOS uptake pathways. Sodium phosphonoformate (PPF) was used as an inhibitor of FR-GST to confirm production of FOS-like enzymes. PCR and sequencing was used to identify *fosA* like genetic variants. All of the isolates were XDR (resistant to β -lactams, aminoglycosides, quinolones, sulfonamides) but remained susceptible to polymyxins. 75 % (82/110) were carbapenem resistant due to the production of a NDM-like enzyme. Resistance to FOS combined with synergy with PPF was seen against 49 isolates (45%) and *fosA5* detected in 60 % of the NDM producers. Plasmid mediated FOS resistance (*fosA5*) is therefore common amongst CRE strains

circulating in the UK, raising concerns on the suitability and likely efficacy of FOS therapies for the treatment of CRE infections.

Author Disclosure Block:

S. Zainal: None. **L. Phee:** None. **H. Abdul Momin:** None. **D. Wareham:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Compensatory Evolution of Mecillinam Resistant Mutants Provides Potential Explanations for Why Clinical Resistance Development Is Slow

Author Block:

E. Thulin, M. Knopp, D. I. Andersson; Uppsala Univ., Uppsala, Sweden

Abstract Body:

Mecillinam is a beta-lactam that is used almost exclusively for treatment of uncomplicated Urinary Tract Infections (UTIs). The mutation frequency to mecillinam resistance (MecR) in the laboratory is very high but despite this fact resistance development in clinical settings has remained slow. We previously showed that even though >40 genes can confer MecR when mutated, only one of them (*cysB*) is found in clinical MecR isolates of *Escherichia coli* (Thulin *et al.* 2015). Based on fitness assays we proposed that the fitness costs of most types of MecR mutations are too high for them to be viable in clinical settings. To better understand how mecillinam resistance evolves, we selected six different MecR mutants for serial passage evolution experiment—*mrda*, *ppa*, *ubiE*, *aspS*, *spoT*, and the clinically important *cysB*—to determine if their low fitness could be genetically compensated. Eight lineages of each mutant were cycled without mecillinam for 400-500 generations, and then fitness (growth rate) and MICs were measured. All lineages of all mutants had increased fitness compared to the parental strain, but the increase in fitness was associated with loss of resistance in all compensated mutants except for the *mrda* mutant lineages. Local DNA sequencing showed that the *ppa*, *ubiE*, *aspS* mutants all had intragenic mutations whereas the *spoT* mutants had compensatory mutations in *relA*. The *cysB* and *mrda* compensated mutants were whole genome sequenced. The compensatory mutations in the *mrda* mutants were point mutations in *nlpI* and *pgsA*, deletion of *sppA*, and a large duplication found in all lineages. The compensatory mutations in the *cysB* mutants were in other genes involved in cysteine biosynthesis, and interestingly one type, *ydjN* mutation, was also found in clinical strains. Thus, compensation of the fitness cost of mecillinam resistance is generally associated (5/6 cases) with a complete loss of resistance, suggesting that mecillinam resistance and high fitness are often genetically incompatible with each other.

This finding provides an additional potential explanation for why the frequency of mecillinam resistance has remained low in clinical isolates.

Author Disclosure Block:

E. Thulin: None. **M. Knopp:** None. **D.I. Andersson:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Insight into the Mechanisms of Carbapenemase Resistance through Animations of Globular Protein Movement Employing Atomic Torsion Mode Analysis

Author Block:

D. Pantazatos¹, E. Mavridou¹, S. THIRUMURUGANANDHAM², M. Tirion³, T. J. Walsh¹; ¹Weill Cornell Med. Coll., New York, NY, ²Yachay Tech Univ., Ibarra, Ecuador, ³Clarkson Univ., Potsdam, NY

Abstract Body:

Introduction: Multi-drug (MD) resistant *K. pneumoniae* (Kp) are a global threat as β -lactamase enzymes (bla) inactivate β -lactam antibiotics. The molecular basis of the extended spectrum often involves point mutations within plasmid mediated bla genes such as TEM52 and KP2 resulting in structural changes in the corresponding enzymes through amino acid substitutions. We aimed to investigate MD-resistance (Kp) upon structural differences and protein movement. For this, we have implemented a novel computational approach in modeling the globular protein movement (GPM) of KPC2 bla and TEM52 bla using Torsion Mode Analysis (TMA). **Methods:** GPM was performed using the Atomic Torsional Movement (ATMAN) Algorithm. ATMAN models protein flexibility by computing the structural symmetry axes, (normal modes) of PDB entries using the rotational degrees of freedom (dihedral angles). The analysis quantifies the energetic cost of all bond-rotations in terms of resultant variations in nonbonded (steric) interactions and intrinsic bond-rotation, as modeled on the standard molecular potential, ENCAD. This uniquely identifies the “degrees of freedom” natural or intrinsic to the atomic structure provided for each protein. By visualizing these motions, activated by the energy in the thermal heat bath, domain motions extending over the breadth of the molecule are seen. **Results:** TEM-52 bla mutations result in significant alterations in the GPM of the enzyme when compared to KPC-2 bla. The 3D GPM demonstrated that mutation-induced altered movements resulted in a >5 fold widening of the active site of TEM-52 bla (MIC>500 mg/L) compared to the KPC-2 bla motions (MIC=1 mg/L). This widened opening of the active site indicates a potential for inactivation of a wider spectrum of antibiotics as the catalytic site is more accessible to larger antibiotics. **Conclusions:** TMA elucidated alterations in structure and movement

surrounding the active site of Kp bla. The 5x widening of the active site in TEM52 lends greater access to diverse antimicrobial structures implicating a potential mechanism for an expanded spectrum of drug resistance. GPM analysis may also serve as a tool for tailored design of bla inhibitors

Author Disclosure Block:

D. Pantazatos: None. **E. Mavridou:** None. **S. Thirumuruganandham:** None. **M. Tirion:** None. **T.J. Walsh:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Functional and Kinetic Characterization of *Pseudomonas aeruginosa* Quinolone Efflux Transporters

Author Block:

D. Wolloscheck, J. Nguyen, H. I. Zgurskaya; Univ. of Oklahoma, Norman, OK

Abstract Body:

Background: Multidrug resistant isolates of *Pseudomonas aeruginosa* pose a serious threat to public health and result in infections that are increasingly harder to treat. Active efflux, one of the primary mechanisms of drug resistance, is typically carried out by RND (Resistance, Nodulation, and Cell Division) efflux pumps. In this study, we characterized two quorum sensing transporters MexEF-OprN and MexGHI-OpmD in their contribution to physiology, their ability to provide resistance against a range of fluoroquinolones, and the kinetics of efflux. **Methods:** Genes encoding efflux pumps were cloned into a broad host expression vector and expressed in *P. aeruginosa* PAO1 lacking the four major constitutively expressed efflux pumps $\Delta mexAB-oprM$ $\Delta mexCD-oprJ$ $\Delta mexJKL$ $\Delta mexXY$. Pigment production and biofilm formation were measured by absorbance spectroscopy. Susceptibilities to different antibiotics were analyzed by measuring MIC (minimum inhibitory concentration) values in cells grown in liquid media. Kinetic measurements of efflux were carried out using varying concentrations of fluorescent probes that have binding sites inside the cell. **Results:** Our results show that pyocyanin production is severely decreased when MexEF-OprN but not MexGHI-OpmD is overexpressed. MIC measurements showed that overexpression of MexEF-OprN increases resistance to a broad range of antibacterial compounds, whereas MexGHI-OpmD has a narrow substrate specificity and primarily decreases MICs of fluoroquinolones. The unique fourth component MexG of MexGHI-OpmD is not required for this activity of the transporter. Surprisingly despite different substrate specificities, the two pumps provide similar levels of fluoroquinolone resistance. Testing 18 structurally diverse fluoroquinolones showed that the MICs were raised by roughly the same factor when comparing the efflux deficient strain with and without MexEF-OprN overexpression. Our kinetic studies provide a quantitative comparison of biochemical properties of these two transporters. **Conclusion:** MexEF-OprN and MexGHI-OpmD are similar in the

mechanism of efflux but differ in their substrate specificities. Although both transporters are important for quorum sensing, their physiological functions are likely different. Efflux pumps do not discriminate between structurally diverse fluoroquinolones.

Author Disclosure Block:

D. Wolloscheck: None. **J. Nguyen:** None. **H.I. Zgurskaya:** None.

Session Number:

128

Session Title:**New insights on Emerging Resistance Mechanisms in Gram-negatives****Publishing Title:*****Pseudomonas aeruginosa* Outer Membrane Proteome: Role of Simple Porins****Author Block:****J. M. Buyck**, P. Saint Auguste, C. Schleberger, D. Bumann; Biozentrum, Univ. of Basel, Basel, Switzerland**Abstract Body:**

Pseudomonas aeruginosa is a major opportunistic pathogen. Because of its high intrinsic resistance to antibiotics and the emergence of additional resistance mechanisms, it has become one of the most dangerous infectious agents in hospital-acquired infections. It is often assumed that the *P. aeruginosa* outer membrane simple porins crucially determine antibiotic translocation and efficacy. Indeed, inactivation of one of its 35 different simple porins, OprD, decreases *P. aeruginosa* susceptibility specifically to carbapenems. However, if similar links also exist between other antibiotics and simple porins remains largely unclear. To determine which porins *P. aeruginosa* expresses, we have developed a sensitive targeted mass spectrometry-based proteomic approach. The results showed that diverse *P. aeruginosa* strains including human clinical isolates, express only a rather small subset of simple porins under various *in vitro* conditions. As expected, OprF was the dominant simple porin. In addition, five other simple porins (OprD, FadL, OprG, OprQ, OprE) were commonly detected. Six other porins were detected at low and variable levels (Tsx, OpdQ, OpdC, OpdH, OpdP, OprB). All other porins were below our detection threshold of ca. 30 molecules per cell. To assess the functional relevance of these porins, we generated a series of single or multiple gene deletions on PA14 strain. Antimicrobial susceptibility testing revealed that none of the simple porins other than OprD affected minimal inhibitory concentrations (MIC) of diverse antibiotics. Surprisingly, even a delta34 strain lacking all simple porins had identical MIC values compared to parental PA14 (except for carbapenems where the delta34 strain phenocopied the *oprD* mutant). This strain grew normally on rich media but had severe growth defects on minimal media containing single carbon sources. Interestingly, we did not detect any compensatory expression of cryptic porins in various multiple knock-outs. These results suggest a role of simple porins in nutrient uptake, but do not support current models of antibiotics membrane translocation. In addition of simple porins, alternative

translocation routes likely exist. These findings have major implications for the mechanism of antibiotic action against *P. aeruginosa* and developments of urgently needed novel drugs.

Author Disclosure Block:

J.M. Buyck: None. **P. Saint Auguste:** None. **C. Schleberger:** None. **D. Bumann:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Comparative Analysis of Antibiotic Resistance Patterns of Nontyphoid *Salmonella* Clinical Isolates Using Next-Generation Sequencing for Discovering Novel Genetic Mutation Targets

Author Block:

K-C. Wang¹, W-H. Chou², C-M. Chang², W-C. Chang², S-B. Fang³; ¹Shuang Ho Hosp., Taipei Med. Univ., Taiwan, Taiwan, ²Coll. of Pharmacy, Taipei Med. Univ., Taipei, Taiwan, ³Shuang Ho Hosp., Taipei Med. Univ., Taipei, Taiwan

Abstract Body:

Background: Non-typhoid *Salmonella* (NTS) is one of the important enteropathogens worldwide. However, the problem of antibiotic resistance in NTS is serious in Taiwan. Therefore, we conducted this study using the next-generation sequencing (NGS) to identify novel genetic mutations in NTS. **Methods:** A total of 19 *Salmonella* clinical isolates were collected in TMU-SHH in Taiwan, including 2 ceftriaxone-resistant strains and 1 ciprofloxacin-resistant strain. The NGS was performed using the illumina[®] MiSeq. The genomic data were analyzed to examine presence genes or genetic mutations of *Salmonella* reported in the PubMed, CARD, and ResFinder. In addition, the BLAST-translated amino acid sequences were also compared between the antibiotic-resistant and antibiotic-susceptible strains. **Results:** First, our data demonstrated presence of plasmid-mediated *bla*_{CMY-2} and *ampC* in the 2 ceftriaxone-resistant strains and absence of these two genes in the other 17 ceftriaxone-susceptible strains. Second, in the ciprofloxacin-resistant strain, we discovered one novel frameshift mutation in *parC* g.1307delA that leads to totally different translation in amino acid sequence in the only one ciprofloxacin-resistant strain and another novel point mutation in *parE* g.1031G>T that changed the amino acid sequence. Furthermore, the point mutation in *parC* g.170C>G and another previously reported point mutation in *gyrB* g.2044del were also found in one of the 18 ciprofloxacin-susceptible strains, suggesting that *parC* g.170C>G and *gyrB* g.2044del are not major genetic mutations contributing to ciprofloxacin resistance. **Conclusions:** The NGS provided detailed information in the genome of our NTS clinical isolates that facilitates comparative analysis of gene and amino acid sequences between antibiotic-resistant and antibiotic-susceptible strains. Moreover, two novel genetic mutations in

parC g.1307delA and in *parE* g.1031G>T are identified as potentially decisive genetic loci to ciprofloxacin resistance in NTS, which warrants further confirmation in their phenotypes by generating recombinant mutated NTS strains.

Author Disclosure Block:

K. Wang: None. **W. Chou:** None. **C. Chang:** None. **W. Chang:** None. **S. Fang:** None.

Session Number:

128

Session Title:

New insights on Emerging Resistance Mechanisms in Gram-negatives

Publishing Title:

Characterization of Mobile Genetic Elements in Antibiotic Resistant *Salmonella enterica* Isolates from Food Animals

Author Block:

E. A. McMillan¹, S. K. Gupta², L. Williams³, T. Jove⁴, C. R. Jackson², M. McClelland⁵, J. G. Frye²; ¹Univ. of Georgia, Athens, GA, ²USDA-ARS, Athens, GA, ³Providence Coll., Providence, RI, ⁴Univ. Limoges, Limoges, France, ⁵Univ. of California, Irvine, CA

Abstract Body:

Background: Antibiotic resistance (AR) is a major concern for the agricultural industry in the U.S. and globally. It is complicated by the fact that AR genes are often located on mobile genetic elements (MGEs) resulting in their spread among bacteria. In order to investigate the relationship between AR and MGEs, we identified AR genes, plasmids, and integrons in the genomes of *Salmonella enterica* isolated from animal sources. **Methods:** *Salmonella enterica* (n=194) isolated from beef and dairy cattle, chicken, swine, turkey, and their meat products representing 84 diverse serovars were selected based on their differing AR phenotypes. Isolates were sequenced using an Illumina HiSeq. Draft genomes were assembled using A5 and annotated with Prokka. Resistance genes were identified using ARG-ANNOT. Plasmid sequences were identified through *in silico* PCR identifying replicon and relaxase genes. Integrons were identified using INTEGRALL. **Results:** The 194 isolates had a total of 883 resistance genes detected and at least one gene was detected per isolate. Plasmids were detected in 157 of 194 isolates. The most prevalent replicons detected were A/C, colE, F, HI1, HI2, and I. AR genes were detected on 110 plasmid sequences and 71 of those contained multiple AR genes. Class 1 integrons were detected in 66 isolates; no class 2, 3, or 4 integrons were detected. AR genes were found in 62 integrons, 55 of which had multiple AR genes detected. Twenty-six of the integrons were located on IncI1 plasmids and two on IncHI2 plasmids. **Conclusions:** Most isolates contained plasmids, integrons, or both. Many of the mobile elements and AR genes have been previously found in *Salmonella*, but not in animal associated isolates or in the rare serovars analyzed. The identification of AR genes on the same contiguous sequence as plasmid and/or integron genes demonstrates linkage of MGEs and AR in these food animal associated *Salmonella*. Additionally, the

localization of class 1 integrons on IncI1 and HI2 plasmids indicates the complex structure of MGEs and may suggest a mechanism for AR genes accumulation on plasmids. Future work will identify other MGEs and determine their relationships to AR.

Author Disclosure Block:

E.A. McMillan: None. **S.K. Gupta:** None. **L. Williams:** None. **T. Jove:** None. **C.R. Jackson:** None. **M. McClelland:** None. **J.G. Frye:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Using Yeast-Three Hybrid to Identify Targets of MMV Malaria Box Compounds That Inhibit Growth of Apicomplexan Parasites

Author Block:

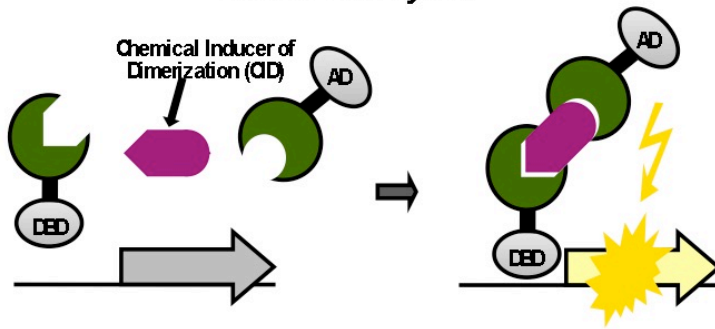
J. E. Foderaro¹, F. Tran², N. J. Westwood², G. E. Ward¹; ¹Univ. of Vermont, Burlington, VT, ²Univ. of St. Andrews, St. Andrews, United Kingdom

Abstract Body:

Our long-term goal is to understand the mechanisms of infection of *Toxoplasma gondii* using small-molecule chemical probes. Using a fluorescence-based assay, our lab screened 400 previously identified small-molecule inhibitors of the related parasite, *Plasmodium falciparum*, for an effect on *T. gondii* growth. We identified a number of potent hits, including a 2,4-diamino-quinazoline chemical scaffold (MMV006169; IC₅₀=1.15µM) that strongly inhibits *T. gondii* intracellular replication with no evidence of toxicity to mammalian cells. Extensive structure-activity analyses with *T. gondii* identified a number of analogs with changed potency and altered replication phenotypes. These structure-activity analyses provided the information necessary to synthesize a bivalent chemical inducer of dimerization (CID) containing MMV006169 for use in yeast three-hybrid (Y3H) experiments. Yeast growth competition assays show that this CID is capable of entering the yeast nucleus, as required for Y3H screening. Identification of the biologically relevant target(s) of these compounds by Y3H analysis is currently underway and will be confirmed using reverse genetics approaches. The discovery of compounds that inhibit the growth of multiple apicomplexan parasites suggests that they may be

directed against conserved targets important to the apicomplexan parasite life cycle.

Yeast Three-Hybrid



Author Disclosure Block:

J.E. Foderaro: None. **F. Tran:** None. **N.J. Westwood:** None. **G.E. Ward:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Adherence to Artemisinin Combination Prescription Guidelines and Health Workers Views Towards the Guidelines, a Case Study of an Urban Health Center in Uganda

Author Block:

I. Mugerwa; Central Publ. Hlth.Lab., Kampala, Uganda

Abstract Body:

Introduction: In 2012 the Uganda Ministry of Health with World Health Organization initiated new malaria treatment guidelines using Artemisinin -Based Combination Therapy, this was due to the increased resistance to first line antimalarial drugs. This study aimed at finding out adherence to the standard Artemisinin -Based Combination Therapy prescription guidelines and health workers views towards the guidelines among the patients diagnosed with malaria in Kisugu Health Center III a suburb of Makindye Division of Kampala District Uganda. Therefore successful implementation of this policy depends on the adherence potentials of health workers.**Rationale:** Due to increasing resistance of malaria parasites towards many drug regimens, the rampant change of anti-malarials has been the core paramount reason for conducting this study, to find out whether the Health Workers are adhering to the current World Health Organization Malaria treatment guidelines.**Method:** Using the pharmacy register, 356 prescriptions for ACTs were reviewed retrospectively and data for the months of August and September 2014 was correlated with the laboratory records for those particular months. This followed key informant interviews of three health workers (two Clinical Officers and one Laboratory Technician).**Results:** The majority of patients who got ACTs were never sent to the laboratory (n=302, **84.8%**). Of the 54 (15.2%) sent to the laboratory, the majority 40 (**74.1%**) tested negative for malaria parasites but were given ACTs. Only 14 (25.9 %) of the 54 sent to the laboratory and also received ACTs, tested positive for malaria parasites. Majority of the patients in this study who got ACTs (n= 342, 96.1%) did not adhere to the new Malaria Treatment Guidelines, MoH, 2012. Majority of the patients who got ACTs at this facility were, children between 0-9years (n=288, 80.9%Health workers also admitted to practice presumptive prescription of ACTs in patients with clinical symptomatic malaria with their explicit reasons contrary to the

guidelines.**Conclusion:** The study confirmed non adherence to the new ACT policy treatment guidelines with explicit reasons for non-adherence. However a research in Public facilities similar to Kisugu Health center III, is highly recommended to broaden the evidence with a bigger sample size.

Author Disclosure Block:

I. Mugerwa: None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Characterisation of New Chemical Scaffolds for the Treatment of Human African Trypanosomiasis

Author Block:

A. J. Jones¹, M. Kaiser², V. M. Avery¹; ¹Eskitis Inst. for Drug Discovery, Brisbane, Australia, ²Swiss Tropical and Publ. Hlth.Inst., Basel, Switzerland

Abstract Body:

Background: *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are the aetiological agents of Human African Trypanosomiasis (HAT), a neglected, parasitic disease prevalent in sub-Saharan Africa. The number of cases of HAT has declined by over 80% in the last 20 years due to the introduction of rigorous surveillance and treatment programs. However, to achieve the goal of eliminating HAT as a public health problem by 2020, new molecules are needed as currently available drugs have limited efficacy, poor safety profiles and protracted, impractical administration schedules. This abstract describes the characterisation of a novel class of promising anti-trypanocidal compounds. **Methods:** A novel class of anti-trypanocidal compounds was previously identified through a HTS whole cell screening campaign against *T.b. brucei*. Analogues were evaluated against *T.b. brucei* and the human infective subspecies *T.b. gambiense* and *T.b. rhodesiense* to build basic structure activity relationships and aid prioritization of lead compounds. Lead compounds underwent physicochemical and metabolism assessment and development of resistant strains was initiated to gain insights into possible molecular targets. **Results:** Following evaluation of over >30 structural analogues, 3 compounds were prioritized based on anti-trypanocidal activity and medicinal chemistry properties. The compounds exhibited IC₅₀ values ranging from 0.32 to 4.83 µM against the human infective subspecies *T.b. rhodesiense* and *T.b. gambiense* (Table 1). Preliminary metabolism studies in human and murine microsomes revealed extensive non-NADPH mediated degradation that will need to be addressed. **Conclusions:** The compounds identified in this study are potent, selective trypanocidal agents. Preliminary studies are in progress to identify the molecular target of the compounds to allow the development of more potent analogues with improved metabolic stability that can be progressed along the drug discovery pipeline for HAT.

	IC₅₀ μM				
Compound	<i>T.b. brucei</i>	<i>T.b. rhodesiense</i>	<i>T.b. gambiense</i> K048	<i>T.b. gambiense</i> STIB930	L6
1	6.77 ± 1.51	4.83 ± 2.1	6.11 ± 1.23	1.26 ± 0.28	48.15 ± 3.18
2	3.09 ± 0.76	0.32 ± 0.18	4.71 ± 0.71	3.23 ± 0.93	62.75 ± 4.31
3	0.04 ± 0.01	4.36 ± 1.52	0.56 ± 0.05	1.11 ± 0.43	1.67 ± 0.59

Author Disclosure Block:

A.J. Jones: None. **M. Kaiser:** None. **V.M. Avery:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Within-Host Selection of Drug Resistance in a Mouse Model of Repeated Incomplete and Inadequate Malaria Treatment: Dose Dependent Selection of Atovaquone Resistance Mutations

Author Block:

S. Nuralitha¹, J. E. Siregar¹, D. Syafruddin¹, S. Marzuki¹, A. I. M. Hoepelman²;
¹Eijkman Inst. of Molecular Biology, Jakarta, Indonesia, ²Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract Body:

Background: The evolutionary selection of malaria parasites within individual host plays a critical role in the emergence of drug resistance, but still not well understood. We have recently developed an animal model based repeated incomplete treatment (RICT) of *Plasmodium berghei* infected mice, investigated within-host resistance selection to antimalarial drugs, and reported the rapid development of resistance to atovaquone and pyrimithamine [Nuralitha et al., 2016. AAC. In Press]. To examine the dynamics of within-host selection of antimalarial drug resistance, we have now compared the selection of atovaquone resistance mutants under two different causes of treatment failure—Repeated incomplete treatment with therapeutic dose (RICT), and Repeated inadequate treatment associated with sub therapeutic dose (RIAT). **Methods:** Both RICT and RIAT are based on cycles of incomplete treatment of *P. berghei*-infected mice, except the dose of atovaquone used in RIAT was sub-therapeutic (0.1 mg kg⁻¹BW) instead of therapeutic (14.4 mg kg⁻¹ BW) dose in RICT. **Results:** The number of treatment cycles for the development stable resistant phenotype during RIAT was 2.00±0.00 cycles (n= 9), which is not statistically different from that of RICT (2.57±0.85 cycles; n=14; p=0.0591). Significant longer treatment time and shorter recovery time were observed in the first cycle treatment for RIAT compared to that for RICT. All mutations underlying atovaquone resistance selected by RIAT was found to be M133I and L144S, in the Qo₁ domain of the mitochondrial *cyt b* gene. In contrast those selected by RICT (Y268N/C, L271V, K272R, L271V in Qo₂ domain and V284F) are in the Qo₂ domain or its neighboring sixth transmembrane region. Resistance parasites resulting from RICT were in all cases found to be of homogenous populatios, while in RIAT mixed

populations of parasites were observed in most cases. **Conclusions:** The development of within-host resistance to Atovaquone is dose dependent. Exposure of mixed populations of resistance parasites found following RIAT to the higher therapeutic dose of RICT revealed further insights into the dynamic of within host selection of resistance to antimalarial drugs.

Author Disclosure Block:

S. Nuralitha: None. **J.E. Siregar:** None. **D. Syafruddin:** None. **S. Marzuki:** None. **A.I.M. Hoepelman:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Intestinal Parasitosis in Relation to CD4+ T Cells Levels and Anemia among HAART Initiated and HAART Naive Pediatric HIV Patients in Model Art Center, Addis Ababa, Ethiopia

Author Block:

H. Mengist; Wollega Univ., Nekemte, Ethiopia

Abstract Body:

Background: Intestinal parasites (IPs) are major concerns in most developing countries where HIV/AIDS cases are concentrated and almost 80% of AIDS patients die of AIDS-related infections. In the absence of highly active antiretroviral therapy (HAART), HIV/AIDS patients in developing countries unfortunately continue to suffer from the consequences of opportunistic and other intestinal parasites. **Methods:** A cross-sectional study was conducted among HAART initiated and HAART naive pediatric HIV/AIDS patients attending a model ART center at Zewditu Memorial Hospital between August 05, 2013 and November 25, 2013. Stool specimen was collected and processed using direct wet mount, formol-ether concentration and modified Ziehl-Neelsen staining techniques. A structured questionnaire was used to collect data on socio-demographic and associated risk factors. CD4+ T cells and complete blood counts were performed using BD FACScalibur and Cell-Dyn 1800, respectively. Logistic regressions were applied to assess any association between explanatory factors and outcome variables. P values < 0.05 were taken as statistically significant. **Results:** The overall prevalence of IPs was 37.8% where 27.8% of HAART initiated and 45.5% of HAART naive pediatric HIV/AIDS patients were infected ($p < 0.05$). *Cryptosporidium* species, *E. histolytica/dispar*, *Hook worm* and *Taenia* species were IPs associated with CD4+ T cell counts <350 cells/ μ L in HAART naive patients. The overall prevalence of anemia was 10% in HAART and 31.7% in non-HAART groups. The prevalence of IPs in non-HAART patients was significantly associated with eating unwashed/raw fruit, open field defecation and diarrhea. IPs significantly increased in rural ($P < 0.05$). **Conclusion:** The overall prevalence of intestinal parasites significantly differed by HAART status and *cryptosporidium* species were found only in HAART naïve patients with low CD4+ T

cell counts. Anemia was also more prevalent and significantly associated with IPs in non-HAART patients.

Author Disclosure Block:

H. Mengist: None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

A Study to Determine the Prevalence and Risk Factors of *Toxoplasma gondii* among the Pregnant Women Visiting Public and Private Clinics in Lahore, Pakistan

Author Block:

T. Ijaz¹, S. Aslam¹, S. Ijaz²; ¹Mayo Hosp., King Edward Med. Univ., Lahore, Pakistan, ²Shalamar Med. and Dental Coll., Lahore, Pakistan

Abstract Body:

Background: *Toxoplasma gondii* (*T. gondii*), a parasite, extensively distributed in the nature, causing Toxoplasmosis, a zoonotic disease. It particularly infects pregnant women which may transmit the infection to their fetus. *T. gondii* is responsible for considerable morbidity and mortality of developing fetus. The transmission of Toxoplasma from infected mother to fetus may lead to abortions, still birth and other complications. In our society human beings have close interaction with the live stock therefore the issue of toxoplasmosis is important. In Pakistan, sero-prevalence of *T. gondii* and its various risk factors are not considerably reported. Keeping in view the importance of congenital toxoplasmosis, this study was conducted to evaluate the seroprevalence and different risk factors of *T. gondii* infection in the pregnant women of Lahore, Punjab who visited public and private clinics for their antenatal checkup. **Methods:** This prospective and observational study was conducted during the year 2012-2013. For the purpose, a total of 100 pregnant women who volunteered for collection of blood and interview were selected and blood samples were collected from them. Blood was analyzed for the presence of IgG and IgM antibodies against *T. gondii* by using Enzyme Linked Immunosorbent Assay (ELISA) technique. The results of ELISA test and data of several risk factors were interpreted by using statistical analysis. **Results:** The results showed that 31% women were positive for Toxoplasma-IgG antibodies, 5% were positive for Toxoplasma-IgM antibodies and 2% were positive for both IgG and IgM antibodies against Toxoplasma. The overall prevalence rate was found to be 34%. There was an association of Toxoplasmosis in women who were illiterate, belonging to the upper class, consuming raw milk, with poor hygiene. An increase in Toxoplasmosis was found in women those were having first trimester of their pregnancy. The data showed that 93% of the women were ignorant of the knowledge about toxoplasmosis.

Conclusion: The prevalence of *T.gondii* among pregnant women is estimated as high along with risk factors like illiteracy, occupation, social status, raw milk etc. Special programs are required to increase the awareness for importance of toxoplasmosis.

Author Disclosure Block:

T. Ijaz: None. **S. Aslam:** None. **S. Ijaz:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

Performance Evaluation of Malaria Microscopists Working at Malaria Slides Rechecking Laboratories for External Quality Assessment in Ethiopia

Author Block:

A. Abebe, H. Asrat, G. Ayana; Ethiopian Publ. Hlth.Inst., Addis Ababa, Ethiopia

Abstract Body:

Background: Microscopic diagnosis of Giemsa stained thick and thin blood films has remained the standard laboratory method for the diagnosis of malaria. The Performance of Malaria Microscopists in all health facilities have been raised concerns by many experts.**Methods:** A cross-sectional study was conducted to assess the performance of 107 Malaria Microscopists who are working at 23 Malaria Rechecking Laboratories in Ethiopia. Data was collected and exported to SPSS version 20 for analysis. Chi-square, sensitivity, specificity, percent agreement, and kappa score were calculated to assess laboratory professionals' performance in detecting and identification of Plasmodium specie. Association was taken as significant at $P < 0.05$.**Results:** A total of 107 study participants were involved in this study, the mean age of the participants was 30 ± 5.04 years. Overall, the sensitivity of participants in detection and species identification of malaria parasites were 96.8% and 56.7%, respectively. The overall agreement on detection and identification of malaria species was 96.8% (Kappa = 0.9) and 64.77% (kappa = 0.33), respectively. The least malaria species which were identified correctly by the participants were *P. malariae* (2.8%) followed by and *P. ovale* (32.7%). Participants at Hospital laboratory had the highest percent agreement (72.3 %, Kappa=0.51) on species identification. Study participants who were participated on malaria microscopy and quality assurance training had a better performance on parasite quantification ($P < 0.001$).**Conclusions:** Agreement of the participants with expert microscopist in the identification of different malaria species and quantification were very low. Therefore, policy backed regular competency assessment and training for malaria microscopists is essential and mandatory to assure proper diagnosis and management of malaria in Ethiopia.

Author Disclosure Block:

A. Abebe: None. **H. Asrat:** None. **G. Ayana:** None.

Session Number:

129

Session Title:

Parasitology and Anti-parasitic Agents

Publishing Title:

A New Polycyclic Xanthone from *Actinomadura* sp Exhibits Potent Antiplasmodial Activity

Author Block:

F. VICENTE, F. ANNANG; FUNDACION MEDINA, GRANADA, Spain

Abstract Body:

Malaria still plagues the world's poorest populations causing thousands of deaths each year mainly children and pregnant women and hence warrants an urgent need for innovation in finding potent lead compounds against *Plasmodium falciparum*. Within the framework of our collaborative program aimed at identifying new antiprotozoal agents from microbial natural products, we report herein the discovery of a new polycyclic xanthone isolated from *Actinomadura* sp. with nanomolar activity against *Plasmodium falciparum*. Whole cell phenotypic High Throughput Screening of about 22, 000 microbial extracts from MEDINA microbial extracts collection against *P. falciparum* using the lactate-dehydrogenase based assay resulted in one *Actinomadura* sp. extract as one of the hits showing potent bioactivity. Bioassay-guided compound purification of this led to the isolation of a new polycyclic xanthone as the bioactive compound. Structural analysis of this new compound using high resolution mass spectrometry and nuclear magnetic resonance spectroscopy revealed significant differences in the structure of this as compared to other members of this family previously described in the literature. The newly identified polycyclic xanthone has a molecular weight of 507 Da, small enough for drug-like compounds, and an IC₅₀ of 9 nM against *P. falciparum*. Although several members of the polycyclic xanthone family with broad spectrum antimicrobial and anticancer activities have been described, the only member of this family reported to date with comparable antiplasmodial activity is simaomicin α . However, cytotoxicity may be an issue and hence the discovery of the new polycyclic xanthenes with comparably potent antiplasmodial activity such as the compound reported here provides an alternative starting point and may give ideas for structural manipulations that may yield more potent and less cytotoxic antiprotozoan compounds from this class of natural products.

Author Disclosure Block:

F. Vicente: None. **F. Annang:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Detection of VIM and KPC Carbapenemases in Two Isolates of *Enterobacter cloacae* from the Same Patient

Author Block:

G. K. Thomson¹, J. W. Snyder¹, Y. Doi², K. S. Thomson¹; ¹Univ. of Louisville, Louisville, KY, ²Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: Three CRE isolates (2 *E. cloacae* and 1 *K. pneumoniae*) were referred to our laboratory for “KPC confirmation”. Only the *E. cloacae* isolates were carbapenemase-producing organisms (CPOs) and each produced two carbapenemases. The production of dual carbapenemases is a novel therapeutic and infection control threat. Therefore tests were initiated to better understand the diagnostic challenges.

Methods: The isolates, G6809 and G6810, were analyzed by MicroScan Walkaway and Vitek 2 supplemented by disk diffusion and Etest. β -lactamases were investigated by tris/EDTA (TE) disk testing, Verigene GN-BC microarray, and IPM bioassays supplemented with boronic acid and TE. Definitive carbapenemase identification for G6809 and genetic analysis was conducted by PCR and sequencing.

Results: The 2 *E. cloacae* CPOs differed in 5 biochemical tests and colony morphology (gray vs white). Both had similar MDR susceptibility patterns that included high-level resistance to ceftazidime-avibactam (CZA) (MIC > 256/4 μ g/ml) and resistance to all other β -lactams. They differed in disk tests with IPM, ATM and CZA with G6810 more resistant to CZA and G6809 more resistant to IPM and ATM. TE tests indicated that both produced a MBL and a class A or D carbapenemase. These were identified by microarray as KPC and VIM. The G6809 carbapenemases were a chromosomal KPC-18 and a plasmid-mediated VIM-1. Subcultures on drug-free media resulted in reductions in CZA MICs to 32/4 and 64/4 μ g/ml respectively for G6809 and G6810. The bioassays indicated that G6809 produced more VIM-1 but less KPC-18 than G6810.

Conclusions: To our knowledge this is the first report of a patient in the US harboring two isolates producing two carbapenemases. The reduction in CZA MICs on subculture suggested loss of the VIM encoding plasmid. A diagnostic challenge was defining the boundary between what constitutes different or identical isolates of the same organism that share the same unique

and rare type of dual carbapenemase production. Most laboratories should be able to detect this dual carbapenemase production in-house by either TE disk testing or molecular testing with a sufficiently broad target range. Testing with only NDM- and KPC-specific PCR would have failed to detect the VIM carbapenemase which could have resulted in inappropriate therapy with CZA.

Author Disclosure Block:

G.K. Thomson: None. **J.W. Snyder:** None. **Y. Doi:** None. **K.S. Thomson:** C. Consultant; Self; BD Diagnostics.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Colistin-Resistant and Carbapenemase-Producing *Klebsiella pneumoniae* Isolates from Bloodstream Infections in a Turkish Hospital; Multiclonal Outbreaks

Author Block:

A. Jayol¹, P. Nordmann¹, Z. Gulay², M. Bicmen³, S. Alpcavus⁴, L. Poirel¹; ¹Univ. of Fribourg, Fribourg, Switzerland, ²Dokuz Eylul Univ. Sch. of Med., Izmir, Turkey, ³dokuz Eylul Univ. Sch. of Med., izmir, Turkey, ⁴dokuz Eylul Univ. Sch. of Med., Izmir, Turkey

Abstract Body:

Background. The recent and large dissemination of OXA-48 and NDM-producing isolates in Turkey is responsible for an increasing usage of colistin. Our study was designed to evaluate retrospectively the occurrence of colistin resistance over a 7-year period among carbapenemase-producing *K. pneumoniae* isolates responsible for septicemia in a Turkish hospital. **Materials/methods** A total of 317 carbapenemase-producing enterobacterial isolates were recovered from blood cultures or catheter samples between 2008 and 2015 and were included in the study. Colistin susceptibility was evaluated using a reference broth dilution technique and the Rapid Polymyxin NP test. Molecular mechanisms responsible for carbapenem and colistin resistance were searched by PCR and sequencing. Genotyping comparison of the isolates was performed by Multilocus Sequence Typing (MLST). **Results** Out the 317 carbapenemase-producing enterobacterial isolates, 42 colistin-resistant *K. pneumoniae* isolates were found using the Rapid Polymyxin NP test that provided results within 2 h. MIC determinations showed that all isolates exhibited MIC values of colistin ranging from 4 to more than 128 mg/l. Genotyping comparison revealed multiple clonal outbreaks with the presence of eleven distinct clones belonging to ST13, ST14, ST35, ST45, ST101, ST273, ST336 and the novel ST2041. Molecular characterization of antibiotic resistance determinants revealed that 64% of the strains produced the carbapenemase OXA-48, 24% produced NDM and 12 % produced both OXA-48 and NDM. The mechanisms responsible for colistin resistance were mutations in PmrB protein or alterations into the mgrB gene. The prevalence of colistin resistance among the carbapenemase-producing *K. pneumoniae* isolates were 5%, 10%, 31%, and 68% in 2012, 2013, 2014 and 2015, respectively, revealing a dramatic increasing rate of the colistin resistance over time. **Conclusion.** This

work identified the large spread of not only OXA-48 but also NDM producers resistant to colistin in Turkey. It showed that multiple ongoing outbreaks due to unrelated colistin-resistant occurred in a same hospital.

Author Disclosure Block:

A. Jayol: None. **P. Nordmann:** None. **Z. Gulay:** None. **M. Bicismen:** None. **S. Alpcavus:** None. **L. Poirel:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Coexistence of a New Allele, *qnrB62*, with *bla_{VIM-2}* Gene on a Novel Complex Class 1 Integron in *Citrobacter freundii*

Author Block:

S. H. Lee, K. S. Park, J. H. Lee, A. M. Karim, M. Park, J. H. Kim, T. Y. Kim; Myongji Univ., Yongin, Korea, Republic of

Abstract Body:

Background: The *qnrB* gene is prevalent in *Citrobacter freundii* clinical isolates. However, there are no reports regarding the coexistence between the *qnrB* and *bla_{VIM-2}* genes. The aims of this study were to access the coexistence of these two genes and the potential of multidrug resistance transfer, and to characterize the mobile element involved. **Methods:** MICs of the antibiotics and an efflux pump inhibitor were determined in a clinical isolate, its transformant and site-directed mutant, according to guidelines of CLSI, by agar dilution method. Molecular characterizations of *qnrB62* gene and its mobile element were performed by PCR amplification, DNA sequencing, PFGE and/or Southern blot analysis. **Results:** Coexistence of *qnrB62* (encoding QnrB62 with Ser198Asn substitution, compared with QnrB5) with *bla_{VIM-2}* gene was detected in a *Citrobacter* clinical isolate resistant to fluoroquinolones and carbapenems. The reduced fluoroquinolone susceptibility is attributable to the *qnrB62*, a Thr83Ile substitution in GyrA and a Ser80Ile substitution in ParC, and efflux pump(s) other than QepA or OqxAB. The genetic context surrounding chromosomal *qnrB62* was a novel complex class 1 integron (In1148::ISCR1::*qnrB62*) containing a unique gene array (*bla_{VIM-2}-aacA4'-8-gucD*) in a new class 1 integron (In1148). An 18 nucleotide deletion at the 3' end of *pspA(Δ18)* upstream of *qnrB62* and an inverted repeat region (IRR2) were detected in In1148::ISCR1::*qnrB62*, indicating past transposition events. **Conclusions:** The fluoroquinolone and carbapenem resistance associated with the In1148::ISCR1::*qnrB62* integron could be related to a potential multidrug resistance horizontal spread that is worrisome worldwide.

Author Disclosure Block:

S.H. Lee: None. **K.S. Park:** None. **J.H. Lee:** None. **A.M. Karim:** None. **M. Park:** None. **J.H. Kim:** None. **T.Y. Kim:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

ST405 NDM-5 Producing *Escherichia coli* in Northern Italy: The First Two Clinical Cases

Author Block:

A. Piazza, Ms¹, I. Bitar, Mr¹, M. Caltagirone, Ms¹, E. Nucleo¹, M. Spalla¹, F. Novazzi¹, E. Oliva², P. Pedroni², **R. Migliavacca**¹, L. Pagani, Mrs¹; ¹Univ. of Pavia, Pavia, Italy, ²AO Desenzano del Garda, Brescia, Italy

Abstract Body:

Background: NDM positive bacteria are now on the top list of carbapenemase producers in some European countries. Dissemination predominantly involves transfer of the *bla*NDM-1 gene, within bacteria resistant to nearly all antibiotics. NDM-1 and -4 have been rarely reported in Italy. **Methods:** Three MDR *E. coli* were recovered, from rectal swab/wound drainage samples of a 60-years-old man and a blood sample of 72-years-old oncological woman. The patients were admitted on March and December 2015 respectively at the surgical unit of Desenzano Hospital, Italy. Although the first patient reported a history of vacation in Thailand, the second one was previously admitted to an Hospital in Verona, where a rectal colonization by a carbapenemase positive strain was ascertained. Identification and antibiotic-susceptibilities were obtained using Autoscan4 System (Beckman Coulter). Phenotypic tests (RoscoDiagnostics), PCR and sequencing for *bla*NDM/CTX-M/*ArmA*/CMY-type genes were performed. PFGE, MLST and PBRT KIT replicon typing have been assessed. **Results:** The three *E. coli* isolates resulted resistant to β -lactams, aminoglycosides, fluoroquinolones, nitrofurantoin, sulfonamides and colistin, retaining susceptibility to tigecycline and fosfomicin (EUCAST breakpoints). Phenotypic test results were confirmed for all the isolates by PCR and sequencing, the latter identifying *bla*NDM-5/TEM-1/CMY-42 genes. Neither *armA* nor *bla*CTX-M-type genes were found. The strains were identical by PFGE. MLST revealed an ST405 lineage; replicon typing indicated IncI1; IncFII groups. **Conclusions:** This is the first report in Italy of three NDM-5/TEM-1/CMY-42 positive ST405 *E. coli* isolates. NDM-5 *E. coli* have already been found in few countries, but never linked to the ST405. The hypothesis of a foreign origin of the first strain is reinforced by the fact that no other similar MDR *E. coli* strains have been isolated in the same period at Desenzano Hospital,

but the identification of a second strain from a different patient could be related to the presence of an ST405 hot spot area in Italy.

Author Disclosure Block:

A. Piazza: None. **I. Bitar:** None. **M. Caltagirone:** None. **E. Nucleo:** None. **M. Spalla:** None. **F. Novazzi:** None. **E. Oliva:** None. **P. Pedroni:** None. **R. Migliavacca:** None. **L. Pagani:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Comparative Kinetic Analysis Of OXA-247 and OXA-438, Novel Variants Of OXA-48-Type Carbapenem-Hydrolyzing Class D β -Lactamases

Author Block:

D. De Belder¹, B. Ghiglione², G. Gutkind², F. Pasterán¹, M. M. Rodríguez², A. Corso¹, S. Gómez¹, **P. Power**²; ¹INEI, ANLIS-Malbrán, Buenos Aires, Argentina, ²Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract Body:

Background: OXA-163 (O163) is a class D β -lactamase closely related to OXA-48 (O48), having a 4-aminoacid (aa) deletion and substitutions D224E/T225P at the vicinity of conserved KTG, that emerged in 2011 and spread in Argentina. Novel variants of the O48 type enzymes with the ability to hydrolyze oxymino-cephalosporins (OC) like cefotaxime (CTX) and ceftazidime (CAZ), and carbapenems (Cb), are increasingly reported, reducing the number of treatment options. The aim of this study was to evaluate the hydrolytic behavior of the novel variants OXA-247 (O247) and OXA-438 (O438) from Argentina. **Methods:** Plasmid-borne *bla*_{OXA-163}, *bla*_{OXA-247}, *bla*_{OXA-438}, and *bla*_{OXA-48} were cloned from clinical isolates or tranconjugants (TC) in suitable vectors. MICs were evaluated by agar dilution and microdilution (CLSI 2015). OXA enzymes were produced in *E. coli* BL21 and purified to homogeneity. Steady-state kinetic parameters were determined by spectrophotometry, in presence and absence of 0.5 mM sodium bicarbonate (NaHCO₃). Plasmid profile was studied with biparental conjugation, S1 nuclease-PFGE and PCR-based replicon typing. **Results:** *bla*_{OXA-163} and *bla*_{OXA-247} are located in a 70-kb IncQ conjugative plasmid while *bla*_{OXA-438} is located in a 56-kb IncII conjugative plasmid. O438 presented different mutations in the vicinity of conserved KTG (214-216): 2-aa deletion (R220-I221) and D224E shift (as in O163) compared to O48. Clinical isolates Kpn163 (O163), Kpn247 (O247), Eco438 (O438) were resistant to Cb, and TCs were >2 fold dilutions vs. acceptor strain. TC163 and TC48 were resistant to OC, unlike TC247 and TC438. Kinetic parameters revealed that k_{cat}/K_m values for CTX in O163, O247 and O438 were lower compared to O48, accompanied by an improvement in the hydrolysis of Cb (lower K_m). For O163, O247 and O438, addition of NaHCO₃ improved k_{cat} values for OC; Cb k_{cat}/K_m were higher than for OC and CAZ K_m were high

(in the mM range) vs. O48. **Conclusion:** Mutations occurring near conserved KTG in OXA-247 and OXA-438 are probably responsible for improved Cb hydrolysis and decreased inactivation of OC. Finally, additional mechanisms are probably involved in the resistance pattern.

Author Disclosure Block:

D. De Belder: None. **B. Ghiglione:** None. **G. Gutkind:** None. **F. Pasterán:** None. **M.M. Rodríguez:** None. **A. Corso:** None. **S. Gómez:** None. **P. Power:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Characterization of CfiA Metallo- β -Lactamase in *Bacteroides fragilis*, in Argentina

Author Block:

M. Litterio¹, D. Cejas², G. Gutkind², M. A. Radice³; ¹Hosp. Garrahan, Buenos Aires, Argentina, ²Univ. de Buenos Aires, Buenos Aires, Argentina, ³Univ. de Buenos Aires, Buenos Aires, Argentina

Abstract Body:

Background: CfiA metallo-beta-lactamase was reported in 3 carbapenem resistant (RC) *Bacteroides fragilis* (Bf) isolates recovered in a surveillance study performed in Argentina during 2006-09. $bla_{CfiA-15}$ was identified in two of them. Our goal was to investigate the presence of bla_{CfiA} in Bf displaying decreased susceptibility to carbapenems (DSC), and to explore the presence of IS in the upstream region of bla_{CfiA} , that may enhance its expression **Methods:** 73 Bf displaying DSC and 3 RC isolates were recovered in the mentioned surveillance study. Screening of bla_{CfiA} was made by PCR using primers GBI-1 and GBI-2. The entire bla_{CfiA} was amplified using primers GBI-1 and GBI-6. Amplicon sequences were compared with the 16 bla_{CfiA} alleles deposited within Genebank. The upstream regions were evaluated by PCR mapping. The presence of promoters were investigated using the on line tool B-PROM **Results:** Comparing the 16 bla_{CfiA} alleles deposited in the Genebank, it was observed that $bla_{CfiA-4, -5, -7, -11}$ had the same nucleotide sequence. Also $bla_{CfiA-6, -16}$, and $bla_{CfiA-12, -13, -15}$ were identical among them. Consequently, we called $bla_{CfiA-4, -5, -7, -11}$, $bla_{CfiA-6, -16}$ and $bla_{CfiA-12, -13, -15}$ as bla_{CfiA-4} , bla_{CfiA-6} and $bla_{CfiA-13}$, respectively. bla_{CfiA} was detected in 4/73 DSC Bf isolates (3527, 3178, 3010 and 3116). bla_{CfiA} sequences obtained from 3527 and 3178 Bf isolates corresponded to new alleles coding for CfiA-2 and CfiA-4, respectively. bla_{CfiA} sequences obtained from 3010 and 3116 Bf isolates corresponded to 2 new CfiA variants, which are related to $bla_{CfiA-13}$. bla_{CfiA} sequences from 2 RC Bf strains, previously categorized as $bla_{CfiA-15}$, also corresponded to $bla_{CfiA-13}$. In the remain RC Bf a new allele of bla_{CfiA-4} was identified. None IS could be found in bla_{CfiA} positive DSC Bf isolates. Promoters (-35 and -10) were identified in 3527 isolate, the only one intermediate to doripenem by CLSI 2009 **Conclusions:** bla_{CfiA} could be present in non carbapenem Bf resistant isolates. According to previous reports, none IS could be identified in DSC Bf

isolates. Before continue increasing complexity, we suggest to stop using those repeated *bla_{CfiA}* alleles which had a previous preeminence, at least until the 16 *bla_{CfiA}* alleles submitted in Genebank have the curation deserved

Author Disclosure Block:

M. Litterio: None. **D. Cejas:** None. **G. Gutkind:** None. **M.A. Radice:** None.

Session Number:**205****Session Title:****Carbapenemases****Publishing Title:****Role of the Disulfide Bridge in the Catalytic Activity and Stability of the GES-5 β -Lactamase****Author Block:**

S. B. Vakulenko¹, C. Smith², Z. Nossoni¹, M. Toth¹, N. Stewart¹; ¹Univ. Notre-Dame, Notre Dame, IN, ²Stanford Synchrotron Radiation Lightsource, Stanford Univ., Menlo Park, CA

Abstract Body:

All class A carbapenemases possess a unique disulfide bridge connecting the two subdomains of these enzymes, and it has been proposed that this disulfide may be responsible for the carbapenemase activity. We investigated the role of the disulfide bridge in the catalytic activity and stability of the GES-5 carbapenemase. To disrupt the disulfide bridge, Cys69Gly, Cys69Leu, and Cys69Met mutants of the GES-5 β -lactamase were constructed. MICs and steady-state kinetics of the mutant enzyme with penicillins were evaluated. X-ray crystal structures of the apo form of the Cys69Gly mutant of GES-5 and its complex with the carbapenem imipenem were determined. Mutant GES-5 with a Cys69Gly substitution retained the ability to produce resistance to β -lactams, although the MIC values for ampicillin, penicillin G, cephalothin, and imipenem decreased 4-, 4-, 16-, and 4-fold, respectively, when compared to those for the wild-type GES-5. The two other substitutions, Cys69Met and Cys69Leu, resulted in a significant decrease in MIC values, dropping them to the background level. The catalytic efficiency (k_{cat}/K_m) of the Cys69Gly mutant enzyme against ampicillin was very similar to that of the wild-type enzyme, while those for penicillin G, cephalothin, and imipenem were 2.3-, 6.0-, and 5.4-fold lower, respectively. Wild-type GES-5 maintained full stability at temperatures up to 45°C and retained some residual activity at 55°C. The Cys69Gly mutant enzyme is less stable than GES-5 at 37°C and completely inactive at 50°C, which could influence the enzyme activity against some substrates. The high resolution structure of the Cys69Gly-GES-5 mutant shows that the structure is unchanged and that the enzyme is capable of binding imipenem in a manner identical to that of the wild-type β -lactamase. Our data show that the disulfide bridge in GES-5 is essential for the enzyme stability, but not the catalytic activity against β -lactam substrates, including carbapenems.

Author Disclosure Block:

S.B. Vakulenko: None. **C. Smith:** None. **Z. Nossoni:** None. **M. Toth:** None. **N. Stewart:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Distantly Located Active-Site Substitutions N106S and D240G Act Synergistically to Rearrange the Active Site and Expand the Substrate Profile of CTX-M β -Lactamases

Author Block:

M. Patel, V. Stojanoski, C. Adamski, B. Fryszczyn, T. Palzkill; Baylor Coll. of Med., Houston, TX

Abstract Body:

The introduction of new β -lactam antibiotics drives the evolution of β -lactamase enzymes. β -lactamases gain the ability to hydrolyze new antibiotics by acquiring mutations leading to amino acid changes within their active site. The introduction of oxyimino cephalosporin antibiotics led to the emergence of the CTX-M family of β -lactamases. The CTX-M enzymes are frequently identified in antibiotic-resistant clinical samples and are named for their enhanced ability to hydrolyze cefotaxime over ceftazidime. Recently, the N106S mutation has been identified in combination with the D240G mutation in clinical isolates. Previous studies have reported that the N106S mutation is detrimental to cefotaxime and ceftazidime hydrolysis when found alone¹. However, N106S allows the enzyme to hydrolyze both antibiotics better than the WT enzyme when found in combination with D240G. The mechanism behind this increased hydrolysis of cephalosporins in the double mutant enzyme has not been studied. The evolutionary role of the N106S substitution in β -lactam antibiotic resistance was studied using the CTX-M-14 β -lactamase as a model system. MICs and steady-state kinetic analysis of the N106S mutant enzyme confirmed a decreased resistance to cefotaxime due to an increased K_m of the enzyme for hydrolysis. The structure of the N106S mutant enzyme in complex with cefotaxime was solved and the results demonstrate that the side chain of the N104 residue loses hydrogen bond contacts with the cefotaxime molecule due to the mutation. When the N104A mutation was introduced into the WT enzyme, a decreased cefotaxime MIC for *E. coli* containing the mutant was observed and the purified mutant enzyme exhibited an increased K_m for cefotaxime hydrolysis, indicating the importance of the N104 residue for cefotaxime catalysis. Next, the structure of the N106S/D240G mutant enzyme with cefotaxime was determined. It revealed a

rearrangement of the Y105 residue to make hydrophobic interactions with the aminothiazole ring of cefotaxime and thereby replace interactions that are lost when N104 rotates out of the active site in the N106S single mutant. Overall, this is an example of how two residues on opposite sides of the active site can work together over a distance to provide increased hydrolysis for oxy-imino cephalosporins.

Author Disclosure Block:

M. Patel: None. **V. Stojanoski:** None. **C. Adamski:** None. **B. Fryszczyn:** None. **T. Palzkill:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Identification Of A Novel Chemical Hit As NDM-1 And IMP-1 Inhibitors Using Surface Plasmon Resonance And *in Vitro* Potency Characterization

Author Block:

D. Andreotti, A. Felici, M. Negri, C. Napolitano; Aptuit (Verona) Srl, Verona, Italy

Abstract Body:

Background: The long history of efficacy proven by β -lactam antibiotics is rapidly diminishing by the world-wide increasing incidence of bacteria Metallo- β -lactamases (MBLs) producers. Identification of novel chemical hits able to inhibit MBLs is a challenging area of investigation. A novel, rapid and sensitive methodology for the identification of potential new NDM-1 and IMP-1 inhibitors based on surface plasmon resonance (SPR) has been applied. Furthermore, the compounds have been evaluated using a specific set of differently permeable *E. coli* strains and expressing NDM-1 or IMP-1. **Methods:** (His tag)-NDM-1 and IMP-1 MBLs have been cloned, overexpressed and purified to homogeneity. Evaluation of efficiency of immobilization on different Biacore T200[®] chips, pH, buffer strengths and interaction time have been evaluated using different standard compounds in order to identify the most appropriate assay conditions. The K_D values of a set of compounds mainly designed to bind the Zn^{+2} present in the active pocket of the NDM-1 and IMP-1 MBLs have been measured. A $K_D < 30 \mu M$ on at least one between NDM-1 or IMP-1 with a Ligand Efficiency > 0.3 were considered acceptable criteria for testing these compounds in the Nitrocefin assay. *In vitro* activity of selected compounds was measured with *E. coli* BW25213 WT, $\Delta tolC$, and $\Delta bamB$ isogenic strains. **Results:** Under optimized experimental conditions a pool of properly designed diketopiperazine derivatives was tested against both NDM-1 and IMP-1 immobilized enzymes and compared to Captopril (IMP-1 $K_D = 1.5 \mu M$, NDM-1 $K_D = 18.6 \mu M$). Among these compounds a few have met the desired criteria with the best one showing a $K_D = 13.5 \mu M$ and $LE = 0.4$ against IMP-1, activity confirmed in the Nitrocefin IMP-1 assay with $IC_{50} = 12.4 \mu M$. Additionally, MIC values confirmed the effectiveness of the hit. **Conclusions:** A sensitive and reliable assay based on SPR methodology for the identification of novel hits active against IMP-1 and NDM-1 has been developed. The methodology has been proven to be fast and reproducible permitting

to identify a novel chemical template with MBLs inhibitory activity. These results have been confirmed by both Nitrocefin assay and MIC determination. Design and synthesis of analogue compounds are currently ongoing.

Author Disclosure Block:

D. Andreotti: None. **A. Felici:** None. **M. Negri:** None. **C. Napolitano:** None.

Session Number:

205

Session Title:

Carbapenemases

Publishing Title:

Tolerance (TOL) to Ceftazidime/Avibactam (C/A), Plazomicin (PLZ) And Colistin (COL) among *Klebsiella pneumoniae carbapenemase* -Producing *K. pneumoniae* (KPC-KP)

Author Block:

G. Haidar¹, C. J. Clancy¹, L. Chen², B. Kreiswirth², M. H. Nguyen¹; ¹Univ. of Pittsburgh, Pittsburgh, PA, ²The Publ. Hlth.Res. Inst. at the Intl. Ctr. of Publ. Hlth., Newark, NJ

Abstract Body:

Introduction: Antibiotic TOL is distinct from resistance (R) and may contribute to treatment failure. C/A and PLZ have anti-KPC-*Kp* activity, but data on TOL are lacking. **Methods:** TOL rates (TR) for 19 ST258 KPC-*Kp* isolates (KPC-2, 15; KPC-3, 4) were defined as number of cells recovered 24h after exposure (MH medium, 37°C) to 10X MIC of C/A, PLZ and COL, divided by starting inoculum. R was defined by viability of TOL cells after re-plating on MH agar with 10X MIC of drug. Whole genome sequencing (Illumina MiSeq) and genome wide association analysis (Plinkon) was performed. **Results:** Median C/A, PLZ, and COL MICs were 1 µg/mL (range 0.25-8), 1 µg/mL (1-2) and 1 µg/mL (1-16), respectively. All isolates were C/A sensitive (S); 94% were COL-S. 84%, 95%, and 21% of isolates were TOL to C/A, COL and PLZ respectively. Among TOL isolates, median TR was greater for COL (8%, range 0.01% - 2.4x10⁵%) than C/A (1%, 0.02% - 8%) or PLZ (0.02%, 0.02% - 0.026 %) (p=0.006). PLZ TR was significantly lower than C/A (p <0.0001). TOL was not correlated with MICs or KPC type. PLZ- or C/A-R did not emerge; 33% (6/18) of isolates developed COL-R (p=0.001). Strains with IncFIB(pQII) plasmid had higher C/A TR (p =0.01); strains with IncR or IncX3 had higher COL TR (p=0.04 and 0.03, respectively). Non-synonymous single nucleotide polymorphisms (SNPs) in 6 genes, 7 genes and 107 genes were significantly associated with TOL to C/A, PLZ and COL, respectively. For each drug, TOL-associated SNPs were found in ≥2 genes encoding constituents of histidine kinase two component regulatory systems (HK TCS) and other genes encoding transcriptional regulators. For COL TOL, SNPs were identified in 4 HK sensor (including *pmrB*) and 13 transcriptional regulator genes. **Conclusions:** PLZ was associated with very

low TOL and no R among KPC-*Kp*, consistent with rapid bactericidal activity. TOL to C/A was higher, but not associated with R. TOL and R to COL were most prominent. Associations between plasmids and TOL suggest that some relevant genes are plasmid-mediated. SNPs within TCS genes suggest that environmental sensing and stress response regulation contribute to TOL. Further studies are needed to determine the relevance of SNPs, and the mechanisms and clinical significance of TOL.

Author Disclosure Block:

G. Haidar: None. **C.J. Clancy:** None. **L. Chen:** None. **B. Kreiswirth:** None. **M.H. Nguyen:** None.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

The Role of Molecular Types and Antimicrobial Susceptibility Patterns of *Clostridium difficile* Isolates in Different Epidemiological Settings

Author Block:

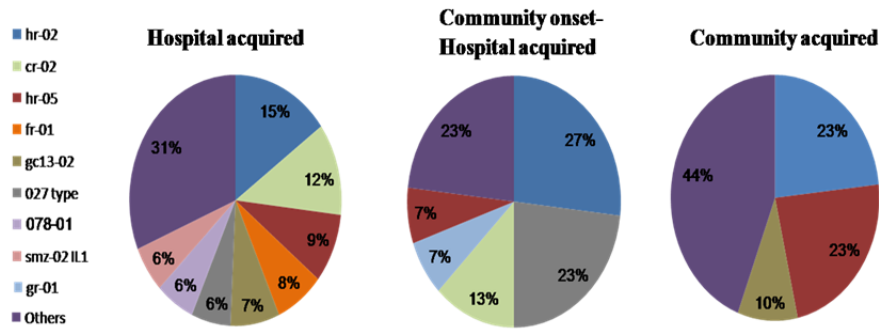
T. Miller-Roll¹, W. Na'amnih¹, D. Cohen², Y. Carmeli¹, **A. Adler, M.D.**¹; ¹Tel-Aviv Sourasky Med. Ctr., Tel-Aviv, Israel, ²Tel Aviv Univ., Tel-Aviv, Israel

Abstract Body:

Background: The aims of this study were to examine the correlation between the molecular type and the antimicrobial susceptibility results of *C. difficile* isolates with a) the source of acquisition and b) the occurrence of the CDI episode (first episode vs. recurrence). **Methods:** This prospective study was conducted at the Tel-Aviv Sourasky Medical Center during 2011-2014. All cases of a) community-acquired (CA) CDI (n=43) and control cases from community-onset, hospital acquired (CO-HA) CDI (n=56), HA-CDI (n=67) and b) 32 cases of recurrent CDI were included. *C. difficile* isolates were analyzed by ribotyping and SlpA typing and tested for susceptibility to vancomycin and metronidazole. **Results:** The most common types were SlpA hr-02 (21%), SlpA hr-05/ribotype-014 (12%), ribotype 027 (10%) and cr-02 (10%). The ribotype 027 was most common in the CO-HA group (23% vs. 4.5%, p<0.001) and the hr-05 type was more common in the CA group (23% vs. 8.8%, p=0.013) (figure). Non-susceptibility (NS) to metronidazole was found only in the ribotype-027 isolates (39%) and NS to vancomycin was found in the ribotype-027 isolates (61%) and the cr-02 type (82%). Most recurrences (n=25, 78%) were identified as relapse and 7 cases (22%) were re-infection. Recurrence cases were mostly hospital-acquired and were caused by the same strains that were common overall. NS to metronidazole and vancomycin were relatively common in re-infection episodes (4/14 in both). **Conclusions:** The composition of *C. difficile* strains was similar in the different settings. Our study raises the question regarding the role of NS to

metronidazole and vancomycin in recurrent infections.

Major (>3) *C. difficile* types by acquisition source



Author Disclosure Block:

T. Miller-Roll: None. **W. Na'ammih:** None. **D. Cohen:** None. **Y. Carmeli:** None. **A. Adler:** None.

Session Number:

206

Session Title:

***Clostridium difficile* Epidemiology and Control**

Publishing Title:

***Clostridium difficile* Infection in Eastern China: Clinical Manifestation in Relation to Risk Factors, Genotypes, and Antimicrobial Susceptibility Profiles**

Author Block:

D. Jin¹, C. Huang¹, Y. Luo¹, J. Cai¹, J. Ye¹, W. Fang², X. Wang³, Y-W. Tang⁴, J. Jiang¹; ¹Zhejiang Provincial Ctr. for Disease Control and Prevention, Hangzhou, China, ²First Affiliated Hosp., Zhejiang Univ., Hangzhou, China, ³Hangzhou First People's Hosp., Hangzhou, China, ⁴Mem. Sloan-Kettering Cancer Ctr., New York, NY

Abstract Body:

Background: *Clostridium difficile* infection (CDI) is a major cause of antibiotic-associated diarrhea worldwide, but few studies on transmission and risk factors have been reported from China. **Methods:** A one-time cross-sectional study was conducted in 2 hospitals in eastern China from 1 June 2012 to 30 September 2015. Stool specimens collected from consecutive hospitalized patients with diarrhea were cultured for *C. difficile* and the isolates analyzed for the presence of toxin genes, *tcdC* gene deletion, genotyping, and antimicrobial susceptibility testing. CDI was defined by IDSA/SHEA guidelines, severities ranged from 1 to 6 were determined based on blinded medical record review. **Results:** A total of 432 *C. difficile* isolates were recovered from 3,953 patients (10.9%); the average CDI severity score of 2.61 ± 1.01 . Of the 411 isolates with further analysis, 282 $tcdA^+B^+$, 94 $tcdA^-B^+$ and 35 $tcdA^-B^-$ were detected. Among genotypes determined by multilocus sequence analysis (ST) and PCR ribotyping, ST37/ribotype 017 (16.2%) was the dominant genotype with CDI severity score of 5 ($P < 0.001$). Three ribotypes (017, 001, and 012) and four STs (ST37, ST3, ST54, and ST2) were predominant, and associated with significantly different antimicrobial resistance profiles between genotypes ($\chi^2 = 32.47-62.15$, $p < 0.001$). Independent factors associated with CDI included patients over 55 years old (OR, 95%CI: 26.80, 18.76-38.29), previous hospitalization (12.42, 8.85-17.43), previous antimicrobial treatment within 8 weeks (150.56, 73.11-310.06), hospitalization stay over three days before sampling (2.34, 1.71-3.22), chemotherapy (3.31, 2.22-4.92), and abdominal surgery (4.82, 3.54-6.55). **Conclusions:** *C. difficile* is circulating in hospitalized patients with diarrhea in eastern China with a CDI prevalence of 10.9%. Advanced age, chemotherapy, abdominal surgery, previous hospitalization, antibiotic administration and extended hospitalization are all risk factors for CDI. CDI severity is generally mild to moderate, with severe cases associated with ST37/ribotype 017.

Author Disclosure Block:

D. Jin: None. **C. Huang:** None. **Y. Luo:** None. **J. Cai:** None. **J. Ye:** None. **W. Fang:** None. **X. Wang:** None. **Y. Tang:** None. **J. Jiang:** None.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

Seasonality of *C. difficile* Infection (Cdi) Case Rates: Myth or Reality?

Author Block:

K. Davies¹, G. Davis¹, F. Barbut², C. Eckert², N. Petrosillo³, R. Pisapia³, E. Reigadas-Ramirez⁴, E. Bouza⁴, F. Berger⁵, M. Herrmann⁵, M. Wilcox¹; ¹Univ. of Leeds, Leeds, United Kingdom, ²Hôpital Saint-Antoine, Paris, France, ³Natl. Inst. for Infectious Diseases, Rome, Italy, ⁴Hosp. Gregorio Marañón, Madrid, Spain, ⁵Univ. of Saarland, Homburg, Germany

Abstract Body:

Background: To compare testing practices (CDI testing intensity and protocol) between countries and healthcare institutions, exploring the relationship with seasonal CDI variation. **Methods:** Data on CDI testing methodology, tests/month, patient bed days (pbds)/month and CDI case demographics were collected from 180 hospitals (Hs) in 5 countries (France 38, Germany 37, Italy 38, Spain 30 and UK 37) from April 2014 to March 2015. CDI testing and incidence were compared between countries and Hs using toxin detecting CDI testing algorithms (TCTA) (i.e. GDH/toxin or NAAT/toxin) vs non-TCTA methods (other algorithms, standalone toxin detection methods, or methods not detecting toxin e.g. NAAT alone). Data were compared between summer (Jun-Aug) and winter (Dec-Feb). **Results:** Case definition and testing varied between Hs and countries. TCTA methods were used in 89% of UK Hs, 33% Spain, 42% Italy, 8% Germany and 26.3% France. Germany Hs had the highest rate of non-toxin detection methods (30/37, 81%). Testing density and case incidence were significantly different between countries: mean tests/10,000pbds per H per month (T/PBDs/H/M), France 34.4, Germany 52.2, Italy 63.9, Spain 83.3, UK 96.0 (ANOVA $p < 0.001$); mean cases/10,000pbds per H per month (C/PBDs/H/M), France 3.2, Germany 6.1, Italy 6.9, Spain 5.2, UK 2.55 (ANOVA $p < 0.001$). Winter testing density and incidence were not significantly higher than summer in any country except Italy (Italy: mean summer 57.2 vs 78.8 winter T/PBDs/H/M, $p = 0.041$; mean summer 6.6 vs 10.1 winter C/PBDs/H/M, $p = 0.017$). There was no seasonal variation in testing density or incidence in Hs that used TCTA methods (mean summer 119.2 vs 102.4 winter T/PBDs/H/M, $p = 0.11$; mean summer 9.6 vs 8.0 winter C/PBDs/H/M, $p = 0.27$). Incidence was significantly higher in winter in Hs using non-

TCTA methods, mean 13.5 vs 10.0 summer C/PBDs/H/M ($p=0.049$), despite no significant increase in testing (mean summer 119.8 vs 115.8 winter T/PBDs/H/M, $p=0.63$). **Conclusions:** Reported CDI rates only increase in winter in countries where testing increases concurrently or in Hs using non-TCTA testing methods for diagnosis of CDI.

Author Disclosure Block:

K. Davies: I. Research Relationship; Self; Sanofi Pasteur. **G. Davis:** I. Research Relationship; Self; Sanofi Pasteur. **F. Barbut:** I. Research Relationship; Self; Sanofi Pasteur. **C. Eckert:** I. Research Relationship; Self; Sanofi Pasteur. **N. Petrosillo:** I. Research Relationship; Self; Sanofi Pasteur. **R. Pisapia:** I. Research Relationship; Self; Sanofi Pasteur. **E. Reigadas-Ramirez:** I. Research Relationship; Self; Sanofi Pasteur. **E. Bouza:** I. Research Relationship; Self; Sanofi Pasteur. **F. Berger:** I. Research Relationship; Self; Sanofi Pasteur. **M. Herrmann:** I. Research Relationship; Self; Sanofi Pasteur. **M. Wilcox:** H. Research Contractor; Self; Sanofi Pasteur.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

A US Based National Sentinel Surveillance Study for the Susceptibility and Epidemiology of *Clostridium difficile* Associated Diarrheal Isolates: 2013-14

Author Block:

D. R. Snyderman¹, L. A. McDermott², N. V. Jacobus², J. Chang², S. Stone², J. Wick², C. M. Thorpe², E. J. C. Goldstein³, R. Patel⁴, S. G. Jenkins⁵, B. A. Forbes⁶, S. Johnson⁷, D. N. Gerding⁷; ¹Tufts Med. Ctr. and Tufts Univ. Sch. of Med., Boston, MA, ²Tufts Med. Ctr., Boston, MA, ³R.M. Alden Res. Lab., Culver City, CA, ⁴Mayo Clinic, Rochester, MN, ⁵Weill Cornell Med. Ctr., New York, NY, ⁶Virginia Commonwealth Univ. Med. Ctr., Richmond, VA, ⁷VA Chicago Hlth.Care System, Chicago, IL

Abstract Body:

Background: We have previously reported on a US based surveillance study from 6 geographically dispersed medical centers for the susceptibility and epidemiology of *C. difficile* isolates collected in the US with specific attention to susceptibility to fidaxomicin. This analysis encompasses data from 2013-14. **Methods:** *C. difficile* isolates or stools from patient with *C. difficile* toxin positive antibiotic associated diarrhea were referred to a central laboratory. Susceptibilities were determined by agar dilution method (M11- A8) against the 10 agents listed below. All isolates had toxin gene profiling, a random sample of approximately 30% of isolates, stratified by center, had REA typing performed. **Results:** To date, toxin gene profiling was performed on 94.3% (895/949) of isolates. Toxin A and toxin B encoding genes *tcdA/tcdB* were detected in 97% (868/871) of isolates, 3% were non-toxigenic. Binary toxin genes were detected in 25.5% (221/868) of isolates where toxin was detectable. Among the sample of 352 isolates REA typed, 17% (60/352) were BI, 16% (56/352) Y, and 13% (46/352) DH. In comparison to 2011-2012, BI incidence has declined, whereas DH incidence has increased. **Conclusions:** Fidaxomicin showed excellent *in vitro* activity against *C. difficile* isolates, independent of toxin gene profiles or REA type, and fidaxomicin activity has not changed over the 4 years of the survey.

Results: A Summary of the Susceptibilities of 949 Isolates Against 10 Antimicrobial Agents

Agent	Range	MIC ₅₀	MIC ₉₀	% Resistance (CLSI)
Fidaxomicin	≤0.004-1	0.25	0.5	NA
Rifaximin	≤0.004->4	0.015	0.12	NA
Rifampin	≤0.004->4	≤0.004	0.15	NA
Tigecycline	0.06-1	0.12	0.25	NA
Vancomycin	0.25-8	1	4	NA
Imipenem	≤0.12-16	4	8	4.4
Moxifloxacin	≤0.5->16	2	16	25.6
Metronidazole	≤0.06-4	0.5	2	0
Clindamycin	0.5->16	4	>16	28.0
Chloramphenicol	≤0.5-≥16	4	8	0

Author Disclosure Block:

D.R. Snyderman: C. Consultant; Self; Merck, Cubist, Summit PLC, BioK+, MedImmune, Chimerix. **E. Grant:** Investigator; Self; Merck, Cubist, Actelion, Summit PLC, Tetrphase. **L. Speaker's Bureau:** Self; Cubist. **L.A. McDermott:** None. **N.V. Jacobus:** None. **J. Chang:** None. **S. Stone:** None. **J. Wick:** None. **C.M. Thorpe:** None. **E.J.C. Goldstein:** E. Grant Investigator; Self; Merck, Cubist, Actelion, Summit PLC. **J. Scientific Advisor (Review Panel or Advisory Committee):** Self; Merck, Cubist, Summit PLC, BioK+. **L. Speaker's Bureau:** Self; Merck, Cubist, Forest, BioK+. **R. Patel:** C. Consultant; Self; Curetis. **E. Grant:** Investigator; Self; Pfizer, Pocared, nanoMR, BioFire, Curetis, Check-Points, 3M, Cubist/Merck, Hutchinson Biofilm Medical Solutions, Accelerate Diagnostics, bioMerieux, Bruker, Abbott, Nanosphere, Siemens, BD. **S.G. Jenkins:** None. **B.A. Forbes:** None. **S. Johnson:** C. Consultant; Self; BioK+. **D.N. Gerding:** C. Consultant; Self; Merck, Shire, Cubist, Rebiotix, Sanofi Pasteur, Summit, DaVoltera, Actelion. **E. Grant:** Investigator; Self; CDC, US Dept of Veterans Affairs Research Service.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

Epidemiology of a National Cohort of Veterans with *Clostridium difficile* Infection (Cdi): Initial vs. Recurrent Disease

Author Block:

H. Morrill, T. Timbrook, Y. Wang, K. LaPlante, A. Caffrey; Providence VAMC, Providence, RI

Abstract Body:

Background: CDI is a major public health burden. Differences in clinical characteristics and outcomes between initial and recurrent CDI are not well described. **Methods:** We conducted a retrospective cohort study among adult Veterans a first CDI episode between 2010-2014, defined as a positive stool sample for *C. diff* toxin(s) or an ICD-9 code for CDI (008.45), and receipt of ≥ 2 days of CDI treatment (PO or IV metronidazole [MTZ], PO or PR vancomycin [VAN], or fidaxomicin [FID]) and no CDI episodes in prior year. 1st and 2nd recurrence were defined as a subsequent CDI episode within 30 days of the end of treatment for each occurrence. Differences between initial CDI episode vs. 1st recurrence and 1st vs. 2nd recurrence were assessed using Fisher's exact or χ^2 tests and Wilcoxon Rank Sum test. **Results:** Initial CDI was identified in 54,144 pts (83.2%), 1st recurrence in 8,646 pts (13.3%), and 2nd recurrence in 2,284 pts (3.5%). Median age increased with each occurrence, respectively (66 IQR [59-77] vs. 68 [62-79] $p < 0.001$; vs. 69 [63-81] $p < 0.001$). Similarly, many comorbidities had significant increases in prevalence with each occurrence. MTZ monotherapy was used in 81.8% of initial episodes. In 1st recurrence, MTZ use decreased to 53.3%, while VAN (8.1% to 23.5%) and VAN+MTZ combination therapy (10.7% to 22.7%) use increased. These trends continued in 2nd recurrence (MTZ 36.4%, VAN 38.5%, VAN+MTZ 24.1%). FID was used in $< 1\%$ of all cases. Median length of stay was greater for initial CDI vs. 1st recurrence (9 IQR [5-21] vs. 6 [3-15] days; $p < 0.001$). 30-day all-cause mortality rates were similar for initial CDI vs. 1st recurrence (10.1% vs. 10.2%; $p = 0.72$) and 1st vs. 2nd recurrence (9.3%; $p = 0.19$). The 30-day recurrence rate was lower in initial CDI (16.0%) vs. 1st recurrence (26.4%; $p < 0.001$) and lower for 1st vs. 2nd recurrence (30.9%; $p < 0.001$). **Conclusions:** CDI continues to be a major public health concern among Veterans and there are important differences between initial and recurrent disease. In our

cohort of Veterans, shorter length of stay, similar mortality rates and higher recurrence rates were observed with recurrent CDI compared to initial disease.

Author Disclosure Block:

H. Morrill: N. Other; Self; Supported in part by a VA VISN-1 CDA and has received research funding from Merck. **T. Timbrook:** None. **Y. Wang:** N. Other; Self; Supported in part by Merck. **K. LaPlante:** N. Other; Self; Research funding or acted as an advisor, or consultant for Cubist, Davol, Forest, and Pfizer Inc. **A. Caffrey:** N. Other; Self; Received research funding from Merck and Pfizer Inc.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

New Variants of Hypervirulent NAP1 Strain of *Clostridium difficile* Emerging and Spreading in the Province of Ontario, Canada

Author Block:

G. Broukhanski, A. Wang; Publ. Hlth.Ontario Lab., Toronto, ON, Canada

Abstract Body:

Background: There are various methods of typing *Clostridium difficile* (CD), identified as an “Urgent Threat” by the CDC, including recently internationally-standardized method of capillary ribotyping (CR, Fawley et al, 2015). To be widely accepted this method requires development of criteria for interpretation of the results. We ribotyped a collection of NAP1 CD strains to see if any variants of ribotype 027, associated with this strain, can be detected and if there are any changes over time in distribution of these variants across Ontario. **Methods:** 774 CD strains, isolated in the province over the last 5 years and identified as NAP1 by PFGE, were ribotyped using capillary electrophoresis on 3130xl Genetic Analyzer. Fragments were visualized with PeakScanner software, ribotyping profiles classified with BioNumerics and distribution of variants across time and location examined using Google Maps. **Results:** There were 16 variants of a “classic” ribotype 027, the one prevalent in 2010, when only 1 of 76 isolates (1.3%) had slightly different ribotype. Proportion of 027 variants increased over years to 17/40 isolates (42.5%) in 2014. One of the variants, which is associate with a major CD outbreak in Ontario in 2011, currently represents majority of variant strains and keeps spreading across the province. **Discussion & Conclusions:** NAP1 strain is comprised of a number of related CD strains which can be distinguished by the CR. The emergence of variants may be caused by the pressures of better infection control measures. Most of the variants are unique, but one of them is replacing the “classic” 027 variant. Due to its increasing occurrence, this novel variant should be further studied and monitored, for it may be better adapted to survive improved methods of disinfection or has features associated with higher transmissibility.

Author Disclosure Block:

G. Broukhanski: None. **A. Wang:** None.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

A Computer Based Automatic Isolation Intervention to Reduce In-Hospital Transmission of *Clostridium difficile* Infection

Author Block:

N. Nachimuthu¹, A. Ballard², F. Reid², H. Nguyen², A. Sirajuddin², J. Butler², J. Katz², L. Ostrosky¹; ¹Univ. of Texas Hlth.Sci. Ctr., Houston, TX, ²Mem. Hermann Hlth. care system, Houston, TX

Abstract Body:

Background: Delay in isolation while waiting for results in patients in whom CDI is suspected may account for small clusters of in-hospital transmission. The goal of our study was to compare the rate of clusters/in-hospital transmission of CDI after introduction of an automatic computer- based isolation intervention. **Methods:** We conducted a pilot study on patients in whom *CDI* was suspected between 1/2015 and 6/2015. The intervention consisted of substituting our standard order for stool *C. difficile* PCR with an order set that had contact precautions pre-checked when ordering the test. NHSN-defined CDI rates, proportion of clustered cases, and time to isolation were calculated and compared to the year immediately preceding the intervention. **Results:** The table below shows the major outcomes of our intervention. While the overall CDI rate was not impacted, we found a statistically significant ($p<0.05$) 10-fold decrease in the proportion of clustered cases. There were 15 small clusters of CDI involving 38 patients in 2014 compared to 1 cluster involving 2 patients during the intervention. Time from *C. difficile* PCR order to isolation was around 1 hour in the intervention group compared to 4.3 days in the pre- intervention cohort. Regarding unnecessary isolation, we found that only 70/755 (9.2%) of patients tested during the intervention phase had confirmed CDI. Patients with a negative PCR had isolation discontinued within 5 days 44% of the time with an average of 20 isolation days, although other causes for isolation may have been present. **Conclusion:** We describe a simple computer-based intervention that reduces in-hospital transmission/clusters of CDI and time from order to isolation. The cost-benefit of the intervention needs to be further explored as well as ways of targeting high risk patients to avoid unnecessary isolation days. **Table 1. Data and Results**

	Control	Intervention
CDI rate per 1000 days	0.29	0.39
Number and proportion of clustered cases of CDI	38/177 (21.4%)	2/70 (2.8%)
Time from <i>C. difficile</i> PCR to isolation (days)	4.3	0.05

Author Disclosure Block:

N. Nachimuthu: None. **A. Ballard:** None. **F. Reid:** None. **H. Nguyen:** None. **A. Sirajuddin:** None. **J. Butler:** None. **J. Katz:** None. **L. Ostrosky:** C. Consultant; Self; Astellas, Merck, Scynxis, Cidara. **E. Grant:** Investigator; Self; Merck, Astellas, Pfizer, Scynexis, T2 Biosystems, Meiji, Immunetics. **L. Speaker's Bureau:** Self; Merck, Pfizer.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

Soap Typing: Universal Cloud-Based Bacterial Strain Typing for Real-Time Infection Control

Author Block:

A. E. Budding¹, M. van der Bijl¹, C. M. J. E. Vandenbroucke-Grauls¹, P. H. M. Savelkoul²; ¹VU Med. Ctr., Amsterdam, Netherlands, ²Maastricht Univ. Med. Ctr., Maastricht, Netherlands

Abstract Body:

Background: Antibiotic resistant pathogenic bacteria from a diverse range of species commonly cause hospital outbreaks. Current typing methods do not provide the speed and universal applicability that is needed for real-time monitoring of these outbreaks. Moreover, lack of standardization presents a major pitfall in national and international comparison of epidemiological data. Here we demonstrate a universal cloud-based bacterial strain typing approach that can be applied in any laboratory within a day. **Methods:** For this, we chose a modernized amplified fragment length polymorphism (AFLP) approach. We developed a 10 minute DNA isolation approach and standardized the restriction/ligation and PCR with bulk-produced mastermixes. Fragment analysis was performed by capillary gel electrophoresis, resulting in digital profiles. Profiles were uploaded to the cloud-based analysis algorithm that regularized and stored resulting data. We coined the procedure Standardized One-day Automated Polymorphism (SOAP) typing and tested it for its universal application on 50 different bacterial species. We compared SOAP typing to NGS based typing in 14 *Clostridium difficile* strains and performed the full procedure independently on a panel of strains from three different bacterial species in two different laboratories. **Results:** SOAP typing could be performed within a single working day and resulted in high-quality profiles in 50 different bacterial species. *C. difficile* strains that were identical with SOAP typing showed at most a single SNP variation in the NGS based analysis. Reproducibility of SOAP typing was very good between laboratories for all strains from the three species that were tested. Detected variation was almost entirely attributable to low-level background noise that could easily be adjusted for by the software. **Conclusions:** We conclude that 1. SOAP typing can be performed in a single-day for all bacterial species. 2. SOAP typing shows the same

resolution as NGS-based typing. 3. SOAP typing is highly reproducible between laboratories.

Author Disclosure Block:

A.E. Budding: A. Board Member; Self; IS-Diagnostics. K. Shareholder (excluding diversified mutual funds); Self; IS-Diagnostics. **M. van der Bijl:** None. **C.M.J.E. Vandebroucke-Grauls:** None. **P.H.M. Savelkoul:** A. Board Member; Self; IS-Diagnostics. K. Shareholder (excluding diversified mutual funds); Self; IS-Diagnostics.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

Impact of *Clostridium difficile* Colonization on the Subsequent Development of *C. difficile* Colitis in a Hematology Oncology Inpatient Population -A Prospective Cohort-

Author Block:

J. Haque, N. A. Ledebor, L. Michaelis, M. Graham, P. Hari, T-L. Mackey, K. Acker, R. Long, B. Shaw, J. N. Wainaina, S. Sara Revolinski, M. Mary Horowitz, S. Munoz-Price; Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: We aimed to determine the prevalence of asymptomatic *C. difficile* carriers in our inpatient hematology oncology (heme-onc) units and its subsequent impact on the clinical diagnosis of *C. difficile* colitis. **Methods:** This quality improvement project was performed as an Infection Control intervention in 3 inpatient hematology oncology units at Froedtert Hospital, a 700-bed teaching facility in Milwaukee, WI. From March 1, 2015 to December 28, 2015, all consecutive patients admitted to those 3 units underwent surveillance cultures upon admission and weekly thereafter. Stool samples were placed in selective agar plates (ChromID *C. difficile*, bioMerieux, Marcy-l'Etoile, FR) and incubated overnight. *C. difficile* colitis was diagnosed clinically by managing teams based on symptoms (i.e. diarrhea) and positive NAAT. Clinical teams were blinded to the surveillance status of patients. Patients with diagnosis of *C. difficile* colitis prior to at least 1 surveillance culture were excluded. Relative risks (RR) and confidence intervals were calculated. Stratified analyses were also done by bone marrow transplant (BMT) status. **Results:** A total of 511 unique patients underwent at least one surveillance culture and 81 (15.8%) were positive at least once. Forty nine patients (9.6%) developed *C. difficile* colitis. The relative risk of developing *C. difficile* colitis among surveillance positive patients was 4.32 compared with surveillance negative patients (RR: 4.32; 95% CI: 2.596-7.2; p<0.0001). Stratification based on BMT status showed a relative risk of developing *C. difficile* colitis based on surveillance status of 4.8 (95% CI: 2.13 - 10.8; p=0.0002) for BMT patients and a relative risk of 4.04 (95% CI: 2.09-7.81; p<0.0001) for non-BMT patients. **Conclusions:** Being an asymptomatic *C. difficile* carrier appears to be

strongly associated with the subsequent clinical diagnosis of *C. difficile* colitis in the heme-onc population, regardless of their BMT status.

Author Disclosure Block:

J. Haque: None. **N.A. Ledeboer:** None. **L. Michaelis:** None. **M. Graham:** None. **P. Hari:** None. **T. Mackey:** None. **K. Acker:** None. **R. Long:** None. **B. Shaw:** None. **J.N. Wainaina:** None. **S. Sara Revolinski:** None. **M. Mary Horowitz:** None. **S. Munoz-Price:** C. Consultant; Self; Xenex and Clorox. L. Speaker's Bureau; Self; Ecolab.

Session Number:

206

Session Title:

Clostridium difficile Epidemiology and Control

Publishing Title:

Clostridium difficile Colitis: Does It Matter When Colonization Occurs?

Author Block:

J. Haque, N. Ledebor, L. Michaelis, M. Graham, P. Hari, T-L. Mackey, K. Acker, R. Long, J. N. Wainaina, S. Revolinski, B. Shaw, M. Horowitz, S. Munoz-Price; Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: We aimed to determine the differential impact on *C. difficile* colitis of the timing of *C. difficile* colonization: present at the time of admission versus acquisition during hospitalization. **Methods:** This project was performed as part of an Infection Control intervention in 3 inpatient hematology oncology units at Froedtert Hospital, a 700-bed teaching facility in Milwaukee, WI. From March 1, 2015 to December 28, 2015, all consecutive patients admitted to those 3 units underwent surveillance cultures upon admission and weekly thereafter. Stool samples were placed in selective agar plates (ChromID *C. difficile*, bioMerieux, Marcy-l'Etoile, FR) and incubated overnight. *C. difficile* colitis was diagnosed clinically by managing teams based on symptoms (i.e. diarrhea) and positive NAAT. Teams were blinded to surveillance culture results. Patients with diagnosis of *C. difficile* colitis prior to at least 1 surveillance culture were excluded. Present at the time of unit admission meant that the stool culture within 48 hours of admission was positive. Acquisition during hospitalization meant that the initial culture(s) was negative; however, at least one subsequent culture was positive. Relative risks (RR) and confidence intervals were calculated based on timing of acquisition. **Results:** A total of 511 unique patients underwent at least one surveillance culture and 81 (15.8%) grew *C. difficile*. Out of the 81 surveillance positive patients, 50 (61.7%) were found to be positive upon admission to the unit and 31 (38.2%) acquired *C. difficile* during unit admission. The relative risk of developing *C. difficile* colitis among patients colonized at the time of admission was 3.82 compared with those who remained surveillance negative (RR: 3.82; 95% CI: 2.06-7.1 ; p<0.0001). The risk of *C. difficile* colitis among patients that acquired *C. difficile* during hospitalization was 5.13 times the risk observed in patients who remained surveillance negative (RR: 5.13; 95% CI: 2.74-9.62; p<0.0001). **Conclusions:** Almost two thirds of our patients were positive upon

admission to the unit; however, the risk of *C. difficile* colitis was higher if acquisition of *C. difficile* occurs during unit admission.

Author Disclosure Block:

J. Haque: None. **N. Ledeboer:** None. **L. Michaelis:** None. **M. Graham:** None. **P. Hari:** None. **T. Mackey:** None. **K. Acker:** None. **R. Long:** None. **J.N. Wainaina:** None. **S. Revolinski:** None. **B. Shaw:** None. **M. Horowitz:** None. **S. Munoz-Price:** C. Consultant; Self; Xenex and Clorox. **L. Speaker's Bureau:** Self; Ecolab.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Transcriptional Regulator MepR Is Involved in the Mechanism of Resistance to Tigecycline in *Staphylococcus aureus*

Author Block:

A. N. G. Dabul¹, J. S. Avaca-Crusca¹, D. V. Tyne², M. S. Gilmore², I. L. B. C. Camargo¹; ¹Physics Inst. of São Carlos, Univ. of São Paulo, São Carlos, Brazil, ²Harvard Med. Sch., Boston, MA

Abstract Body:

Tigecycline is an antimicrobial agent derived from the tetracyclines which possesses high affinity to the bacterial ribosome. During surveillance in a Brazilian hospital in 2009, it was observed that 13 out of 36 isolates from patients infected by methicillin-resistant *Staphylococcus aureus* (MRSA) were tigecycline-resistant (TIG^R) showing Minimal Inhibitory Concentration (MIC) > 0.5 mg/L, even though the use of this drug by the medical staff only started in 2011. All 13 isolates were ST105.A susceptible strain, SA43 (MIC = 0.25 mg/L) was submitted to *in vitro* selection of resistant mutants with increasing concentrations of tigecycline during 15 days. The following samples had their genomes sequenced by Illumina MiSeq platform and their variants were detected using CLC Genomics Workbench v.8.5: Experiment A days 2 (MIC = 0.25 mg/L), 5 (MIC = 1 mg/L), 7 (MIC = 2 mg/L) and 10 (MIC = 4 mg/L), Experiment B days 2 (MIC = 0.25 mg/L), 6 (MIC = 1 mg/L), 7 (MIC = 2 mg/L) and 10 (MIC = 4 mg/L), and Experiment C days 2 (MIC = 0.25 mg/L), 4 (MIC = 1 mg/L), 6 (MIC = 2 mg/L) and 7 (MIC = 2 mg/L). The variants were detected comparing A5, A7 and A10 to A2; B6, B7 and B10 to B2; and C4, C6 and C7 to C2. Some mutations were observed in the *mepR* gene (transcriptional regulator) in all TIG^R strains sequenced, either leading to a premature stop codon or to an amino acid substitution. The latest isolated strains (A7, A10, B10, C6 and C7) presented amino acid substitutions also in the gene coding for an efflux pump *mepA*. Both genes are part of the operon *mepRAB*, previously reported as possibly involved in the mechanism of resistance to tigecycline. To verify if these mutations led to efflux of the tigecycline molecule, the same strains had their MIC to tigecycline determined in the presence and in the absence of three efflux pumps inhibitors:

Verapamil, Reserpine and Carbonyl cyanide-m-chlorophenylhydrazone. All TIG^R strains had at least a 4-fold decrease in their tigecycline MIC in the presence of Verapamil, confirming the efflux. Moreover, relative gene expression of *mepA* was accessed by qPCR, allowing us to demonstrate that the mutations in *mepR* lead to over-expression of *mepA* what is known to result in tigecycline resistance.

Author Disclosure Block:

A.N.G. Dabul: None. **J.S. Avaca-Crusca:** None. **D.V. Tyne:** None. **M.S. Gilmore:** None. **I.L.B.C. Camargo:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Genetic Determinants and Role of Carbapenems in Acquisition of Ceftaroline Resistance in Mrsa

Author Block:

M. Fatouraei¹, M. Martinez Moral¹, M. Roch¹, I. Yun², W. Rose³, **A. E. Rosato**¹;
¹Houston Methodist Res. Inst., Houston, TX, ²Univeristy of wisconsin, Madison, WI,
³Univ. of Wisconsin, madison, WI

Abstract Body:

Background: MRSA represents a worldwide public health challenge, with significant clinical impact on individuals with chronic diseases such as cystic fibrosis (CF). Chronic pulmonary infection with persistent MRSA is thought to confer CF patients a worse overall clinical outcome resulting in increased rate of declined lung function. Ceftaroline (CPT) targets PBP2a (mecA) and is currently indicated for the treatment of community acquired pneumonia. Although the incidence of CPT resistance in clinical isolates is still relatively low, we have described CPT intermediate- and highly-resistant strains. We demonstrated both in vivo and in vitro the striking observation that pre-exposure to carbapenems in the context of patients with demonstrated chronic diseases may predispose to CPT resistance, determining serious clinical implications. Methods: A collection of 250 MRSA strains (either normal or SCV phenotypes) adults and children CF patients were obtained from Seattle Children's Research Institute and University of Wisconsin. Whole genome sequencing (WGS) and RNA-Seq were used to genotyping and differential gene expression analyses. Metabolic adaptations were evaluated by metabolic analysis. Cell wall associated factors (e.g. PBPs) were evaluated by HPLC and localization studies. Results: WGS revealed that acquisition of CPT resistance followed a sequential process of mutation selection: MRSA strains carrying (F254Y)- thyA polymorphism and exposed to imipenem (IPM) introduced mutations in PBP2 (S707L) while further exposure to CPT resulted in mutations in the mecA gene (Y446N). These mutations were linked to changes in the HPLC profile of muropeptides with marked differences in both cell wall (CW) and cross-linking (increase of a monomeric peptidoglycan (Gly)₄), and genes associated to CW synthesis, cell division and

metabolism. Conclusions: Our observation suggest that thyA polymorphisms are a precursor event predisposing to development of IPM resistance, and followed of CPT resistance even in the absence of CPT therapy. This highlights the discovery of additional mutations in other-than-those in PBP2a signaling pathways, explaining the worldwide existence of CPT resistant strains in patients not previously exposed to this antibiotic.

Author Disclosure Block:

M. Fatouraei: None. **M. Martinez Moral:** None. **M. Roch:** None. **I. Yun:** None. **W. Rose:** None. **A.E. Rosato:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Small-Rna and Mrna Comparative Transcriptome of Dap-R-Hvisa *Staphylococcus aureus* by Rna-Seq

Author Block:

V. Cafiso, S. Stracquadanio, G. Pigola, A. Ferro, S. Stefani; Univ. of Catania, Catania, Italy

Abstract Body:

Daptomycin (DAP) is the last-resort treatment for heterogeneous Vancomycin-Intermediate-*Staphylococcus aureus* (hVISA) and Vancomycin-Intermediate-*S.aureus* (VISA). DAP-resistance onset, also linked to reduced vancomycin susceptibility, is a public health issue. We analyzed the small-RNA and mRNA comparative transcriptome of DAPR-hGISA (1C) and DAPR-qVISA (3B) clinical isogenic isolates vs their DAPS-VSSA (1A) or hDAP-hGISA (3A) counterparts by Illumina Rna-seq, bioinformatics (Rockhopper) and filtering analysis. Single DAP-R vs DAP-S pairs **mRNA transcriptome**, evidenced that: i) the 1C strain had **13** 5'-UTRs and **3** 3'-UTRs, **8** sense and **19** antisense mRNAs, **341** differentially expressed protein coding genes, **1057** likely operons and **538** multigene operons vs 1A; ii) the 3B strain had **99** 5'-UTRs and **70** 3'-UTRs, **11** sense and **22** antisense mRNAs, **210** differentially expressed protein coding genes, **1122** likely operons and **547** multigene operons vs 3A. **Small-RNA transcriptome** of the same pairs, evidenced that: i) 1C had **10** 5'-UTRs and **5** 3'-UTRs, **447** sense and **852** antisense small-RNAs, **220** differentially expressed protein coding genes, **1039** likely operons and **533** multigene operons vs 1A; ii) 3B had **38** 5'-UTRs and **11** 3'-UTRs, **814** sense and **1655** antisense small-RNAs, **416** differentially expressed protein coding genes, **1069** likely operons and **544** multigene operons vs 3A. Statistically significant filtering analysis of the data for sorting small-RNAs, mRNAs or their encoded proteins up or down-regulated in both DAP-R vs DAP-S strains highlighted: i) **31** antisense small-RNAs for targets including 5, 16 and 23S ribosomal RNAs, tRNA, metabolic genes, transporters, oxidative stress, cell-wall biosynthesis; ii) **38** small-RNA encoded proteins including TCSTS YycFG, transporters, metabolic enzymes, a virulence factor, metabolic and transcriptional regulators, dynamin-like proteins, Hsp40 chaperone,

DNA replication proteins, cell envelope biogenesis; iii) no mRNA regulators; iv) **15** mRNA encoded proteins for a TCRS, metabolism, transport, cell-wall and rRNA biosynthesis. This is the first study to take a full snapshot of the comparative transcriptome of DAPR-hGISA MRSA highlighting their RNAome signatures.

Author Disclosure Block:

V. Cafiso: None. **S. Stracquadanio:** None. **G. Pigola:** None. **A. Ferro:** None. **S. Stefani:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Comparative Genome Analysis of the Daptomycin Resistant *Streptococcus anginosus* Strain J4206 Associated with Breakthrough Bacteremia

Author Block:

M. Rahman¹, S. V. Nguyen¹, K. A. McCullor¹, C. J. King¹, J. H. Jorgensen², W. M. Mcshan¹; ¹Univ. of Oklahoma Hlth.Sci. Ctr., Oklahoma City, OK, ²Univ. of Texas Hlth.Sci. Ctr. at San Antonio, San Antonio, TX

Abstract Body:

Streptococcus anginosus is an opportunistic human pathogen associated with brain and liver abscesses. The daptomycin resistant strain J4206 was isolated from a patient suffering from breakthrough bacteremia and septic shock at UTHSC at San Antonio. Daptomycin is a lipopeptide that targets the bacterial cell membrane in the presence of calcium, and this resistance is a complex phenomenon involving multiple alterations in the cell membrane and cell wall. We compared the J4206 genome with those of two previously reported daptomycin-susceptible *anginosus* strains, SA and J4211, to identify unique features that might be associated with daptomycin resistance. Strain J4211 was isolated from the same facility as J4206 while strain SA was selected following BLAST search results that showed strong sequence similarity to J4206. The J4206 genome was sequenced using the Illumina MiSeq platform and annotated by the RAST pipeline. Genomic comparisons were done using the CGView server and the software package Geneious. The J4206 genome is 2,001,352 bp long with a GC content of 38.62%. The genome contains multiple mobile genetic elements such as SanCI (*Streptococcus anginosus* Chromosomal Island), transposons, and integrative conjugative elements; however, the vancomycin resistance element *vanG* found in strain SA is missing. The J4206 genome encodes a cluster of capsular polysaccharide genes for choline metabolism and transport that are absent in the J4211 genome. Choline is a positively charged molecule that might be involved in neutralizing cell surface charges that are crucial for daptomycin resistance. Further, the J4206 genome contains two additional genes encoding a unique sortase enzyme and LPXTG-target protein that are involved in cell wall modification. This comparative genomic study indicates presence of unique genes in

the J4206 genome that are involved in cell surface modification and thus might contribute to the acquisition of daptomycin resistance.

Author Disclosure Block:

M. Rahman: None. **S.V. Nguyen:** None. **K.A. McCullor:** None. **C.J. King:** None. **J.H. Jorgensen:** None. **W.M. Mcshan:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

CDP-Diglyceride Synthase a Mediates High-Level Daptomycin Resistance in *Streptococcus Mitis*

Author Block:

N. N. Mishra¹, R. Seepersaud², D. N. Alvarez¹, T. T. Tran³, B. A. Bensing², C. Garcia-de-la-Maria⁴, J. M. Miro⁴, M. J. Rybak⁵, C. A. Arias³, A. S. Bayer¹, P. M. Sullam²; ¹LA Biomed at Harbor UCLA Med. Ctr, Torrance, CA, ²UCSF and VA Med. Ctr., San Francisco, CA, ³Univ of Texas Med. Sch., Houston, TX, ⁴Hosp. Clinic-IDIBAPS, Barcelona, Spain, ⁵Wayne St. Uni., Detroit, MI

Abstract Body:

Background: *S. mitis* is an important cause of endocarditis and sepsis syndromes in neutropenic cancer patients. This organism can rapidly develop high-level and durable daptomycin-resistance (DAP-R) *in vitro* and *in vivo* after DAP exposures. Using comparative whole genome sequencing between a DAP-susceptible *S. mitis* strain and its DAP-R derivative, we identified mutations in two genes involved in the synthetic pathway of two important cell membrane (CM) phospholipids (PLs): phosphatidyl glycerol [PG] and cardiolipin [CL]. The genes encode CDP-diglyceride synthase A (CdsA) and phosphatidylglycerol synthase (PgsA). Of note, CdsA is upstream of PgsA in the PL synthetic pathway. Here we describe the impact of deleting *cdsA* on key CM properties. **Methods:** Mutant generation: allelic replacement of *cdsA* with *cat*. MICs - E-Test. CM PLs: PG; CL; and phosphatidic acid (PA) by 2D-TLC and chemical quantification. CM fluidity - Polarization spectrofluorometry using 1,6-diphenyl-1,3,5-hexatriene (DPH) CM orientation. Relative surface charge - Cytochrome C binding. **Results:** Deletion of *cdsA* caused a marked defect in growth. Despite this altered fitness, the mutant was highly DAP-R (MIC >256 ug/ml) (parental *S. mitis* 351 DAP MIC = 1.5 ug/ml). Of note, the *cdsA* mutant exhibited a virtual disappearance of both PG and CL, with maintenance of phosphatidic acid (PA). Additionally, the *cdsA* mutant demonstrated a significantly greater positive surface charge and increased CM fluidity vs. the 351 parental strain. **Conclusions:** Deletion of *cdsA* encoding a critical enzyme in the synthetic pathway of PG and CL resulted in DAP-R, despite a high fitness cost. This

mutation recapitulated virtually all the signature phenotypic changes seen in DAP-R *S. mitis* strains generated by serial *in vitro* passage in DAP, suggesting that the SNPs in *cdsA* identified in the above high-level DAP-R variant likely represent loss-of-function mutations. The mechanism(s) by which the *cdsA* mutation yields DAP-R and impacts CM fluidity and surface charge are currently under investigation.

Author Disclosure Block:

N.N. Mishra: E. Grant Investigator; Self; Merck Pharmaceuticals. **R. Seepersaud:** None. **D.N. Alvarez:** None. **T.T. Tran:** None. **B.A. Bensing:** None. **C. Garcia-de-la-Maria:** None. **J.M. Miro:** None. **M.J. Rybak:** N. Other; Self; consulting, speaker or have supported research from Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company and is partially supported by NIAID R01AI12400-01 and R21 AI109266-01. **C.A. Arias:** N. Other; Self; Forest Role(s): Investigator, Speaker's Bureau. **A.S. Bayer:** N. Other; Self; Research grants from: NIH-NIAID; ContraFect Corp; Theravance Pharmaceuticals. **P.M. Sullam:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Selective Binding of Antibiotic Mediates Daptomycin Resistance in *Streptococcus mitis*

Author Block:

T. T. Tran¹, C. Garcia de la Maria², N. Mishra³, J. Miro², M. Rybak⁴, P. Sullam⁵, A. Bayer³, C. Arias¹; ¹Univ Texas Med Schl, Houston, TX, ²Hosp Clinic-IDIBAPS, Barcelona, Spain, ³LA Biomed Res Inst, Torrance, CA, ⁴Anti-Infective Res Lab, Wayne State, Detroit, MI, ⁵VA Med Cntr, San Francisco, CA

Abstract Body:

Background: Daptomycin (DAP) resistance (R) in *S. mitis* develops rapidly *in vitro* and *in vivo*, resulting in isolates with very high DAP MICs ($\geq 256 \mu\text{g/ml}$). In other Gram-positive species, the mechanisms of DAP resistance are *i*) repulsion of the DAP molecule from the surface and *ii*) diversion of DAP from critical septal areas which involve rearrangement of phospholipid (PL) microarrays in the cell membrane (CM). However, the mechanistic bases for high-level and durable DAP -resistance observed in *S. mitis* are not known. **Methods:** We used *S. mitis* clinical strain 351 (MIC 0.5 $\mu\text{g/ml}$) and 2 DAP-R derivatives obtained *in vivo* after DAP exposure using a rabbit experimental endocarditis model (DAP MICs $\geq 256 \mu\text{g/ml}$). In order to evaluate the mechanism of DAP resistance, we exposed *S. mitis* cells to BODIPY-labeled DAP (BDP-DAP) at concentrations of 4 and 64 $\mu\text{g/ml}$. Fluorescence intensity was quantified by normalizing it to protein content of the sample. Additionally, we used 10-*N*-nonyl acridine orange (NAO) stain to visualize the distribution of anionic PL microdomains. **Results:** Anionic PL microdomains of parental DAP-S *S. mitis* 351 were visualized as intense linear staining at the bacterial septum and polar sites, as previously described in other organisms. In contrast, NAO-stained domains were absent in the CM of DAP-R derivatives suggesting marked remodeling of CM PLs. Interestingly, DAP-R derivatives showed increased fluorescent intensities compared to their parental *S. mitis* 351 ($P < 0.001$) at all BDP-DAP concentrations. Single-cell imaging of bacterial cells treated with BDP-DAP (64 $\mu\text{g/ml}$) showed intense binding the fluorescent DAP in few selective cells while antibiotic binding was completely absent in neighboring cells. Selective BDP-DAP binding

accounted for the high fluorescent intensity observed in DAP-R derivative compared to the DAP-susceptible parental **Conclusions:** DAP-R in *S. mitis* is associated with important remodeling of CM PLs and selective strong binding of the antibiotic to few cells, sparing the majority of the population from cell damage caused by the antibiotic. This strategy appears to be a novel mechanism of resistance against the antimicrobial peptide attack

Author Disclosure Block:

T.T. Tran: None. **C. Garcia de la Maria:** None. **N. Mishra:** E. Grant Investigator; Self; Merck. **J. Miro:** None. **M. Rybak:** N. Other; Self; consulting, speaker or have supported research from Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company and is partially supported by NIAID R01AI12400-01 and R21 AI109266-01. **P. Sullam:** None. **A. Bayer:** N. Other; Self; Research grants from: NIH-NIAID; ContraFect Corp; Theravance Pharmaceuticals. **C. Arias:** C. Consultant; Self; Theravance, Cubist, Bayer. **E. Grant Investigator;** Self; Theravance. **F. Investigator;** Self; Forest. **J. Scientific Advisor (Review Panel or Advisory Committee);** Self; Cubist, Bayer. **L. Speaker's Bureau;** Self; Forest, Theravance, Pfizer, Astra-Zeneca, the Medicines Company, Cubist, Novartis.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Transcriptional Analysis of the *Liafsr* Cluster in Daptomycin-Resistant *Enterococcus faecalis*

Author Block:

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¹Univ. El Bosque, Bogota, Colombia, ²Univ. of Texas Hlth.Sci. Ctr., Houston, TX, ³Univ. de Chile, Santiago de Chile, Chile

Abstract Body:

Background: Daptomycin, a bactericidal lipopeptide antibiotic, is a first line agent for the treatment of severe enterococcal infections. Substitutions in LiaFSR, a three component regulatory system that controls cell envelope homeostasis, have been shown to be mediators of DAP resistance in *E. faecalis*. Using RNA Seq, we previously identified the cluster *liaXYZ* that is strongly regulated by LiaR. Additionally, we showed that LiaR tetramers bind with high affinity to DNA upstream of *liaXYZ*. In this work, we performed a transcriptional analysis of the *liaFSR* and *liaXYZ* clusters in *E. faecalis*.

Methods: The following *E. faecalis* strain pairs were compared to evaluate changes in *liaXYZ* and *liaFSR* expression, **i)** S613 vs R712, a clinical strain pair of DAP-S and DAP-R, respectively, recovered before and after DAP treatment, **ii)** TM vs TM Δ *liaR*, a DAP-R derivative of S613 containing 3 allelic replacements in genes involved in DAP resistance and its *liaR* deletion derivative, respectively, **iii)** OG1RF and a *liaR* deletion derivative (OG1RF Δ *liaR*). RNA extraction from 3 biological replicates was performed and cDNA was generated. Quantitative qRT-PCR was performed in the absence of DAP and relative expression ratios (Log₂ fold change) were calculated by normalizing to *gyrB* expression.

Results: Compared to DAP-S S613, expression of *liaFSR* and *liaXYZ* clusters in R712 was increased about ~2 and ~4 fold, respectively ($p < 0.05$). In contrast, expression of both clusters was markedly reduced in *liaR* deletion mutants of TM and OG1RF. In TM Δ *liaR*, *liaF* expression decreased ~2 fold, whereas *liaS* decreased 9 fold compared to TM. Transcript levels of the *liaXYZ* cluster decreased ca. 5 fold ($p < 0.05$). Expression of both clusters were not significantly affected in the OG1RF Δ *liaR* compared to OG1RF, except for *liaX* and *liaS* which showed a decrease of 2.64 and 3.91 fold, respectively,

($p < 0.05$). **Conclusions:** Our findings support LiaR-mediated differential transcriptional regulation of a novel gene cluster *liaXYZ* that mediates cell membrane adaptation against DAP and antimicrobial peptide “attack” in enterococci. Complete characterization of this cluster and their gene products would be crucial in the elucidation of the mechanistic basis of antibiotic resistance in enterococci.

Author Disclosure Block:

S. Rincon: None. **D. Panesso:** None. **M. Latorre:** None. **T. Appel:** None. **J. Reyes:** None. **T.T. Tran:** None. **C.A. Arias:** C. Consultant; Self; Theravance, Cubist, Bayer. **E.** Grant Investigator; Self; Theravance. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Cubist, Bayer. **L.** Speaker's Bureau; Self; Forest, Theravance, Pfizer, Aztrazeneca, Cubist, The medicine company role, Novartis,.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Adaptive Mutations in Liar are Sufficient to Confer Daptomycin Resistance in *Enterococcus faecium* by Inducing Phosphorylation-Independent Activation of the Liafsr-Mediated Membrane Stress Response

Author Block:

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Abstract Body:

The cyclic lipopeptide daptomycin (DAP) has become one of the last remaining options for the treatment of multidrug-resistant enterococcal infections. The Trp73 to Cys substitution in the response regulator LiaR is commonly found in clinical isolates of *E. faecium*. We have determined the crystal structure of activated LiaR by X-ray crystallography at 3.2 Å resolution (space group P31; a=b=68.03, c=276.95 Å, α=β=90, γ=120°) and then used DNase footprinting by automated capillary electrophoresis, sedimentation equilibrium analytical ultracentrifugation, and microscale thermophoresis to understand how activated LiaR binds specific DNA sequences upstream of the *liaFSR* and *liaXYZ* operons. The crystal structure revealed that the activated LiaR is a dimer poised to make interactions with its target DNA. Non-activated LiaR exists as a monomer in solution with a $K_d > \sim 1600$ M. Addition of BeF_3^- (a phosphomimetic) strongly promotes dimer formation (K_d 10-20 M). The LiaR^{W73C} substitution promotes phosphorylation-independent dimerization of the LiaR protein with $K_d=182$ M. *Efm* LiaR^{W73C} binds about 20-fold tighter than wild type LiaR to the upstream regulatory regions of the operons controlled by LiaR. Our study revealed that the adaptive substitution W73C, even in the absence of phosphorylation, activates LiaR to undergo a phosphorylation-like self-dimerization event that allows LiaR to recognize and activate upstream regulatory regions that extend beyond the predicted consensus sequence. These results elucidated a mechanistic basis for how mutations in LiaR confer increased resistance to DAP through alterations in signaling.

Author Disclosure Block:

M. Davlieva: None. **A. Tovar-Yanez:** None. **K. DeBruler:** None. **P. Leonard:** None. **M. Zianni:** None. **C. Arias:** None. **S. Yousif:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Staphylococcus aureus Genetic Factors Contributing to Antibiotic Tolerance

Author Block:

A. S. Nuxoll, K. Lewis; Northeastern, Boston, MA

Abstract Body:

Antibiotic therapy is often unsuccessful against chronic infections, largely due to antimicrobial tolerance. Despite important implications to human health, the mechanism behind tolerance is poorly understood. Tolerance is mediated by persister cells, which are formed by all major pathogenic bacteria. Chronic infections including indwelling devices, osteomyelitis, cystic fibrosis, endocarditis, and deep-seated infections are associated with biofilms. Many antibiotics are proficient at killing growing cells and we rely on the immune system to clear the remaining, dormant persisters. Biofilms are especially problematic, as persisters are protected from the immune system. Recent advances on persister formation were made with studies in *E. coli* implicating toxin-antitoxin (TA) modules in antibiotic tolerance, through decreased protein synthesis and energy levels. However, deletion of the TA modules in *S. aureus* has no measureable effect on persister formation demonstrating that antibiotic tolerance is dependent on a different mechanism. While virtually nothing is known about persister formation in *S. aureus*, we have made significant advances in the field recently. We find that the entire population in stationary growth are antibiotic tolerant and persisters formed during exponential growth enter stationary phase growth early. Through a whole-genome screen we found that pyruvate dehydrogenase, PdhB, a critical enzyme for central metabolism, and global regulators CcpA and CodY affect persister formation. CcpA and CodY sense the nutritional status of the cell and regulate numerous central metabolic genes associated with the transition to stationary growth. Furthermore, interruption of late TCA cycle genes resulted in a high persister (hip) phenotype. *S. aureus* clinical isolates exhibiting decreased TCA cycle activity also had a hip phenotype. These findings support the notion that the aforementioned central metabolism components are critical for persister formation.

Author Disclosure Block:

A.S. Nuxoll: None. **K. Lewis:** None.

Session Number:

207

Session Title:

Deciphering New Mechanisms of Antimicrobial Resistance and Tolerance in Gram-positive Bacteria

Publishing Title:

Linking the σ^B Mediated Bacterial Stress Response to Antibiotic Tolerance in *Listeria monocytogenes*

Author Block:

I. Takeuchi, **T. D. Curtis**, L. Gram, G. Knudsen; Technical Univ. of Denmark, Kgs. Lyngby, Denmark

Abstract Body:

The foodborne pathogen *Listeria monocytogenes* is capable of survival and growth in a wide variety of environmental conditions. It can also survive high concentrations of antibiotics by forming persister cells, thought to be mediated largely by chromosomally encoded Toxin/Antitoxin (TA) systems. TA systems may also have a regulatory role, which has been indicated by a potential regulatory network between the alternative sigma factor B (σ^B) and an adjacent *mazEF* TA module in *Staphylococcus aureus*. In *L. monocytogenes*, σ^B is responsible for the transcription of many stress and virulence genes, and transcriptomic studies indicate that a homologous *mazEF* TA module is also part of the σ^B operon. The potential relationship between the general stress response and TA systems could shed light on our understanding of how bacteria tolerate antibiotics, and in order to determine if such a regulatory network exists in *L. monocytogenes*, as well as the effect on antibiotic tolerance, we constructed a series of in-frame deletion mutants in *mazE* (antitoxin), *mazF* (toxin), σ^B , and the ATP-dependent protease *clpP*, which activates toxins by degrading their cognate antitoxin. When log-phase cultures were exposed to super-MIC levels of norfloxacin over 48 hours, $\Delta mazE$ was as sensitive as the wild type (4.80 ± 0.45 and $5.13 \pm 0.56 \log_{10}$ CFU/ml, respectively), while $\Delta mazF$ and $\Delta clpP$ survived more poorly (4.69 ± 0.02 and $3.03 \pm 0.27 \log_{10}$ CFU/ml, respectively). $\Delta sigB$ survived slightly better ($6.00 \pm 0.53 \log_{10}$ CFU/ml), albeit not significantly so, suggesting that σ^B may repress the transcription of *mazEF* or other TA systems, which is currently being confirmed using RT-qPCR. Killing experiments on stationary phase cultures or those incubated in nutrient limited media yielded similar results to those using log-phase cells reported above. In conclusion, we demonstrate that the MazF toxin and ClpP protease have significant roles in the tolerance towards antibiotics of *L.*

monocytogenes, that the relative antibiotic susceptibility between the tested mutants is not affected by stationary phase or nutrient limitation, and also indicate that σ^B may negatively regulate the expression of *mazEF* in a manner similar to *S. aureus*.

Author Disclosure Block:

I. Takeuchi: None. **T.D. Curtis:** None. **L. Gram:** None. **G. Knudsen:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Ocular Candidiasis in Patients with Candidemia

Author Block:

T. Ueda, Y. Takesue, K. Nakjima, K. Ichiki, A. Doita, Y. Wada, T. Tsuchida, Y. Takahashi, M. Ishihara, T. Kimura; Hyogo Coll. of Med., Hyogo, Japan

Abstract Body:

Background: Ocular candidiasis is a major complication of candidemia. At our institution, ophthalmological examinations are routinely performed in patients with candidemia based on the bundle approach (Takesue Y et al., JAC, 2015). **Methods:** The incidence of ocular candidiasis among patients with candidemia, the timing of diagnosis, the vitreous body involvement rate, antifungal use, and clinical outcomes were reviewed in non-neutropenic adult patients with ocular candidiasis that were treated between April 2007 and June 2015. **Results:** Among 98 candidemia patients, 94 underwent ophthalmological examinations, and ocular candidiasis was detected in 25 patients (26.6%). Eighteen patients were diagnosed at the initial examination (2.7 ± 1.5 days after the confirmation of candidemia), and 7 further patients were diagnosed at follow-up examinations (12.0 ± 7.3 days). Vitreous body involvement was detected in 8 of 25 patients (32.0%). Among the 21 conscious patients, 8 complained of visual abnormalities (38.1%). *Candida albicans* was the most commonly detected causative agent in the patients' blood specimens, and the *C. albicans* isolation rates of the patients with and without ocular candidiasis differed significantly (92.0% vs. 38.0%, $P < 0.001$). The patients with ocular candidiasis had significantly higher β -D-glucan values (282.7 ± 213.6 pg/mL vs. 104.1 ± 175.4 pg/mL, $P < 0.001$). Treatment success (an improvement in the patient's clinical data and the resolution of ocular disease confirmed by an ophthalmological examination) was achieved in 18 of 25 patients. The 28-day mortality rate was 5/25 patients (20%). Fluconazole, voriconazole, and liposomal amphotericin B were administered as initial antifungals to 10, 4, and 11 patients, respectively. None of the patients received intravitreal injections of antifungals or underwent vitrectomy. The mean duration of antifungal therapy in the patients who survived more than 28 days was 50.9 ± 33.0 days. **Conclusions:** The incidence of candidiasis (26.6%) was concomitant with previous reports. However, a higher frequency of vitreous body involvement was

experienced. In addition, a considerable number of patients who were not diagnosed with ocular candidiasis at the initial examination exhibited the condition during follow-up ophthalmological examinations.

Author Disclosure Block:

T. Ueda: None. **Y. Takesue:** None. **K. Nakjima:** None. **K. Ichiki:** None. **A. Doita:** None. **Y. Wada:** None. **T. Tsuchida:** None. **Y. Takahashi:** None. **M. Ishihara:** None. **T. Kimura:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Isavuconazole (Isv) Serum Levels and Clinical Outcomes in Immunocompromised Patients

Author Block:

J. Sexton, B. Tegtmeier, J. Ito, S. Dadwal, J. Kriengkauykiat; City of Hope, Duarte, CA

Abstract Body:

Background: Studies have not suggested target ISV levels that correlate to clinical outcomes or side effects (SE). The purpose of this study was to associate trough levels with response and SE in patients with invasive fungal infection (IFI). **Methods:** Retrospective study from April to September 2015. SE analysis included patients who received ISV ≥ 7 days with trough levels. Clinical analysis only included patients with proven or probable IFI. Demographics, 14-, 30-, and 90-day response, ALT, total bilirubin, serum creatinine (Cr), and QTc were collected. Responders were patients with complete or partial response, nonresponders were those with stable or progressive disease or death. **Results:** Of the 37 patients who met criteria for clinical analysis, all had an underlying hematologic malignancy, 21 (56.7%) had hematopoietic cell transplant, 8 (21.6%) received radiation within 30 days of starting ISV, 7 (18.9%) underwent induction chemotherapy, and 15 (40.5%) were on active immunosuppression. Median trough levels for responders at 14, 30, and 90 days were 2.6, 2.5, and 2.5 mcg/mL, respectively, vs 2.5, 2.6, and 2.8 mcg/mL for nonresponders, $p=NS$. Patients with trough of ≥ 3 mcg/mL had a 14-day complete or partial response of 46.7% vs 36.4% for patients with trough < 3 mcg/mL, $p=.73$. Day 30 and 90 responses also did not differ. 62 patients met criteria for SE analysis. Change in baseline for trough < 2.5 vs ≥ 2.5 mcg/mL: 54% of patients vs 54% had 30% increase for ALT, 58% vs. 46% had 30% increase for bilirubin, 46% vs 41% had 30% increase Cr, while 7.9% vs 5.4% had decrease in QTC. All p -values NS. At trough ≥ 5 mcg/mL, proportion of patients with ALT ≥ 3 times upper normal limit was higher (15% trough < 5 vs 25% ≥ 5 mcg/mL, $p=.60$), and increase in ALT from baseline was also higher (median change 1.4 times for trough < 5 vs 2.6 for trough ≥ 5 mcg/mL, $p=.15$). **Conclusions:** There is no association between ISV trough levels and response to therapy. Patients with trough levels of ≥ 5 mcg/mL may experience higher SE.

Author Disclosure Block:

J. Sexton: None. **B. Tegtmeier:** None. **J. Ito:** L. Speaker's Bureau; Self; Astellas, Merck. **S. Dadwal:** None. **J. Kriengkauykiat:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Isavuconazole Prophylaxis (Isa Px) Among Solid Organ Transplant (Sot) Patients (Pts): Preliminary Impressions on Dosing and Interactions with Tacrolimus

Author Block:

R. M. Rivosecchi¹, C. N. J. Clancy¹, R. K. Shields¹, L. Clark¹, A. Humar¹, C. R. Ensor¹, B. A. Falcione¹, R. Venkataramanan², M-H. Nguyen¹; ¹UPMC Presbyterian, Pittsburgh, PA, ²Univ. of Pittsburgh Sch. of Pharmacy, Pittsburgh, PA

Abstract Body:

Background: ISA is approved for treatment of invasive aspergillosis and mucormycosis (IM). The use of ISA as px has not been evaluated. Our SOT program began universal ISA px following a cluster of IM cases among pts receiving voriconazole (VOR) px. There are no definitive recommendations for FK dosing among pts receiving ISA. We describe our preliminary experience using FK in SOT pts receiving ISA. **Methods:** This was a retrospective review of SOT pts from Sept. to Dec. 2015. Pts received ISA 372 mg Q 8h for 6 doses, then 372 mg Qd. Px duration was 4 mos for lung (Lu) and 1 mo for other SOT. FK dose was at the discretion of the treating physician with titrations based on daily monitoring during the early post-transplant period. **Results:** 95 SOT pts have received primary ISA px. We describe 41 pts who received ISA and FK (Kidney (K) transplant, 18; Liver (Li), 10; Lu, 10; Heart (H), 2; Li-K, 1), and who were followed for ≥ 2 mos post-SOT. Median age was 59 yrs, and 66% were men. 5 pts had received VOR and 2 fluconazole previously. Among the remaining 34 pts, median times to first therapeutic FK levels were 6d (K), 6d (Lu), 5d (Li) and 2.5d (H). Median (SD) FK concentration/dose (C/D) ratios, in (ng/mL)/(mg/kg), were 110 (± 85) at d7 and 110 (± 216) at d14. In 26% (9/34) of pts, there was a downward trend in C/D over 21d. C/D was highest during the first 2d (median 175) and lower on d3 (104, $p=.002$) and d4 (127, $p=.0002$), likely reflecting ISA loading doses. 85% of pts exhibited consistent C/D from d3 to d21, and 15% exhibited an upward trend. Median FK C/D during ISA px was 27% higher than after stopping px ($p=.04$). Of note, 1 pt developed invasive fungal infection (IFI) on ISA px. VOR and posaconazole levels were sub-therapeutic during subsequent treatment, suggesting poor absorption or high clearance in this pt (genetic studies pending). **Conclusions:** Our preliminary data suggest that ISA increases FK levels in

SOT pts by 27%. Clinicians should be aware that FK levels during ISA loading may be higher than at later time points, and that there is inter-pt variability in the effect of ISA on FK levels. Inter-pt variability and occurrence of a IFI suggest a potential role for ISA therapeutic drug monitoring in guiding management.

Author Disclosure Block:

R.M. Rivosecchi: None. **C.N.J. Clancy:** None. **R.K. Shields:** None. **L. Clark:** None. **A. Humar:** None. **C.R. Ensor:** None. **B.A. Falcione:** None. **R. Venkataramanan:** None. **M. Nguyen:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Isavuconazole Prophylaxis (Isa Px) Following A Cluster Of Invasive Mucorales Infections (Imi) In A Solid Organ Transplant (Sot) Program: Clinical Experience And Patient (Pt) Outcomes

Author Block:

M. Nguyen, R. Rivosecchi, R. Shields, B. Falcione, C. Ensor, V. Venkataramanan, A. Humar, C. Clancy; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: ISA is FDA-approved for primary treatment of adults with invasive aspergillosis and IMIs. There are limited data on ISA as px, or on its use in SOT pts. As part of a response to a cluster of 3 IMIs among SOT pts receiving voriconazole (VOR) px at University of Pittsburgh Medical Center (UPMC) in 5/15-9/15, universal ISA px was initiated. **Methods:** We conducted a retrospective review of our clinical experience with 105 SOT pts who received ISA px from 9/6/15-12/31/15. **Results:** IMIs occurred in liver (Li), lung (Lu) and heart (H) transplant (Tx) pts (1 each). The incidence of IMIs among SOT pts from 1/15-9/15 was 1.2% (3/254), including 2.4%, 2% and 6.2% among Li, Lu and H Tx pts, respectively. 98 SOT pts have received ISA as primary px (renal (R), 48; Li, 19; Lu, 18; H, 7; others, 6). 7.1% (7) were colonized with moulds pre-SOT (*A. fumigatus* (Af) culture +, 6; pathology, 1). 33% (6) of LuTx and 2.1% (1) of RTx pts were colonized. Other risk factors for invasive fungal infections (IFI) included renal failure (R, 27% (13/48); others, 20% (10/50)) and re-operation (19.4% (19/98)). 1 LuTx pt (5.6%) developed chest wall Af infection (colonized pre-Tx), and 2 LuTx pts (11.1%) developed Af colonization with \geq stage III ischemia reperfusion injury. No other moulds or yeasts were recovered from pts. An additional 7 LuTx pts have received ISA after developing VOR intolerance (4) or *Aspergillus* colonization despite VOR (2) or posaconazole (POS). While on ISA, 1 pt developed colonization with *Penicillium*, and 1 developed Af colonization with stenosis of a stented airway. Therefore, the overall incidence of IFI with ISA px was 1% (1/105; 4% (1/25) among LuTx pts). ISA was discontinued in 1.9% (2/105) of SOT pts due to possible intolerance (1 LiTx pt with a seizure; 1 LuTx with weight loss). Historic IFI rates among UPMC LuTx and LiTx pts receiving VOR px are 10% and 5%, respectively, and VOR discontinuation rates are 27%

and 2%, respectively. **Conclusions:** There were no further cases of IMI and the incidence of IFIs was extremely low following institution of ISA px in our SOT program. Rates of drug intolerance and discontinuation were lower than historic experience with VOR px. ISA is both effective and well-tolerated as px among SOT pts.

Author Disclosure Block:

M. Nguyen: None. **R. Rivosecchi:** None. **R. Shields:** None. **B. Falcione:** None. **C. Ensor:** None. **V. Venkataramanan:** None. **A. Humar:** None. **C. Clancy:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Multicenter Study of Posaconazole Delayed-Release Tablet Serum Level and Association with Hepatic and Cardiac Toxicity

Author Block:

N. Pettit¹, M. Miceli², C. Rivera³, A. Perissinotti⁴, M. Hsu⁵, P. Narayanan⁶, J. Pisano¹, S. Seo⁵, **A. Paskovaty⁵**; ¹UCM, Chicago, IL, ²U of Michigan, Ann Arbor, MI, ³MAYO, NY, NY, ⁴U of Michigan, NY, NY, ⁵MSKCC, NY, NY, ⁶MAYO, Rochester, MN

Abstract Body:

Background: Posaconazole delayed-release (PDR) tablet formulation achieves a higher serum level over the oral-suspension formulation. **This study evaluated whether higher therapeutic levels are associated with elevated liver function tests as a primary endpoint and prolonged QT interval as a secondary endpoint.****Methods:** This was a retrospective, multicenter study. Patients were ≥ 18 years of age, received posaconazole delayed-release tablets for prophylaxis or treatment of invasive fungal infections with a level obtained between 5-14 days from PDR initiation. AST, ALT, Alkaline Phosphatase (ALK), total bilirubin levels, and QTc were obtained at baseline and by end of study period, and were evaluated using linear and logistic regression, respectively.**Results:** 166 cancer patients including 53(32%) with underlying hepatic dysfunction were evaluated from four centers. The majority [141(85%)] received posaconazole delayed-release tablets for prophylaxis. Median serum posaconazole level was 1250 ng/mL (range 110-4220), obtained after median 6 days of therapy (range 3-14). Increasing levels of serum posaconazole were not significantly associated with changes in liver function measures from baseline. Among 118/166 patients in whom QTc was available, increased levels of serum posaconazole were not significantly associated with likelihood of QTc ≥ 500 at the end of treatment period (OR: 0.96). Per each additional hepatotoxic medication, there was a mean increase in total bilirubin of 0.13 mg/dL (p=0.01) and a mean increase in ALK of 7.1 U/L (p=0.09). Patients with baseline liver dysfunction had higher mean increase in total bilirubin (0.3 vs. 0.5 U/L, p=0.08).**Conclusions: Higher posaconazole levels were not significantly associated with elevation in liver function tests, even in patients with underlying hepatic dysfunction. Concomitant hepatotoxic medications increased the likelihood of elevated total bilirubin and ALK.**

Author Disclosure Block:

N. Pettit: None. **M. Miceli:** None. **C. Rivera:** None. **A. Perissinotti:** None. **M. Hsu:** None. **P. Narayanan:** None. **J. Pisano:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas. **S. Seo:** None. **A. Paskovaty:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Real-world Use of Posaconazole Extended Release Tablets in Patients with Hematologic Malignancy

Author Block:

F. Tverdek, S. Heo, S. Aitken, B. Granwehr, D. Kontoyiannis; Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract Body:

Background: Posaconazole (PCZ) is the preferred mold-active azole for prophylaxis against invasive fungal infection (IFI) in patients with hematologic malignancy (HM). An extended release tablet formulation of PCZ with improved pharmacokinetics versus the suspension has recently become available, but clinical data are limited. The purpose of this study was to examine the real-world pharmacokinetics and prophylactic effectiveness of PCZ tablets in patients with HM. **Methods:** This is a retrospective cohort of adult inpatients (≥ 18 years) with HM who received ≥ 3 days of PCZ tablets from 12/2013 to 10/2015 for primary IFI prophylaxis. Clinical information and concomitant drug use data were collected and correlated with low PCZ serum levels (< 700 ng/mL). Rates of IFIs, defined according to EORTC/MSG criteria, were assessed. Only the first inpatient prophylaxis episode was assessed for patients with multiple episodes. **Results:** 279 patients (mean age 56.3 ± 17.8 y; 56% male) were included. 93.2% had leukemia or myelodysplastic syndrome, 19.7% had prior hematopoietic stem cell transplant (HCT), and 80.7% had active malignancy. 62 patients had PCZ levels obtained with a median (IQR) value of 1380 ng/mL (859 - 1890). 17.8% of levels were low. Among 12 patients with two PCZ levels obtained without dose modification, the median (IQR) variability was 65.4% (28.8 - 156.8). Patients with low levels were younger compared to those with high levels (mean 45.0 ± 17.7 vs 59.4 ± 14.0 y, $p = 0.038$). Classification and regression tree (CART) analysis showed that patients age < 41 y were more likely to have low levels than those ≥ 41 y (50.0% vs 10%, $p = 0.001$, OR 9.0 [95% CI 2.1 - 38.8]). Body weight and body mass index did not correlate with low PCZ levels. Probable IFIs occurred in 5 patients (2%), all with levels > 700 ng/mL. **Conclusions:** We describe the largest series of PCZ tablet prophylaxis to date. PCZ tablets were effective as prophylaxis with 2% probable breakthrough IFIs that occurred exclusively in patients with levels > 700 ng/mL.

Careful assessment of epidemiology for PCZ resistant or tolerant fungi and PCZ therapeutic drug monitoring targets are warranted.

Author Disclosure Block:

F. Tverdek: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas. **S. Heo:** None. **S. Aitken:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas. **B. Granwehr:** E. Grant Investigator; Self; Merck. **D. Kontoyiannis:** I. Research Relationship; Self; Merck, Pfizer, Astellas. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Merck, Astellas, Mylan, T2 Biosystems, F2G.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Outcomes of Posaconazole Oral Suspension Therapy in Lung Transplantation

Author Block:

W. Jeong¹, G. I. Snell², B. J. Levvey², G. P. Westall², C. O. Morrissey², S. Ivulich², C. F. Neoh³, M. A. Slavin⁴, D. C. M. Kong¹; ¹Monash Univ., Melbourne, Australia, ²Alfred Hosp., Melbourne, Australia, ³Universiti Teknologi MARA, Selangor, Malaysia, ⁴Peter MacCallum Cancer Ctr., Melbourne, Australia

Abstract Body:

Background: At our centre, voriconazole (VRC) is the first line antifungal (AF) for pre-emptive therapy post-lung transplant (LTx). VRC is replaced by posaconazole oral suspension (POS) when: patients are intolerant to VRC; VRC trough plasma concentration (C_{\min}) remains sub-therapeutic despite two increases in VRC dose; or pre-emptive therapy for Mucormycete colonisation is indicated. POS is primarily prescribed as the first line AF, when there is concern about potential hepatotoxicity with VRC or a history of squamous cell carcinoma. Despite being increasingly prescribed, the outcomes of POS therapy following LTx remain poorly documented. We aim to describe the outcomes of POS therapy post-LTx at our centre. **Methods:** This is a retrospective cohort study evaluating the safety and efficacy of POS therapy in LTx recipients between January 2009 and December 2015. Patients were followed for up to a year after commencing AF post-LTx. **Results:** A total of 18 patients received POS as first line AF post-LTx. POS was initiated as pre-emptive therapy for post-LTx *Aspergillus* colonisation (n=9), pre-LTx *Aspergillus* colonisation (n=4) and donor colonisation (n=1); empirical therapy for presumed invasive fungal disease (n=3); and treatment of an aspergilloma (n=1). The median (IQR) duration of POS therapy was 120 (74-188) days. 6 months post-commencement of POS, colonising isolate was eradicated in 16 patients (i.e. negative bronchoalveolar lavage culture). Recurrent colonisation occurred in 1 patient (associated with sub-therapeutic POS C_{\min} ; therapy was subsequently changed to VRC), and 1 patient had died. At 12 months, of the 16 patients who had received only POS and were still alive, 1 had recurrent colonisation, whilst the other 15 remained clear of fungal colonisation. Therapeutic drug monitoring was performed in all patients, with the first sample taken [median (IQR)] 7 (5-13) days after commencing POS. The median (IQR) of

initial C_{\min} , at 400mg twice daily dose, was 0.84 (0.46-1.15) mg/L. No severe adverse events requiring cessation of POS were documented. **Conclusion:** Our data suggested that first line POS therapy in LTx recipients was well tolerated and efficacious.

Author Disclosure Block:

W. Jeong: None. **G.I. Snell:** None. **B.J. Levvey:** None. **G.P. Westall:** None. **C.O. Morrissey:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory board for Merck, Sharp and Dohme (MSD). N. Other; Self; Received research grants from Merck, Sharp and Dohme (MSD) and Gilead Sciences; speaker honoraria from Pfizer. **S. Ivulich:** None. **C.F. Neoh:** None. **M.A. Slavin:** N. Other; Self; Received research grants from Merck, Sharp and Dohme (MSD), Pfizer and Gilead Sciences. **D.C.M. Kong:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory board for Merck, Sharp and Dohme (MSD) and Pfizer. N. Other; Self; Received financial support from Pfizer, Novartis, Merck, Sharp and Dohme (MSD) and Gilead Sciences.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Increase in Pan-Triazole Resistance to *Aspergillus*-Active Triazoles in Patients with Hematologic Malignancies

Author Block:

A. M. Tatar¹, N. D. Albert², P. E. Verweij³, J. F. Meis³, R. E. Lewis⁴, D. P. Kontoyiannis²; ¹Rice Univ., Houston, TX, ²The Univ. of Texas MD Anderson Cancer, Houston, TX, ³Radboud Univ. Med. Ctr., Nijmegen, Netherlands, ⁴The Univ. of Bologna, Bologna, Italy

Abstract Body:

Background: Pan-triazole resistance (PTR) is a growing concern with the widespread use of these agents in patients with hematological malignancies. In this study, we screened for PTR in 290 *Aspergillus* unselected clinical isolates recovered from respiratory sources in patients with hematological malignancies at the University of Texas MD Anderson Cancer Center (MDACC), collected from 1999-2015. Since *Aspergillus*-active new triazoles (voriconazole (VRC), posaconazole (PCZ)) were introduced at MDACC after 2002, we evaluated *in vitro* PTR patterns before and after 2002. **Methods:** *Aspergillus* clinical isolates (*A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*) from 1999-2002 (n=183) and from 2003-2015 (n=107) recovered from patients with hematological malignancies at MDACC were screened for resistance to itraconazole (ITRA), VRC, and PCZ using the four-well multidish method[1]. Resistance to all three drugs in the screen was confirmed using the CLSI M38-A2 broth microdilution antifungal susceptibility testing method[2]. Resistance was defined as isolate MIC greater than MIC₅₀ values reported by Pfaller[3]. Changes in PTR of *Aspergillus* after 2002 were compared by species. **Results:** PTR isolates were discovered in all *Aspergillus* species in both periods. A statistically significant increase in the percentage of PTR isolates after 2002 was found in *A. fumigatus* (6/97 vs. 12/53, p=0.0218) and *A. niger* (4/21 vs. 10/15, p=0.0061). No significant changes in PTR rates were observed for *A. terreus* nor *A. flavus* isolates. **Conclusions:** Since the widespread use of triazoles, rates of PTR *Aspergillus* isolates have significantly increased in patients with hematological malignancy at our institution as a species-specific phenomenon. As PCZ and VRC are first-line choices for prophylaxis and treatment for invasive aspergillosis respectively,

this change in resistance patterns might have therapeutic implications. Further studies are needed to capture the clinical correlates of PTR and its genomic determinants.

Author Disclosure Block:

A.M. Tataara: None. **N.D. Albert:** None. **P.E. Verweij:** None. **J.F. Meis:** None. **R.E. Lewis:** None. **D.P. Kontoyiannis:** None.

Session Number:

246

Session Title:

Clinical Mycology

Publishing Title:

Combination Therapy Using Isavuconazole and Micafungin for Treating Murine Mucormycosis

Author Block:

T. Gebremariam¹, A. Alqarihi¹, P. Uppuluri¹, N. Azie², J. Edwards, Jr.¹, **A. S. Ibrahim¹**;
¹Los Angeles BioMed. Res. Inst. at Harbor-UCLA Med. Ctr., Torrance, CA, ²Astellas
Pharma, Northbrook, IL

Abstract Body:

Background: Mucormycosis is a life-threatening infection occurring in predominantly immunocompromised patients. Isavuconazole (ISA) is approved for treatment of invasive mucormycosis. Previously, we determined that *Rhizopus oryzae* possesses the target enzyme for echinocandins and micafungin (MICA) has activity against murine mucormycosis. Here we compared the activity of combination therapy (ISA + MICA) to placebo, either drug alone, or standard therapy of liposomal amphotericin B (LAmB) in treating pulmonary murine mucormycosis. **Methods:** CD-1 mice were immunosuppressed with cyclophosphamide (200 mg/kg, i.p.) and cortisone acetate (500 mg/kg, sq) on day -2 and +3 relative to intratracheal infection with *R. oryzae*. Treatment with ISA at 110 or 215 mg/kg (tid by oral gavage), MICA at 1 mg/kg (qd i.p.), LAmB at 15 mg/kg (qd, i.v.), or combination of ISA + MICA started 16 h post infection and continued through D+4 relative to infection. Survival was the primary efficacy endpoint. **Results:** All treatments significantly improved survival of mice (n= 9 for placebo, ISA at 110 mg/kg, LAmB, MICA, ISA 110 mg/kg + MICA; n=8 for ISA at 215 mg/kg; and n= 7 for ISA 215 mg/kg + MICA) vs. placebo by Log Rank test (Table). The enhancement of survival time by ISA or MICA treatment was equivalent to LAmB. None of the treatment regimens showed superiority over one another. Although combination therapy of ISA + MICA did not enhance survival of mice over monotherapy, antagonism was not detected between the two drugs.

Treatment	% Survival (D +21)	Median Survival time (Days)	P value (vs. placebo)
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Placebo	0	7	
ISA (110 mg/kg)	22	14	0.02
ISA (215 mg/kg)	25	12	0.004
MICA (1 mg/kg)	44	20	0.0013
LAmB (15 mg/kg)	44	17	0.0003
ISA 110 + MICA	22	14	0.03
ISA 215 + MICA	57	>21	0.01

Conclusions: ISA and MICA monotherapy are efficacious in treating pulmonary murine mucormycosis. Combination therapy of these two agents does not demonstrate synergy nor is it antagonistic in this model.

Author Disclosure Block:

T. Gebremariam: None. **A. Alqarihi:** None. **P. Uppuluri:** None. **N. Azie:** D. Employee; Self; Astellas Pharma. **J. Edwards, Jr.:** C. Consultant; Self; Astellas Pharma. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas Pharma. **A.S. Ibrahim:** C. Consultant; Self; Astellas Pharma. E. Grant Investigator; Self; Astellas Pharma. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas Pharma.

Session Number:

246

Session Title:**Clinical Mycology****Publishing Title:****Identification of Caspofungin-potentiating Drug-like Molecules****Author Block:**

S. Tripathi¹, D. Levin², Q. Feng¹, X-C. Li¹, M. Jacob¹, P-S. Donover³, M. Reichman³, A. Clark¹, **A. Agarwal¹**; ¹U. of Mississippi, University, MS, ²Boston U., Boston, MA, ³LIMR Chemical Genomics Ctr., Wynnewood, PA

Abstract Body:

New therapies are needed to potentiate echinocandin antifungals such as caspofungin (CAS) due to their narrow spectrum of activity and emerging drug resistance. Our approach aims to improve CAS activity by exploiting the cell wall integrity pathway (CWIP), which is required for cell wall stress adaptation. Since the transcription factor Rlm1 regulates the CWIP, this pathway can be monitored with a *lacZ* reporter driven by Rlm1-responsive elements. In this study, using this assay, we have identified compounds that improve CAS potency in *Candida albicans*, *C. glabrata*, and *Cryptococcus neoformans*. Given the utility of drug repurposing, we have screened 1,172 FDA-approved drugs and a collection of 8,000 diverse small molecules in an orthogonal-pooled screening format. For the primary screen, *Saccharomyces cerevisiae* cells carrying the promoter-reporter plasmid were grown to log phase, and exposed to either CAS alone or CAS + test compounds. After a 4 h incubation to activate the CWIP, beta-galactosidase (beta-gal) activity was measured. Compounds that inhibited beta-gal activity by at least 50% were considered hits. These were confirmed in dose response assays, and were defined as strong hits if they inhibited beta-gal activity at the primary test concentration and also at two additional 2-fold diluted concentrations. Confirmed compounds were evaluated by checkerboard assays to examine CAS potentiation. The screen with FDA-approved drugs identified 75 hits, 24 of which were confirmed to be strong hits. Among these, the anticoagulant dabigatran exhibited CAS-synergizing activity in *C. albicans* and *C. glabrata*. It also demonstrated additive activity with CAS against a CAS-resistant clinical isolate of *C. glabrata* as well as the CAS-insensitive pathogen *C. neoformans*. The screen with the diverse small-molecule library identified 27 hit compounds, 4 of which were confirmed to be strong hits. Of these, one compound with similarity to a known chitin synthase inhibitor exhibited CAS-synergizing activity

against CAS-resistant strains of *C. albicans* and *C. glabrata*, as well as *C. neoformans*. Our work reveals that CWIP-altering compounds have potential use in combination therapy with CAS, providing a means to eliminate CAS-resistant and CAS-insensitive fungal pathogens.

Author Disclosure Block:

S. Tripathi: None. **D. Levin:** None. **Q. Feng:** None. **X. Li:** None. **M. Jacob:** None. **P. Donover:** None. **M. Reichman:** None. **A. Clark:** None. **A. Agarwal:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Population Pharmacokinetics and Dosing Regimen Optimization of Meropenem in Cerebrospinal Fluid and Plasma in Patients with Meningitis after Neurosurgery

Author Block:

C. Lu¹, Y. Zhang¹, M. Chen², P. Zhong², J. Wu¹, Y. Zhang¹, J. Zhang¹; ¹Inst. of Antibiotics, Huashan Hosp., Fudan Univ., Shanghai, China, ²Dept. of Neurosurgery, Huashan Hosp., Fudan Univ., Shanghai, China

Abstract Body:

Background: Meropenem serves as an important broad-spectrum antibiotic to manage post-neurosurgical meningitis, but its pharmacokinetics in the plasma and cerebrospinal fluid (CSF) in this group is not well known. Our aim was characterizing meropenem population pharmacokinetics (PPK) and target attainment in plasma and CSF and recommending favorable dosing regimens for this patient group. **Methods:** 82 post-neurosurgical meningitis patients were included to receive meropenem of 2g q8h, 1g q8h or 1g q6h. After 3 days' infusion, blood and CSF samples were collected at predefined time points with meropenem concentration determined. The PPK model was developed using NONMEM. Probabilities of Target Attainment (PTA) were predicted via Monte Carlo simulations using the target of unbound meropenem concentrations above the MIC for at least 40% of the dosing interval in plasma, and 50% in CSF at MICs of 0.25 to 8 mg/L. **Results:** A two-compartment model plus another CSF compartment without covariate best described the data. The clearance (CL), inter-central and peripheral compartment clearance (Q1) and inter-central and CSF compartment clearance (Q2) were 22.2 L/h, 1.79 L/h and 0.01 L/h, respectively. The distribution volume of central compartment (V1) and peripheral compartment (V2) were 17.9 L and 3.84 L, while the volume of CSF was fixed at 0.13L and CSF clearance was calculated by observed CSF drainage speed. The uptake factor, multiplier for the transfer of meropenem from central to CSF compartment was 0.172. Simulation results show that the PTAs increase as the infusion duration prolonged and as daily volume of CSF drainage decreases. Under all circumstances of 1g q8h and 1g q6h, the PTAs are <90% in CSF to manage bacteria with MIC > 0.25 mg/L. To get PTA>90%, 2g q8h with infusion 3 hours and CSF drainage speed of 150mL/day can well manage bacteria with MIC up to 4 mg/L in blood and MIC

up to 0.5 mg/L in CSF, while continuous infusion with no CSF drainage can well manage bacteria with MIC up to 8 mg/L in blood and up to 1 mg/L in CSF. **Conclusions:** 2g q8h with infusion duration at least 3h with limited CSF drainage volume (<150mL/day) is recommended to treat post-neurosurgical meningitis patients.

Author Disclosure Block:

C. Lu: D. Employee; Self; Roche R&D Center China Ltd. **Y. Zhang:** None. **M. Chen:** None. **P. Zhong:** None. **J. Wu:** None. **Y. Zhang:** None. **J. Zhang:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Eravacycline (ERV) Pharmacokinetics (PK) and Challenges in Defining Humanized Exposure *In Vivo*

Author Block:

A. K. Thabit, M. L. Monogue, D. P. Nicolau; Ctr. for Anti-Infect. Res. and Dev., Hartford Hosp., Hartford, CT

Abstract Body:

Background: PK studies for novel antimicrobial agents are done to correlate their exposures to the efficacy derived from pharmacodynamic (PD) studies *in vivo*. Protein binding (PB) studies are also conducted as part of PK studies in order to estimate the free fraction of the compound. ERV is a novel fluorocycline that belongs to the tetracycline class of antimicrobials. We aimed to assess the PK profile of ERV including its PB profile through a series of PK studies in the immunocompetent murine thigh infection model. **Methods:** A total of 5 PK studies utilizing single intravenous (IV) doses of 2.5, 5, 6, 7.5, and 10 mg/kg of ERV were conducted on ICR mice infected with *Klebsiella pneumoniae* in both thighs. ERV dosing was started 2 hours post infection. Terminal blood samples were collected in sodium heparin tubes at 8 time points post-dose (5 minutes to 12 hours) where each time point included 8 mice divided into 2 groups. Blood samples from each group of 4 mice were pooled into a single tube. Plasma was separated by centrifugation. PB studies were conducted using the ultrafiltration method. ERV concentrations were determined using a verified method. The mean concentration per time point for each dose studied was calculated. Since $fAUC/MIC$ is the PK/PD parameter that best correlates with the efficacy of tetracyclines, the $fAUC_{0-12}$ of ERV was calculated using the linear trapezoidal rule formula. **Results:** PB of ERV ranged 12.5-97.3% with a mean \pm SD of $71.4 \pm 17.1\%$ and showed atypical nonlinear, inversely proportional concentration-dependence to total drug concentration. Non-specific binding of ERV to the membrane of the ultrafiltration device was negligible. The $fAUC_{0-12}$ of ERV was semi-linear across the dose range studied. None of the doses studied, if given q24h, yielded a $fAUC_{0-24}$ value within the range of the target seen in human. However, when the $fAUC_{0-24}$ of the 2.5 mg/kg dose was calculated based on a q12h regimen, it yielded a value of 1.64 mg·hr/l which most closely mimicked the human phase 1

exposure associated with a 1 mg/kg q12h IV dose. **Conclusion:** Similar to tigecycline, ERV has a unique non-linear protein binding profile that must be accounted for when conducting translational efficacy studies. An ERV IV dose of 2.5 mg/kg q12h was the dose that best simulated the human exposure.

Author Disclosure Block:

A.K. Thabit: None. **M.L. Monogue:** None. **D.P. Nicolau:** A. Board Member; Self; Tetrphase Pharmaceuticals, Inc.. E. Grant Investigator; Self; Tetrphase Pharmaceuticals, Inc..

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

β -Lactam Therapeutic Drug Monitoring (TDM) in Pediatrics

Author Block:

J. Cies¹, W. Moore, II¹, A. Enache², A. Chopra³; ¹The Ctr. for Pediatric Pharmacotherapy, Pottstown, PA, ²Atlantic Diagnostic Lab., Ben Salen, PA, ³NYU Langone Med. Ctr., New York, NY

Abstract Body:

Background: Anti-infective dose personalization using TDM is common for aminoglycosides and glycopeptides. However, use of this practice with β -lactams has not been widely described in pediatrics. The objective is to describe the number of pediatric patients undergoing β -lactam TDM (BLTDM) and determine if dosing adjustments were required. **Methods:** This is a retrospective chart review of pediatric patients that received BLTDM from September 2014-December 2015. β -lactams subject to TDM were ampicillin (AMP), cefazolin (CFZ), cefotaxime (CTX), cefepime (FEP), doripenem (DOR), meropenem (MEM), and piperacillin (PIP). Individual PK parameters were calculated to determine if standard dosing regimens produced a pharmacodynamic (PD) target of at least 40% $fT > 4-6 \times \text{MIC}$. If an organism was not obtained, the MIC breakpoint was used for each drug. Pediatric dosing references (Lexi-Comp, Micromedex and Harriet Lane) were used to determine whether standard published dosing recommendations would have produced the desired PD target. **Results:** 112 patients received BLTDM; 64 (57%) were male and 48 (43%) were female. The median age was 5 yrs (range 3 days-23 yrs) with a median weight of 14.6 kg (range 2.49-116 kg). The anti-infectives tested were 4 (3.6%) AMP, 6 (5.4%) CFZ, 24 (21.4%) CTX, 50 (44.6%) FEP, 2 (1.8%) DOR, 19 (17%) MEM, and 7 (6.3%) PIP. 108 of 112 (96.4%) patients had altered anti-infective serum concentrations: 90 (80.4%) patients had low levels and 18 (16%) had elevated levels. 4 (3.6%) patients were on continuous renal replacement therapy (CRRT), 11 (9.8%) were receiving extra-corporeal life support (ECLS), and 31 (27.7%) of patients had cystic fibrosis (CF). Of the 46 patients receiving CRRT, ECMO, and with CF all had low anti-infective serum concentrations. 4 patients (2 FEP, 2 CTX) had therapeutic serum concentrations with standard pediatric dosing. **Conclusions:** Overall, 96.4% of patients had altered anti-infective serum concentrations. Considering

dosing regimens for pediatrics are determined from studies in healthy volunteers or extrapolated from adult data, these data suggest BLTDM is a potentially valuable intervention to optimize anti-infective exposure. Additional research in specific pediatric populations and the need for assessing clinical outcomes with BLTDM in pediatrics is warranted

Author Disclosure Block:

J. Cies: C. Consultant; Self; Atlantic Diagnostic Laboratories. **W. Moore:** None. **A. Enache:** None. **A. Chopra:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Tissue Distribution and Pharmacokinetics of CD101 in an Immunocompetent Mouse Model of Invasive Candidiasis

Author Block:

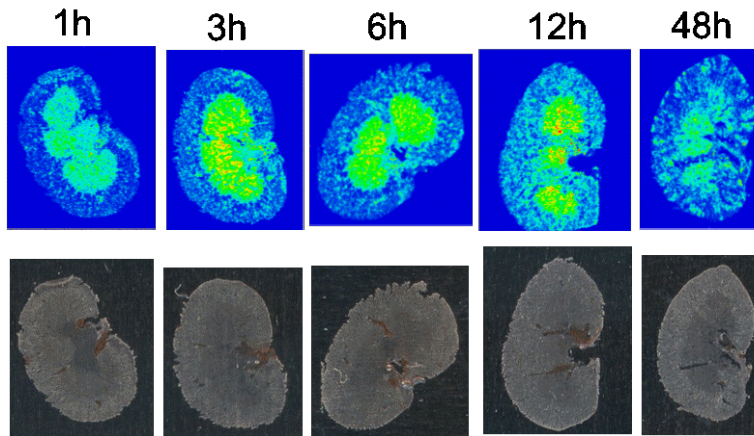
Y. Zhao¹, B. Prideaux¹, P-Y. Chen¹, Y. Nagasaki¹, M-H. Lee¹, G. Hough², V. Ong², D. S. Perlin¹; ¹Publ. Hlth.Res. Inst., Rutgers BioMed. and Hlth.Sci., Newark, NJ, ²Cidara Therapeutics, San Diego, CA

Abstract Body:

Background: CD101 is a novel echinocandin being developed to treat invasive *Candida* infections. Herein, tissue distribution of CD101 was investigated by matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) in an immunocompetent invasive candidiasis mouse model. **Methods:** Female 6-week-old BALB/c mice were IV infected with 2×10^6 CFU of *C. albicans* strain ATCC 90028 on day 0. Single doses of CD101 at 10, 20, 40 or 60 mg/kg were administered at 24h post-infection via IP injection. Blood was collected predose and at 1, 3, 6, 12, 24, and 48h post-dose (3 mice/timepoint/dose) for PK assessment. Plasma concentrations were measured by LC-MS/MS. Kidneys from the 10 mg/kg group at 0, 1, 3, 6, 12, and 48h post-dose time points were analyzed for tissue distribution by MALDI-MSI. **Results:** Maximum plasma concentrations (C_{max}) of CD101 were observed at 1-12h with mean C_{max} values of 23.1, 43.3, 82.3, and 95.8 $\mu\text{g/mL}$ for the doses of 10, 20, 40, and 60 mg/kg, respectively. Corresponding mean values for AUC_{0-t} , where $t=48\text{h}$ post-dose, were 736, 1250, 2380, and 3300 $\mu\text{g}\cdot\text{h/mL}$. Mean half-life was long for each dose, ranging from 29.8 to 52.0h. A unique kidney distribution pattern of CD101 was observed with higher drug signals in the medulla but lower levels of drug reaching the outer cortex. CD101 in the kidney accumulated over time, with strong signals visualized from 3 to 12h. Drug signal decreased slowly after 12h post-dose, yet appreciable signal was still detectable at 48h (**Figure**). **Conclusion:** The exceptional PK properties and prolonged tissue distribution of

CD101 suggest the great potential of CD101 in the treatment of invasive candidiasis.

**MALDI imaging of CD101 in *C. albicans*
infected mouse kidney**



Author Disclosure Block:

Y. Zhao: E. Grant Investigator; Self; Merck. **B. Prideaux:** None. **P. Chen:** None. **Y. Nagasaki:** None. **M. Lee:** None. **G. Hough:** D. Employee; Self; Cidara Therapeutics, Inc. **V. Ong:** D. Employee; Self; Cidara Therapeutics, Inc. **D.S. Perlin:** E. Grant Investigator; Self; NIH, Cidara, Astellas, Merck, Scynexis. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cidara, Merck, Astellas, Scynexis.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

An Inverted-U Relationship between Amoxicillin/Clavulanic Acid Exposure and Colonization by ESBL(+) Bacteria in Non-ICU Population

Author Block:

J. Meletiadis¹, E. Taconelli², G. De Angelis³, G. Restuccia³, L. Preotescu⁴, M. Popoiu⁴, B. Carevic⁵, T. Tomic⁵, Y. Carmeli⁶, A. Lerner⁶, A. Adler⁶, S. Percia⁶, H. Goosens⁷, S. Malhotra-Kumar⁷, C. Lammens⁷, J. Mouton¹; ¹Erasmus MC, Rotterdam, Netherlands, ²Università Cattolica Sacro Cuore, Rome, Italy, ³Università Cattolica Sacro Cuore, Rome, Italy, ⁴Inst. for Infectious Diseases "Matei Bals", Bucharest, Romania, ⁵Clinical Ctr. of Serbia, Belgrade, Serbia, ⁶Sourasky Med. Ctr., Tel-aviv, Israel, ⁷Univ. of Antwerp, Antwerp, Belgium

Abstract Body:

Background: The relationship between exposure and development of antimicrobial resistance is largely unknown for most antibacterial drugs. We correlated (A/C) PK/PD indices (PDI) with colonization by ESBL(+) pathogens in a cohort of non-ICU patients. **Methods:** 263 patients hospitalized in medical and surgical wards of three centers and treated with amoxicillin/clavulanic acid were repeatedly screened during treatment for ESBL(+) pathogens using rectal swabs and selective chromogenic medium. Patients colonized before treatment were excluded. A/C exposure was calculated for each patient using KinFun1.07 Software (Medimatics,NL) and a population pharmacokinetic model with creatinine clearance as covariate (Carrier et al JAC 2013). %fT>MIC, fCmax/MIC, fCmin/MIC and fAUC/MIC were calculated using the resistance breakpoint of 8 mg/l. The PDI were associated with the colonization rate by ESBL(+) pathogens using classification regression tree analysis (CART), X² test and multivariate logistic regression analysis. **Results:** Out of 263 patients, 49 (16%) patients became colonized with ESBLs. All PDIs were significantly associated with colonization, with the strongest association found with fCmax/MIC (X²=14, p=0.0009). CART analysis split the fCmax/MIC ratios at 3.42 and 5.12. The colonization rate was 10%, 44% and 20% at fCmax/MIC<3.42, 3.42-5.12 and >5.12, respectively. Multivariate logistic analysis showed that the fCmax/MIC ratio (X²=12.9, p=0.0004) together with the duration of therapy (X²=9.7, p=0.002) were independent risk factors for colonization. **Conclusions:**

An inverted-U relationship between A/C exposure and colonization by ESBL(+) pathogens was found with the highest risk of colonization observed at intermediate fC_{max}/MIC ratios.

Author Disclosure Block:

J. Meletiadis: E. Grant Investigator; Self; Astellas, Gilead, MSD, Pfizer. **E. Taconelli:** None. **G. De Angelis:** None. **G. Restuccia:** None. **L. Preotescu:** None. **M. Popoiu:** None. **B. Carevic:** None. **T. Tosic:** None. **Y. Carmeli:** None. **A. Lerner:** None. **A. Adler:** None. **S. Percia:** None. **H. Goosens:** None. **S. Malhotra-Kumar:** None. **C. Lammens:** None. **J. Mouton:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Accurin® Nanoparticles Improve the Therapeutic Index of Colistin in Preclinical Models of Infection

Author Block:

D. Zaytseva-Zotova¹, A. Zinchenko¹, T. Levada¹, D. Andreev¹, Y. Shilov¹, E. Safarova¹, A. Horhota², G. Troiano², S. Zale²; ¹BIND (RUS), LLC, Moscow, Russian Federation, ²BIND Therapeutics Inc, Cambridge, MA

Abstract Body:

Background: Due to undesired toxicity issues, primarily nephrotoxicity, after i.v. administration of colistin sulphate (CS), colistin is generally administered as a prodrug, colistin methanesulfonate (CMS). CMS is associated with variable pharmacokinetics (PK) and poor deposition into diseased tissues, and inefficient conversion of the prodrug to the active moiety. The present study was aimed at assessing the application of BIND ACCURIN® platform for encapsulation of colistin into biodegradable polymeric nanoparticles (NPs) to improve control over drug concentration in blood as well as facilitating increased uptake of drug at sites of localized infection. **Methods:** CS was efficiently encapsulated into polylactide-polyethylene glycol NPs with the diameter of 100 nm using a nanoemulsion process. PK was studied after administration of a single dose of NPs to SD rats. Renal toxicity studies were performed in mice to characterize ability of NPs to decrease nephrotoxicity of colistin. Biodistribution and efficacy studies were conducted in *K. pneumoniae* lung and thigh infection models in mice. **Results:** Encapsulation of CS into NPs prolonged the circulation of CS in blood and enhanced the CS concentration profile at sites of infection due to higher deposition of NPs in the inflamed tissue. All CS-NPs were significantly more tolerable after i.v. injection in comparison to free CS. Tubular degradation and necrosis in kidneys were seen on histology after administration of free drug, whereas no changes were found for groups receiving similar doses of the CS-NPs (i.v. and s.c.). Comparable efficacy of conventional drug and CS-NPs was demonstrated in *K. pneumoniae* lung infection model: 33% and 67% of sterilized animals after s.c. and i.v. administration of a single 40 mg/kg dose of CS-NPs compared to 50% for CS given s.c. in the same dose (n=6). **Conclusion:** Encapsulation of colistin into ACCURIN® NPs resulted in

differentiated PK and biodistribution of the drug, increased tolerability and decreased nephrotoxicity with no loss of efficacy in treatment of experimental infection. Thus, the results provide an excellent example of widening of therapeutic window of poorly tolerated anti-infective drugs using ACCURIN® polymeric nanoparticles.

Author Disclosure Block:

D. Zaytseva-Zotova: None. **A. Zinchenko:** None. **T. Levada:** None. **D. Andreev:** None. **Y. Shilov:** None. **E. Safarova:** None. **A. Horhota:** None. **G. Troiano:** None. **S. Zale:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Penicillin-binding Protein (Pbp) Occupancy Patterns Determine Killing of *Pseudomonas aeruginosa* (Pa) at High and Low Bacterial Density

Author Block:

J. B. Bulitta¹, T. Velkov², K. Rogers², J. Shan², A. Oliver³, R. L. Nation², J. D. Boyce², B. T. Tsuji⁴, C. B. Landersdorfer²; ¹Univ. of Florida, Orlando, FL, ²Monash Univ., Melbourne, Australia, ³Hosp. Son Espases, Palma, Spain, Palma de Mallorca, Spain, ⁴SUNY Buffalo, Buffalo, NY

Abstract Body:

Background: All β -lactam antibiotics bind to multiple different penicillin-binding proteins (PBP) with different affinity. Little is known about which PBPs need to be bound and inactivated to kill Pa at high bacterial density. Our aim was to identify PBP occupancy patterns, achieved by cephalosporins or monobactams, which cause rapid killing of Pa at high and low bacterial density. **Methods:** The beta-lactam concentrations required for 50% (IC₅₀) and 90% (IC₉₀) PBP binding were determined for ceftazidime (CAZ), cefsulodin (CSU) and aztreonam (ATM) by the Bocillin FL assay. Static concentration time-kills were performed over 24 h (initial inocula: 10⁶ for wild-type and double-knockout; and 10⁷ CFU/mL for all strains). A range of beta-lactam concentrations was tested against four isogenic Pa strains (PAO1 wild-type, Delta-ampC, Delta-oprM, and Delta-ampC & Delta-oprM) which lacked the AmpC beta-lactamase, efflux by MexAB-OprM, or both. **Results:** IC₅₀ for PBP1a/1b/3 were 0.125/8/<0.125 mg/L for CAZ, 8/0.75/0.5 mg/L for CSU and 2/1/<0.01 mg/L for ATM. IC₉₀ were 2/32/0.5 mg/L for CAZ, >128/8/0.5 mg/L for CSU and 16/16/0.5 mg/L for ATM. All PBP2 IC₅₀ were >128 mg/L. To achieve ≥ 1.5 log₁₀ killing at 4h, the double-knockout strain required 32 (CAZ), 256 (CSU), and 128 mg/L (ATM) at the high inoculum, but only 1 (CAZ), 2 (CSU) or 0.5 mg/L (ATM) at the low inoculum; ≥ 1.5 log₁₀ killing of the wild-type at 4h required >256 mg/L at the high inoculum and 4 (CAZ), 2 (CSU) or 8 mg/L (ATM) at the low inoculum. AZT and CSU at 128 x MIC yielded minimal (<0.5 log₁₀) killing of the double-knockout at 4h at the high inoculum. **Conclusion:** This study provides critical insights on efficacious PBP occupancy patterns against Pa in both high- and low-inoculum phenotype. Rapid initial killing of Pa was achieved by simultaneous

and extensive (>90%) binding of PBP1a, 1b and 3 at high bacterial density, whereas PBP1a (50-90% bound) and PBP3 (>90%) binding or PBP1b (50-90%) and PBP3 (>90%) binding was sufficient at low bacterial density. These new insights hold great promise to optimize novel double beta-lactam combinations against persistent, difficult-to-treat Pa infections.

Author Disclosure Block:

J.B. Bulitta: E. Grant Investigator; Self; Pfizer, Cubist, Trius, Cempra, CSL. **T. Velkov:** E. Grant Investigator; Self; The Medicines Company. **K. Rogers:** None. **J. Shan:** None. **A. Oliver:** E. Grant Investigator; Self; Cubist, Wockhardt. **R.L. Nation:** E. Grant Investigator; Self; The Medicines Company. **J.D. Boyce:** None. **B.T. Tsuji:** E. Grant Investigator; Self; Pfizer, Cubist, Cempra, GSK. **C.B. Landersdorfer:** E. Grant Investigator; Self; Pfizer, Cubist, Trius, CSL.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

***In Vitro* Pharmacodynamics (Pd) of Polymyxin B (Pb) in Combination (Combo) with Zti-01 (Fosfomycin, Fm) Against Polymyxin Susceptible (Pb_s) and Resistant (Pb_r) Carbapenemase-Producing *Klebsiella pneumoniae* (Kp)**

Author Block:

V. H. Lee¹, F. Minhaj¹, J. K. Diep¹, E. J. Ellis-Grosse², C. S. Abboud³, A. Forrest⁴, G. G. Rao¹; ¹SUNY, Buffalo, NY, ²Zavante Therapeutics, Inc, San Diego, CA, ³Inst. Dante Pazzanese de Cardiologia, São Paulo, Brazil, ⁴UNC, Chapel Hill, NC

Abstract Body:

Background: The increasing prevalence of PB_R carbapenemase producing KP emphasizes the urgency for optimization of PB based combos. ZTI-01 is an IV form of FM, the only epoxide antibiotic with a novel MOA that inhibits peptidoglycan biosynthesis by irreversibly inhibiting MurA. We assessed the PD of PB & FM alone & in combo against PB_S & PB_R KP clinical isolates. **Methods:** Three KPC-2 isolates [KPC30 (MIC_{PB}: 0.5mg/L, MIC_{FM}: 8mg/L), KPC9 (MIC_{PB}: 0.5mg/L, MIC_{FM}: 128mg/L) & KPC78 (MIC_{PB}: 64mg/L, MIC_{FM}: 16mg/L)] were used. Time-kill experiments were performed to evaluate the PD of PB (0.5, 1, 2 & 4 mg/L) & FM (75, 150 & 200 mg/L) alone & in combo against an initial inoculum of ~10⁶ CFU/mL. Samples were drawn at 0, 1, 2, 4, 6, 8, 24, 28, 32 & 48h for bacterial quantification. PD effect was assessed by using the log₁₀ of the ratio of CFU at all measured time points compared to the CFU at 0h. **Results:** Against KPC30 (PB_S & FM susceptible) & KPC9 (PB_S & FM resistant), monotherapy (mono) with all PB & FM concentrations (conc) resulted in bactericidal activity at 2h followed by regrowth similar to growth control (GC) beyond 8h. All PB conc in combo with FM resulted in bactericidal activity by 2h. Bactericidal activity was sustained against KPC30, however, against KPC9, only PB 2 & 4 mg/L in combo with FM 150 or 200 mg/L resulted in sustained activity over 48h. PB mono against KPC78 (PB_R & FM susceptible) performed similar to GC. FM mono demonstrated > 2 log reduction by 4h followed by regrowth beyond 8h. PB in combo with lowest FM conc of 75 mg/L resulted in > 2 log reduction by 6h followed by regrowth similar to GC by 28h. All PB conc in combo with FM conc of 150 & 200 mg/L resulted in bactericidal activity by 8h. However, only combo of PB 4 mg/L with FM 200 mg/L resulted in sustained

bactericidal activity over 48h. **Conclusion:** Our results support our hypothesis that PB & FM act synergistically against KPC-producing KP as PB causes outer membrane perturbation in Gram-negative bacteria, allowing for enhanced killing by FM. Further evaluation of these combos in a hollow-fiber system over a longer period of time is warranted for dose optimization & clinical translation.

Author Disclosure Block:

V.H. Lee: None. **F. Minhaj:** None. **J.K. Diep:** None. **E.J. Ellis-Grosse:** A. Board Member; Self; Zavante Therapeutics, Inc. **D. Employee:** Self; Zavante Therapeutics, Inc. **C.S. Abboud:** None. **A. Forrest:** None. **G.G. Rao:** None.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Bacterial Replication Rate Modulation in Combination with Antimicrobial Therapy: Turning the Microbe against Itself

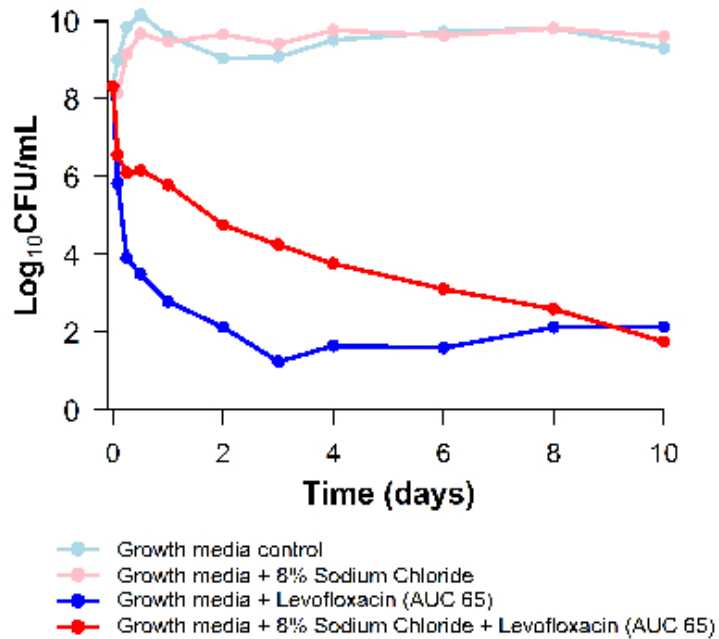
Author Block:

P. G. Ambrose, B. D. VanScoy, H. Conde, J. McCauley, C. M. Rubino, S. M. Bhavnani;
ICPD, Latham, NY

Abstract Body:

Given that most antibiotics require replicating bacteria to exert their effects, increasing the bacterial growth rate in the context of an effective antibiotic exposure may represent an unexploited path to decrease therapy duration. To demonstrate that altering the bacterial growth rate in the context of an antibiotic exposure is coupled with therapy duration, we used a PK-PD infection model in which the media was altered such that bacteria grew at different rates. *S. aureus* ATCC 29213 was exposed to a typical daily levofloxacin (LEV) exposure (free-drug AUC, 65; MIC, 0.125 mg/L; AUC:MIC ratio, 520) in a 10-day hollow-fiber infection model. The initial bacterial inoculum (10^8 CFU/mL) was selected to approximate that observed in high-density infections. Treatment arms and associated control arms differed only in sodium chloride (NaCl) concentration (0 and 8%) within the media (Mueller Hinton II). NaCl concentrations were selected based upon the relationship between increasing NaCl concentration and decreasing bacterial growth rate (not shown). The **Figure** shows the impact of NaCl concentration on bacterial density over time, with and without LEV. In the LEV-containing arm with media in which the challenge isolate grew slowly (NaCl 8%), the time to reaching a bacterial density of 10^2 CFU/mL was 10 days while in the LEV-containing arm with media in which the isolate grew rapidly (NaCl 0%), the time to reaching a the same bacterial density was approximately 2 days. We demonstrated that modulating the bacterial replication rate in the context of an antibiotic exposure is coupled with the rate and extent of bactericidal effects. Our data may provide a *proof-of-*

concept for new adjunctive therapeutic options to antimicrobial agents that reduce



treatment duration.

Author Disclosure Block:

P.G. Ambrose: D. Employee; Self; ICPD. **K. Shareholder** (excluding diversified mutual funds); Self; ICPD. **B.D. VanScoy:** D. Employee; Self; ICPD. **H. Conde:** D. Employee; Self; ICPD. **J. McCauley:** D. Employee; Self; ICPD. **C.M. Rubino:** D. Employee; Self; ICPD. **K. Shareholder** (excluding diversified mutual funds); Self; ICPD. **S.M. Bhavnani:** D. Employee; Self; ICPD. **K. Shareholder** (excluding diversified mutual funds); Self; ICPD.

Session Number:

247

Session Title:

Innovations and Challenges in Pharmacokinetics/Pharmacodynamics (PK/PD)

Publishing Title:

Pharmacokinetic-Pharmacodynamic (PK-PD) Analyses for Efficacy and Safety of Brilacidin (BRI) Using Data from Patients with Acute Bacterial Skin and Skin Structure Infections (ABSSSI)

Author Block:

S. M. Bhavnani¹, J. P. Hammel¹, A. Forrest¹, S. A. VanWart¹, P. Sager², K. J. Tack³, R. W. Scott⁴, D. M. Jorgensen³, P. G. Ambrose¹; ¹ICPD, Latham, NY, ²Stanford Univ., Palo Alto, CA, ³Cellceutix Corp., Beverly, MA, ⁴Fox Chase Chemical Diversity Ctr., Doylestown, PA

Abstract Body:

BRI is a synthetic molecule of a novel class of agents that demonstrates potent antimicrobial activity against Gram-positive organisms, including MRSA. BRI is being developed for the treatment of patients with ABSSSI. Using a previously-developed population PK model for BRI and data from 2 Phase 2 studies of BRI-treated patients with ABSSSI, PK-PD relationships for efficacy and safety were assessed. Patients received 1 of the following IV BRI dosing regimens: 0.75, 0.4, or 1.0 mg/kg on Day 1 followed by 0.35, 0.3, or 0.35 mg/kg q24h for Days 2-5, respectively, single doses of 0.6 or 0.8 mg/kg on Day 1, or 0.6 mg/kg on Day 1 followed by 0.3 mg/kg q24h on Days 2-3. PK-PD relationships for clinical response on Days 2-3, end-of-therapy (EOT), test-of-cure (TOC), change in lesion size on Days 1-6, systolic blood pressure (SBP) and numbness or tingling (N/T) were explored. Univariable and multivariable analyses for efficacy were carried out using chi-square or Fisher's exact tests and logistic regression. Repeated measures multiple linear regression and multivariable autoregressive logistic regression were performed to evaluate independent variables predictive of SBP and N/T, respectively. BRI AUC:MIC ratio and AUC exposure variables were evaluated for efficacy and safety analyses, respectively. Significant PK-PD relationships for clinical response at EOT and TOC and ≥ 20 and $\geq 50\%$ reduction from baseline in lesion area on Days 2 and 3, respectively, were identified ($p \leq 0.027$). A repeated measures multivariable linear regression model for SBP included the following significant variables: 48-h prior AUC, elevated SBP or DBP at screening, history of hypertension, BMI, time of SBP measurement, and study day. An interaction was identified by which the relationship

between SBP and 48-h prior AUC differed by study day. A multivariable logistic regression model for N/T on a given day included the following significant variables: age, study day, prior day occurrence of N/T, prior day AUC and study. PK-PD analyses for efficacy and safety, which demonstrated significant findings, will be useful to support BRI dose selection.

Author Disclosure Block:

S.M. Bhavnani: I. Research Relationship; Self; Cellceutix Corp. **J.P. Hammel:** I. Research Relationship; Self; Cellceutix Corp. **A. Forrest:** I. Research Relationship; Self; Cellceutix Corp. **S.A. VanWart:** I. Research Relationship; Self; Cellceutix Corp. **P. Sager:** C. Consultant; Self; Cellceutix Corp. **K.J. Tack:** C. Consultant; Self; Cellceutix Corp. **R.W. Scott:** C. Consultant; Self; Cellceutix Corp. **D.M. Jorgensen:** D. Employee; Self; Cellceutix Corp. K. Shareholder (excluding diversified mutual funds); Self; Cellceutix Corp. **P.G. Ambrose:** I. Research Relationship; Self; Cellceutix Corp.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:**Added Value of Next Generation Sequencing for MLST Analysis of a *Pneumocystis jirovecii* Pneumonia Outbreak****Author Block:**

E. Charpentier, C. Garnaud, C. Wintenberger, J. Rendu, P. Pavese, T. Drouet, C. Augier, P. Malvezzi, A. Thiébaud, J-B. Murat, M-R. Mallaret, O. Epaulard, **M. Cornet**, S. Larrat, D. Maubon; Grenoble Alpes Univ. Hosp., Grenoble, France

Abstract Body:

Multilocus Sequence Typing (MLST) is an accurate method for strain characterization. If Sanger sequencing has limitations, notably for subpopulation detection, Next Generation Sequencing (NGS) allows both majority and minority variant studies. As mixed strains co-infections are frequent in *Pneumocystis jirovecii* Pneumonia (PCP) we aimed to evaluate the added value of NGS compared to Sanger sequencing in an epidemiological MLST study of PCP. We compared NGS (GS junior +, Roche) and Sanger sequencing on samples from 32 PCP patients diagnosed at Grenoble Alpes University Hospital between 2012 and 2015. Among them, 13 cases corresponded to a putative outbreak among solid organ transplant (SOT) recipients between May 2014 and March 2015 and 19 were control patients with no epidemiological link. A three loci scheme was used for the MLST analysis (*MIT26S*, *CYTB* and *SOD*). New primers were designed to obtain 800 bp amplicons (GS +). Sanger and GS+ data were analyzed with SeqScape or AVA softwares respectively. As no previous report used 800 bp loci, genotypes were named as follows *MIT*: capital letter, *CYT*: number, *SOD*: lowercase. Among the 13 SOT patients, 6 shared the same major genotype: C2a. Transmission map confirmed the probable nosocomial PCP acquisition. The 800bp *MIT* sequence revealed two new SNPs (13505;13543) and a new MNP within an inverted repeat sequence (13554-13560). These new polymorphisms increased significantly the discriminatory power of the *MIT* locus. Proportion of mixed genotype infections was significantly higher with NGS analysis (65%) compared to Sanger (25%). NGS approach revealed infections with more than 3 subpopulations and the presence of ultra-minority strains (1%) among which the C2a genotype. As expected, in this specific context of PCP, NGS outperforms the Sanger approach in terms of subpopulation determination. Longer reads analysis revealed new SNPs of interest for

increased typing discrimination. These findings bring new insight for future epidemiological studies on this non cultivable opportunistic fungus.

Author Disclosure Block:

E. Charpentier: None. **C. Garnaud:** None. **C. Wintenberger:** None. **J. Rendu:** None. **P. Pavese:** None. **T. Drouet:** None. **C. Augier:** None. **P. Malvezzi:** None. **A. Thiébaud:** None. **J. Murat:** None. **M. Mallaret:** None. **O. Epaulard:** None. **M. Cornet:** None. **S. Larrat:** None. **D. Maubon:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

Repeated Outbreaks of *Pneumocystis jirovecii* Pneumonia with Different Genotypes in a Single Renal Transplant Center

Author Block:

A. Takahashi-Nakazato¹, N. Goto², K. Tsuchiya¹, H. Gatanaga¹, S. Oka¹; ¹Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan, ²Japanese Red Cross Nagoya Daini Hosp., Nagoya, Japan

Abstract Body:

Background: Outbreaks of *Pneumocystis jirovecii* pneumonia have been frequently reported for the past two decades. However, whether or not these outbreaks were caused by pathogenic *P. jirovecii* was not determined yet. In a renal transplant center, the first outbreak (33 cases) occurred in 2004-2008 (Transplantation 2009) and was settled with trimethoprim-sulfamethoxazole (ST) prophylaxis. Despite the prophylaxis, two new outbreaks occurred in 2010-2011 (3 cases) and 2013-2014 (10 cases). We analyzed sequences of *P. jirovecii* in the two outbreaks and compared with the previous one.**Methods:** Total DNA was extracted from bronchoalveolar lavage fluid. The internal transcribed spacer (ITS) 1 and 2 which contain region of the nuclear operon (5.8S) of *P. jirovecii* was amplified and sequenced. Genotype (GT) of *P. jirovecii* was determined based on the scores of combination of ITS1 and 2. Results of the alignment were then analyzed by the Clustal W program and the distance matrixes were generated by the neighbor-joining method. Phylogenetic analyses were conducted with Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6).**Results:** In the first outbreak, GT of *P. jirovecii* was Bi as previously reported. At that time, the same GT of *P. jirovecii* was identified from a mouth wash of hospital staff and a swab of out-patient environment. In contrast, two GTs of *P. jirovecii* were identified in the recent outbreaks; one was Eb in three cases in 2010- 2011, and the other was Ne in 10 cases in Nov, 2013- Dec, 2014. Notably, there was a 2-year interval between GT Eb and GT Ne outbreaks. The first surveillance of *P. jirovecii* carrier was carried out in 15 clinical staff members in Dec, 2014, detected GT Ne in one staff. The second surveillance was conducted three months later, resulted all negative. The results in a series of outbreaks strongly suggest the possibility of existence of the super-spreader rather than that of pathogenic *P.*

jirovecii for the outbreak in the susceptible hosts. **Conclusions:** This study documented that the three different GTs of *P. jirovecii* have caused each outbreak in a single renal transplant center for 10 years.

Author Disclosure Block:

A. Takahashi-Nakazato: None. **N. Goto:** None. **K. Tsuchiya:** None. **H. Gatanaga:** None. **S. Oka:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

Hospital Outbreak and Post-Outbreak Investigation of Legionnaires' Disease (LD) Using Whole Genome Sequencing (WGS)

Author Block:

B. Decker¹, L. Chen², A. Sonel¹, M. Nguyen³, B. Kreiswirth², C. Clancy¹; ¹VA Pittsburgh, Pittsburgh, PA, ²PHRI Rutgers NJ Med, Newark, NJ, ³Univ Pittsburgh, Pittsburgh, PA

Abstract Body:

VAP had an LD outbreak due to *L. pneumophila* (Lp) in 2011-12, which ended after instituting water system (WS) hyperchlorination. Sporadic LD cases were diagnosed thereafter (none were definite hospital-acquired). WS culture positivity for Legionella was reduced to $\leq 2\%$. We performed Illumina MiSeq WGS on 47 Legionella isolates that were recovered from patients (pts), pts' homes or VAP WS (3 campuses) in 1982-2015, and determined phylogenetic relationships with 70 published genomes by comparing single nucleotide polymorphisms (SNPs). Pt and WS isolates included 3 Lp subsp. (*pneumophila* (5 clades), *pascullei* (2 clades), *fraseri*). WS *L. longbeachae* (Ll) were also recovered. Isolates defined clades that were distinct from published genomes. Identical Lp *pascullei* were recovered from pts and WS in 2012 (0-2 SNPs). Outbreak isolates differed from 1982 WS isolates by ~ 2300 SNPs. SNPs were concentrated in 3 defined chromosomal regions consistent with recombination, the largest of which (~ 160 kb) included the *lps* locus and resulted in a switch from serotype 5 to 1. One 2012 Lp *pascullei* pt isolate was distinct from outbreak isolates, but identical to an isolate from the pt's home. Data on non-outbreak isolates re-iterated several themes: a) a number of identical Lp isolates were recovered from pts' samples, homes and WS from different campuses; b) identical Lp isolates persisted in the WS for ≥ 8 years; c) other Lp isolates evolved by mutations and chromosomal recombination; d) various closely-related isolates of Ll, usually a soil organism, were recovered from WS at different campuses; and e) sequence based typing, in general, was a useful proxy for WGS. Our LD outbreak was caused by Lp *pascullei* that evolved over 30 years from an endemic WS isolate. Numerous disease-causing Legionella isolates persisted for years within the hospital WS despite remediation, many of which were genetically indistinguishable from isolates at

other campuses and in the community. A lack of evolution in some isolates may be consistent with persistence or latency within sequestered sites such as biofilms. Legionella clonality in a geographic region may limit WGS as a lone investigative tool. Our findings emphasize a need for classical epidemiological approaches to enhance molecular data.

Author Disclosure Block:

B. Decker: None. **L. Chen:** None. **A. Sonel:** None. **M. Nguyen:** None. **B. Kreiswirth:** None. **C. Clancy:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

Outbreaks and Pseudo Outbreaks Produced by *Achromobacter* Spp. in Two Hospitals in Argentina

Author Block:

M. Papalia¹, G. Greco², V. Alexander², L. Clara², M. Vallejos³, G. Gutkind¹, **M. Radice¹**;
¹Univ Buenos Aires, CABA, Argentina, ²Hosp Privado, CABA, Argentina, ³Hosp Privado, Bs As, Argentina

Abstract Body:

Background: *Achromobacter* spp. are non-frequent nosocomial and community pathogens able to cause infections in immunocompromised patients. Even if only very recently the different species within the genus started to be effectively discriminated, *A. xylosoxidans* constitutes the most frequently isolated species among them, and has already been implicated in nosocomial outbreaks associated with contaminated aqueous solutions. The aim of this study was to characterize the microorganisms from 2 independent nosocomial outbreaks or pseudo outbreaks that occurred in Buenos Aires, in 2015. **Methods:** We received 17 *Achromobacter* spp. isolates recovered at 2 hospitals (H) in Buenos Aires. Ten isolates were recovered at H1 (7 from clinically relevant samples and 3 from intravenous injectable vials). Seven were isolated at H2 from clinically relevant samples that were interpreted in most cases as contamination (was not present in gram stain, and grew only in broth with late development). Species identification was performed by *nrdA* amplification and sequencing, and MLST. RAPD-PCR (using the previously described primer 270) and *XbaI*-PFGE were conducted to evaluate the relationship among these isolates. **Results:** All isolates from H1 were identified as *Achromobacter* genogroup 20. On the basis of PFGE typing, the injectable vial isolates and 5 from clinical samples displayed identical restriction patterns, and the remaining 2 were closely related, suggesting an association between the outbreak and the microorganisms recovered from the vials. In H2: Except for one (identified as *A. xylosoxidans*) all were also identified as *Achromobacter* genogroup 20. Three of them displayed identical PFGE pattern, and another a very similar pattern, possibly related to the other 3. RAPD-PCR technique was not useful to look for genetic relationships among the isolates as it displayed a low discriminatory power. **Conclusion:** *XbaI*-PFGE

genotyping method proved to be useful to investigate clonal relationship among *Achromobacter* spp. isolates, showing a high discriminatory power and reproducibility. This constitutes the first report of *Achromobacter* genogroup 20 nosocomial outbreaks or pseudo outbreaks, showing that other *Achromobacter* species than *A. xylosoxidans* may be implicated.

Author Disclosure Block:

M. Papalia: None. **G. Greco:** None. **V. Alexander:** None. **L. Clara:** None. **M. Vallejos:** None. **G. Gutkind:** None. **M. Radice:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

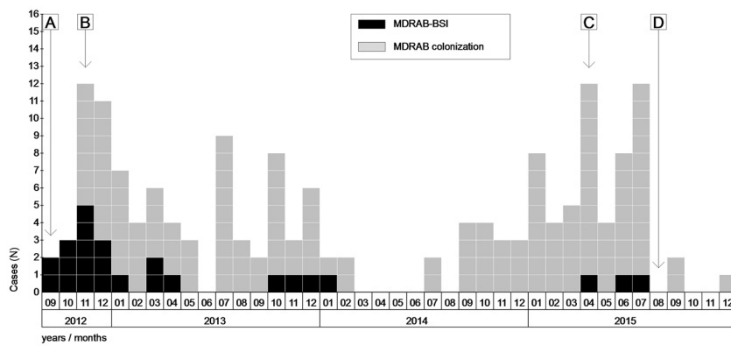
Containment of *Acinetobacter baumannii* outbreak in Latvian Neonatal Intensive Care Unit

Author Block:

A. Gramatniece¹, M. Saule¹, I. Silamikelis², E. Dimina¹, I. Zahare³, D. Fridmanis², I. Zahare¹, J. Klovins², U. Dumpis¹; ¹Pauls Stradins Clinical Univ. Hosp., Riga, Latvia, ²Latvian BioMed. Res. and Study Ctr., Riga, Latvia, ³Children's Clinical Univ. Hosp., Riga, Latvia

Abstract Body:

Neonatal Intensive Care Unit (NICU) patients have an increased risk for multidrug resistant *A. baumannii* (MDRAB) healthcare associated infections (HAIs). We implemented HAI surveillance system in Stradins University Hospital to monitor the HAI rates, detected MDRAB outbreak and investigated it using whole genome sequencing. All neonates admitted to NICU from 09.01.2012 to 12.31.2015 were included in the surveillance. Case-control study was performed to identify the risk factors for acquisition of MDRAB. Whole genome sequencing was used to differentiate phenotypically similar strains from adult ICU and NICU. The surveillance included 989 newborns. Significant ($p < 0.05$) risk factors for acquisition of MDRAB blood stream infection (BSI) were mechanical ventilation, use of central venous catheter and nasogastric tube. Phylogenetic analysis of full genome sequences showed that isolates from adult ICU and NICU were distinct, ruling out inter-ward transmission. Introduction of surveillance (Fig 1, A) and infection control (B) decreased the incidence of MDRAB. High colonization rates reappeared in April 2015 (C) and ward was closed for 2 weeks (D) for profound cleaning after which no MDRAB-BSI cases were registered. Complete renovation was not possible due to budget constraints. **Figure 1**



Our findings indicate that surveillance with additional infection control measures significantly reduced HABSI rates and largely contained an outbreak of MDRAB even if an environmental source was not identified. We have shown that whole genome sequencing can provide valuable information on MDRA outbreak epidemiology.

Author Disclosure Block:

A. Gramatiece: None. **M. Saule:** None. **I. Silamikelis:** None. **E. Dimina:** None. **I. Zahare:** None. **D. Fridmanis:** None. **I. Zahare:** None. **J. Klovins:** None. **U. Dumpis:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

Survey of an Outbreak of Vancomycin-resistant Enterococci via Fourier-transform Infrared Spectroscopy Compared to Molecular Biology Methods

Author Block:

N. Mauder¹, B. Müller², **M. Kostrzewa**¹, M. Wasner²; ¹Bruker Daltonik GmbH, Bremen, Germany, ²MVZ Labor Dessau GmbH, Dessau-Rosslau, Germany

Abstract Body:

Background: Vancomycin resistant enterococci (VRE) are an emerging threat and became a major problem in the United States and Europe, i.e. because VRE possess an intrinsic resistance to many antibiotics commonly used. The high discriminatory power of Fourier-transform infrared spectroscopy (FTIR) has been shown for a variety of microorganisms. To address the needs for an economical and robust method for strain typing to aid clinical epidemiology and surveillance this method was retrospectively used to analyze the occurrence of VRE in one hospital. **Methods:** 248 VRE isolates were collected in a clinical environment. Every isolate was checked for presence of *hyl* and *esp* genes, and the resistance type was determined. About fifty percent of the isolates were also typed by MLST. Three independent FTIR measurements were performed. Each time isolates were cultivated on sheep blood agar at 37°C for 24 h. An inoculum of the confluent part of a pure culture was suspended in 70% ethanol and spotted on silicon sample plates as quadruplicates. After drying the spots were measured using a TENSOR 27 plus HTS-XT module (Bruker Optics, Ettlingen, Germany) in transmission mode. Second derivative spectra were windowed to 800-1300 /cm, vector-normalized and cluster-analyzed. Specificity of cluster assignment was checked via cross-validation. **Results:** 10 different MLS types were identified. Regarding the genes *hyl*, *esp*, and the resistance type these MLS types were assumed to be of different homogeneity. The MLS type of the presumed outbreak cluster was very homogeneous with a Simpson's diversity index 1-D of 0.07, while other MLS types were quite heterogeneous and reached a value of up to 0.75. IR clustered the VRE isolates into 13 groups including few subgroups. 90% of the outbreak MLS type isolates are found in an IR cluster only containing this one MLS type. 10% cluster into two other IR clusters, one containing rare *esp* negative isolates of this MLS type. **Conclusions:** Infrared spectroscopy proved to be a

powerful method for typing of enterococci showing a great congruence with MLS typing. Some discrepancies between MLS types and IR groups can be explained by heterogeneity within the MLS types. After all FTIR might also recognize differences not identified by the tested molecular markers.

Author Disclosure Block:

N. Mauder: D. Employee; Self; Bruker Daltonik GmbH. **B. Müller:** D. Employee; Self; MVZ Labor Dessau GmbH. **M. Kostrzewa:** D. Employee; Self; Bruker Daltonik GmbH. **M. Wasner:** D. Employee; Self; MVZ Labor Dessau GmbH.

Session Number:

248

Session Title:**Nosocomial Outbreaks****Publishing Title:****Whole Genome Sequencing to Track Outbreaks of Vancomycin-Resistant Enterococci in Real-Time****Author Block:**

A. C. Ong, **P. Mc Gann**, R. Maybank, E. Snesrud, Y. I. Kwak, R. J. Clifford, M. Hinkle, E. Lesho; WRAIR, Silver Spring, MD

Abstract Body:

Although whole genome sequencing (WGS) is increasingly employed, obstacles remain, especially for clinical laboratories needing urgent turnaround times (TAT) to support infection control. Here, NGS was used to investigate 2 potential outbreaks of vancomycin resistant *Enterococcus faecium* (VRE), in real-time, enabling an accurate assessment of TAT. Two suspected outbreaks of VRE at separate medical facilities were reported. The first involved 3 VRE cultured from 3 patients housed at the same ICU over 5 weeks. The second involved 5 VRE cultured from 5 patients over 4 weeks. All isolates were sequenced on an Illumina Miseq desktop sequencer. Including overnight culturing, a TAT of just 48.5 hours for a report was achievable. WGS provided a wealth of information that was not obtainable using other typing methods, including refining the source of transmission and providing insights into antibiotic susceptibilities from WGS data. Analysis of the first outbreak indicated that all 3 isolates were indistinguishable except for one SNP, suggesting nosocomial transmission. Furthermore, SNP-based analysis indicated that transmission most likely occurred in two separate events from patient #1 to patient #2 and #3, rather than a linear transmission from patient #1 to patient #2, and then to patient #3, as was suspected from the timeframe of the outbreak. In contrast, all 5 isolates from outbreak 2 were separated by at least 20 SNP's, suggesting no nosocomial transmission had occurred. Notably, PFGE was unable to distinguish between 2 strains, which would have resulted in outbreak protocols being implemented unnecessarily. In both outbreaks, there was 100% concordance between antibiotic resistance gene content and phenotype, though point mutations in genes encoding drug targets were essential in correlating resistance to the β -lactams and fluoroquinolones. We demonstrate that clinically relevant TAT's, particularly for priority requests, are achievable using WGS in the clinical setting and can provide an unprecedented level of resolution for outbreak

investigations. Furthermore, we highlight the pitfalls associated with predicting antibiotic susceptibilities from gene content alone.

Author Disclosure Block:

A.C. Ong: None. **P. Mc Gann:** None. **R. Maybank:** None. **E. Snesrud:** None. **Y.I. Kwak:** None. **R.J. Clifford:** None. **M. Hinkle:** None. **E. Lesho:** None.

Session Number:

248

Session Title:

Nosocomial Outbreaks

Publishing Title:

Outbreak Of *klebsiella Pneumonia* Vim St-14 In Northern Ireland

Author Block:

M. Pathiraja¹, E. Mckle¹, N. Damani¹, E. Porter¹, A. McCorry¹, K. Hopkins², J. Turton²;
¹Craigavon Area Hosp, Armagh, United Kingdom, ²Publ. Hlth.England, London, United Kingdom

Abstract Body:

Until 2014 all cases of carbapenemase-producing Enterobacteriaceae in our acute hospital were sporadic cases linked to travel or to other healthcare facility outbreaks. Clinical isolates from three patients in a double sided 65 bed Surgical Unit, including from a bacteraemia, were found to be *K. pneumoniae* ST14 producing VIM-2 using molecular methods. The index case had no known link with this strain in the UK or abroad and had less than 48 h prior admission to a tertiary unit in Belfast. Subsequent two cases were identified over 15 months accounting for separate epidemiologically linked transmissions (Fig.1). Both cases were missed on initial screening, due to insensitivity of the method used and being discharged, respectively. Outbreak investigation revealed that although all cases were linked to the Surgical unit, none of the patients were in direct contact in a single bay. The transmission was most likely to have occurred across isolation rooms and across separate surgical wards due to sharing of staff, beds, mattresses etc. As a part of control measures multiple screening rounds and environmental samples were done including re-admissions to identify those who were exposed. Over 500 patients were screened with no new cases detected. Non-compliance with infection prevention control (IPC) practices and overuse of broad-spectrum antibiotics especially carbapenems were identified. Contact IPC measures and review of antibiotic prescribing with restriction of carbapenems, audits and feedback were implemented. A six month follow up found no further cases. This outbreak of *K. pneumoniae* ST-14 carrying VIM-2 was successfully controlled by implementation of contact IPC precautions, screening and antibiotic stewardship program.

Index	Ward 4S	VIM KP bacteraemia	Tigercyc line, source control	recovery					
		Contact period							
Case 2	Ward 4 S		Screen neg	home		Ward 4N		VIM KP abdominal pus drainage	
							Contact period		
Case 3						Ward 4S		No screen	VIM KP abdominal pus drainage
	31/03/14	09/04/14	16/04/14	19/04/14		04/03/15	09/03/15	27/03/15	07/07/15
	Fig 1. Timeline of outbreak								

Author Disclosure Block:

M. Pathiraja: None. **E. Mckle:** None. **N. Damani:** None. **E. Porter:** None. **A. McCorry:** None. **K. Hopkins:** None. **J. Turton:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Reduction In Unnecessary Antipseudomonal Antibiotics Through Implementation Of A Risk Stratification Guideline For Community Acquired Pneumonia

Author Block:

R. Feldman, S. Revolinski, C. Dang, **A. Huang**; Froedtert & The Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: The IDSA guidelines for treatment of healthcare associated pneumonia (HCAP) recommend treatment with an antipseudomonal antibiotic (APabx) for patients with healthcare exposure presenting from the community that may be at risk for multi drug resistant organisms (MDRO), however, this strategy has been shown to be a poor predictor of MDRO pneumonia. We devised a treatment algorithm to increase specificity of identifying patients at risk for MDRO community acquired pneumonia (CAP) utilizing stratification of risk. **Methods:** A treatment guideline and order set for CAP were implemented at an academic medical center. Risk factors were chosen using national epidemiologic data and treatment was stratified by quantity of risk factors and severity of illness. A single-center, pre and post intervention analysis was conducted on all patients treated via IDSA guidelines from Dec 2013 - Feb 2014 and all patients treated according to the new institutional guidelines from Dec 2014- Feb 2015. The primary outcome was need for antibiotic escalation after 24 hours of empiric therapy. Secondary outcomes included 30-day mortality, 30-day readmission, and APabx use. **Results:** 444 patients were evaluated: 186 patients pre and 258 post intervention. There was more influenza and less exacerbation of chronic lung disease in the post-intervention group. There were no statistically significant differences in the pre and post intervention group in need for antibiotic escalation (6.5 vs. 9.3%, $p=0.28$), 30-day mortality (7.5 vs. 6.2%, $p=0.58$), or 30-day readmission (16.1 vs. 12.0%, $p=0.22$). A 12% reduction in APabx occurred in patients treated for non-severe CAP. Patients receiving double coverage for pseudomonas was reduced from 36% to 9.2% ($p<0.01$). The institutional algorithm exhibited better specificity and predictive values for MDRO detection than IDSA guidelines and maintained identical sensitivity. The strongest risk factors for prediction of resistant organism were history of resistant pathogen (odds ratio [OR] 4.4, 95% CI 1.08-18.3) and

need for ICU admission (OR 4.07,95% CI 1.21-13.71).**Conclusions:** Utilization of a CAP treatment algorithm based on quantity of risk factors and severity of illness reduced APabx use without negatively impacting treatment outcomes.

Author Disclosure Block:

R. Feldman: None. **S. Revolinski:** None. **C. Dang:** None. **A. Huang:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Evaluation on the Use of Antibiotics for Surgical Prophylaxis among Patients Receiving Antibiotics for an Active Infection

Author Block:

C. Reuter, T. Hudson, C. Nguyen, J. Li; Ochsner Med. Ctr., New Orleans, LA

Abstract Body:

Background: Patients treated for an active or suspected infection often receive additional prophylactic antibiotics prior to surgical procedures, which may or may not be necessary. The purpose of this study is to determine the appropriateness of antibiotics for surgical prophylaxis among patients already receiving antibiotics for an active or suspected infection. **Methods:** This was a single-center, retrospective cohort study that included patients greater than 18 years old who were receiving antibiotics for an active infection and had surgery between January through March 2015. Appropriateness of overall antibiotic coverage was defined based on either gaps or redundancy in spectrum of coverage. Appropriate spectrum of coverage was based on the type of surgery and guideline recommendations. Microbiologic history within 90 days was also evaluated to determine if additional coverage was warranted (e.g. vancomycin for methicillin-resistant *Staphylococcus aureus*). Outcomes comparing patients receiving inappropriate coverage (IC) versus appropriate coverage (AC) included 30-day readmission, hospital length of stay (LOS), 14-day all-cause mortality, and antibiotic-related adverse events (AEs). **Results:** This study included 499 patients with an overall rate of appropriate antibiotic coverage of 65%. Baseline characteristics of the IC (n=173) and AC (n=326) groups were generally similar, including age (mean = 56 years), comorbidities, and microbiologic history. The IC group had more clean surgeries compared to the AC group (46% vs. 28%, p<0.001). There was no difference between the IC and AC groups in 30-day readmission (32% vs. 31%, p=0.96), LOS (24 vs 24 days, p=0.98), 14-day all-cause mortality (10% vs. 6%, p=0.12); Rate of AEs was greater in the IC group (3% vs. 0.6%, p=0.05). **Conclusions:** Approximately 35% of prophylactic antibiotics were prescribed inappropriately, with an associated increase in adverse events, but no difference mortality or readmission.

Author Disclosure Block:

C. Reuter: None. **T. Hudson:** None. **C. Nguyen:** None. **J. Li:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Impact of an Antimicrobial Stewardship Program on Antibiotic Resistance: A Patient-Level Perspective

Author Block:

S. S. L. TANG, W. H. L. LEE, J. Teo, T-P. Lim, M. P. CHLEBICKI, A. L. KWA;
SINGAPORE GENERAL Hosp., SINGAPORE, Singapore

Abstract Body:

Background: Antimicrobial Stewardship Programs (ASP) seek to promote appropriate antibiotic (abx) use & slow spread of resistance. Institutional-level measure of ASP effectiveness in reducing abx resistance is complex & subject to influence by various external factors. This study aims to quantify impact of ASP interventions on subsequent development of infections caused by organisms that are at least multidrug-resistant (MDR) from a patient-level perspective. **Methods:** In our hospital, all patients prescribed parenteral ciprofloxacin, piperacillin-tazobactam (PTZ) or a carbapenem undergo ASP review using the prospective audit-feedback mechanism. This is a retrospective study of all patients with ASP intervention to narrow empirical cover, de-escalate or discontinue abx between January 2014 and June 2015, & had 14-day reinfection, 30-day infection-related mortality or hospital readmission. We compared the incidence of clinically significant MDR Gram-negative bacteria & *Clostridium difficile* infection between those with intervention accepted (recommendation adopted within 48hrs of intervention) & the rejected group. **Results:** A total of 324 patients were included; their demographics & clinical characteristics were comparable. PTZ was most commonly prescribed (n=239, 74%), followed by carbapenems (n=78, 24%). Intervention to discontinue abx, de-escalate & narrow empirical cover was made in 215 (66%), 57 (18%) & 52 (16%) patients respectively. The overall intervention acceptance rate was 70% (n=228). Subsequent infection by MDR organism was approximately 5-fold more frequent when ASP interventions were rejected (9% vs. 2%, p=0.003); this difference was most apparent with *Clostridium difficile* infection, which demonstrated a statistical trend (0.4% vs. 3.2%, p=0.08). Patients in the accepted group also had significantly shorter duration of abx therapy (3 vs. 6 days, p<0.001) & overall length of hospital stay (15 vs. 21 days, p=0.03). There were no significant differences in reinfection, infection-related mortality

& readmission rates in both groups. **Conclusions:** ASP recommendations are protective against subsequent infections caused by MDR organisms & are associated with shorter duration of abx therapy & hospital stay, without compromise in patient safety outcomes.

Author Disclosure Block:

S.S.L. Tang: None. **W.H.L. Lee:** None. **J. Teo:** None. **T. Lim:** None. **M.P. Chlebicki:** None. **A.L. Kwa:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Early Experience with T2candida and Antifungal Utilization at a Large Community Health System

Author Block:

M. E. Patch¹, K. Napholz², F. Cioffi¹, M. Saunders¹, S. Estrada¹; ¹Lee Mem. Hlth.System, Fort Myers, FL, ²Univ. of Toledo, Toledo, OH

Abstract Body:

Background: T2Candida is a qualitative rapid diagnostic test for the identification of candidemia and detects the top 5 species of *Candida* from whole blood samples in 3-5 hours. In the United States, Candidemia accounts for the 4th leading cause of nosocomial bloodstream infections and *Candida* species are the most common cause of invasive fungal infections. The mortality rate for candidemia is estimated between 15-25% and as high as 47% and is estimated to add around \$40,000 in hospital costs. Early addition of appropriate antifungals in patients with candidemia as well as discontinuation of unneeded therapy can improve outcomes by decreasing mortality and adverse events.**Methods:** This study was conducted as a 2 phase retrospective chart review. Phase 1 was completed from September to November 2014 to describe outcomes of patients with candidemia including time to initiation of appropriate antifungal therapy. Phase 2 was completed from September to November 2015 to describe the impact of T2Candida test on timing of initiation of appropriate antifungals for patients with candidemia.**Results:** The T2Candida panel was positive in 5/101 patients, 4 *C. albicans*/*C. tropicalis* and 1 *C. parapsilosis*. Patients with a positive result had initiation of appropriate antifungal coverage in an average of 5 hours compared to 40 hours for candidemia patients prior to T2Candida implementation. Antifungal therapy was not initiated in 36 patients and stopped after one dose in 15 patients with negative T2Candida results. Patients with continued therapy after a negative result received an average of 3 days of antifungals compared to an average of 9 days of therapy for patients receiving micafungin prior to T2Candida implementation.**Conclusions:** A positive T2Candida result allows the provider to rapidly ensure a patient is treated with appropriate antifungals while a negative T2Candida result, with a 99.4% specificity, will enable

providers to avoid or discontinue unnecessary antifungals. These results are predicted to reduce mortality as well as toxicity, resistance, and cost associated with antifungal use.

Author Disclosure Block:

M.E. Patch: None. **K. Napholz:** None. **F. Cioffi:** None. **M. Saunders:** None. **S. Estrada:** C. Consultant; Self; T2 Biosystems.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Do European Medical Students Feel Prepared to Prescribe Antibiotics Responsibly?

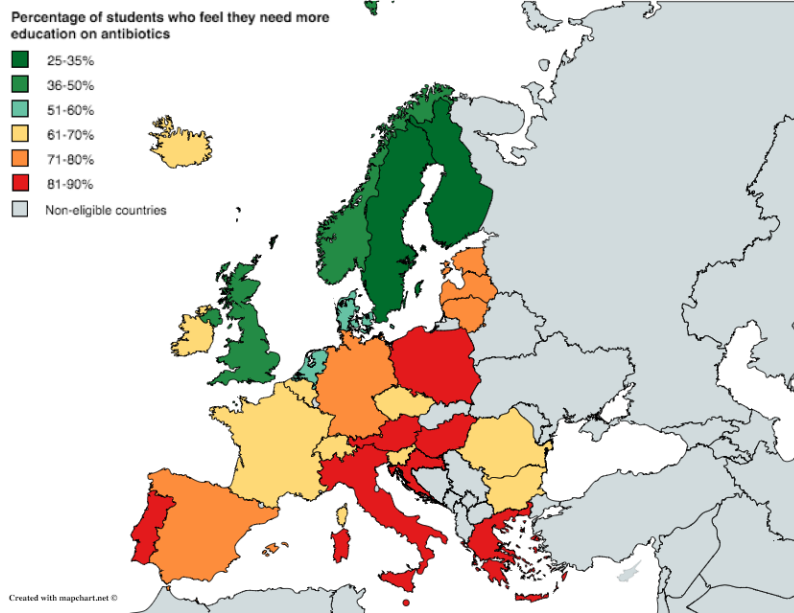
Author Block:

O. J. Dyar¹, D. Nathwani², I. C. Gyssens³, C. Stålsby Lundborg¹, C. Pulcini⁴, ESGAP; ¹Karolinska Inst.t, Stockholm, Sweden, ²Ninewells Hosp. and Med. Sch., Dundee, United Kingdom, ³Radboud Univ. Med. Ctr., Nijmegen, Netherlands, ⁴Université de Lorraine, Nancy, France

Abstract Body:

Background: We conducted a Europe-wide study to assess how prepared medical students felt on a range of topics related to antibiotic use. **Methods:** All final year students at European schools were eligible to complete an online survey developed by international experts in antibiotic stewardship. Email invitations were sent through country coordinators, with the survey accessible from 16/1-31/12 2015. **Results:** 7398 students responded from all 29 countries (22% response rate across schools). Over 90% of students felt sufficiently prepared to recognise clinical signs of infection and interpret biochemical markers of inflammation. Over half did not feel prepared to use surgical antibiotic prophylaxis principles (51%), to decide the shortest adequate duration of therapy (53%), nor to challenge senior doctors' prescribing when they felt antibiotics were not indicated (50%). Students thought the most effective teaching methods were discussions of clinical cases, small-groups, infectious diseases placements and peer teaching. Only 33% of students felt they had received sufficient teaching on antibiotics to practice as a doctor. Students in Southern and Eastern Europe expressed higher needs for further education than those in Northern Europe ($p < 0.01$). **Conclusions:** In the largest ever study of student preparedness on any topic, we found that most final year European medical students still feel they need more education on antibiotics. High value teaching methods were identified, as well as current areas of curriculum strength and weakness.

The results will be used throughout Europe to support improvements in education.



Author Disclosure Block:

O.J. Dyar: None. **D. Nathwani:** None. **I.C. Gyssens:** None. **C. Stålsby Lundborg:** None. **C. Pulcini:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Probability of Target Attainment and Clinical Outcome of Alternative Dosed Cefepime

Author Block:

R. L. AKINS, M. D. Smith; Methodist Charlton Med. Ctr., Dallas, TX

Abstract Body:

Background: Alternative dosed β -lactams have shown to improve probability of target attainment (PTA) based on pharmacodynamics $60\% fT > MIC$; thus improve clinical success and lower mortality. Modeling has demonstrated alternative dosed cefepime (FEP) may provide more suitable PTA, particularly in less susceptible pathogens ($MIC \geq 4mg/L$). Our institution has employed alternative FEP dosing based on infectious indication. Objective was to determine PTA of each alternative regimen and clinical outcome. **Methods:** All patients receiving $\geq 72h$ FEP from Jan 1-Dec 31, 2015 were included. Following data was collected: + culture and organism, MIC, clinical indication, dosing, escalation of therapy (failure), adverse events and mortality. PTA was calculated based on population FEP pharmacokinetics and MICs. Following alternative dosing

Indication	Creatinine Clearance (CrCl) Cockcroft-Gault (ml/min):					
	≥ 50	30-49	10-29	<10/Dialysis	CVVHDF (1-1.5L/h)	CVVHDF (2-2.5L/h)
CNS Infections, Neutropenic Fever, Patients weight ≥ 120 kg	2g Q8h	2g Q12h	1g Q8h	2g Q24h	2g Q12h	2g Q8h
Severe Sepsis /Septic Shock, HCAP/HAP/VAP, Osteomyelitis, Intra-abdominal Infection, Pyelonephritis, Bacteremia, SSTI	1g Q6h	1g Q8h	1g Q12h	1g Q24h	1g Q8h	1g Q6h
UTI	1g Q8h	1g Q12h	1g Q24h	1g Q24h	1g Q8h	1g Q6h

strategy was employed:

Results: Total of 334 patients received FEP $\geq 72h$. 101 had + cultures (30% with $MIC \geq 4mg/L$). *P. aeruginosa* and *E. coli* were most common organisms with MIC range $\leq 2 - \geq 16mg/L$ for each. PTA of 100% was obtained for all alternative FEP regimens except: $CrCl \geq 50$ - 2gmQ8h and 1gmQ6h ($MIC \geq 16mg/L$) and 1gmQ8h ($MIC \geq 8mg/L$); $CrCl$ 30-49 - 1gmQ12h ($MIC \geq 16mg/L$); $CrCl$ 10-29 - 1gmQ24h ($MIC \geq 16mg/L$). Primary

indication was HCAP followed by SSTI and severe sepsis. 4% were considered failure secondary to therapy escalation. Mortality was 4.8% overall with no significant difference ($p>0.05$) despite PTA (100%) in 5/16 patients with + cultures. No adverse events were noted. **Conclusions:** Clinical success was achieved in 96% patients utilizing alternative dosed FEP. Alternative dosing achieved 100% PTA for all organisms with MIC ≤ 8 mg/L in all but 1 regimen. No adverse events occurred with alternative dosing. Mortality rates were low with no deaths in patients with PTA less than 100% (range 51-86%).

Author Disclosure Block:

R.L. Akins: F. Investigator; Self; Merck, Theravance Biopharma. **M.D. Smith:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Improved Antibiotic Stewardship and Treatment of *Staphylococcus aureus* Bacteremia through Implementation of a Nucleic Acid Microarray Identification System and Lean Process Management

Author Block:

H. L. Cox, M. M. Richey, A. J. Mathers, W. M. Novicoff, **J. C. Eby**; Univ. of Virginia, Charlottesville, VA

Abstract Body:

Background: Identification of bacteria and their resistance gene determinants by nucleic acid-based rapid diagnostic systems has the potential to improve management of *Staphylococcus aureus* bacteremia (SAB). In order to realize this potential, we implemented a nucleic acid microarray (NAM) for detection of *S. aureus* in blood cultures in conjunction with a quality improvement (QI) bundle. **Methods:** Clinical outcomes for patients with SAB were compared before and after initiation of the QI bundle and NAM. The bundle included mandatory Infectious Diseases (ID) consultation. In patients with SAB identified by NAM, ID consultation was triggered directly from the microbiology laboratory by the positive NAM result, reducing waste in the consultation process. A standard response by ID consultants, and an educational program for staff were also included in the bundle. All patients ≥ 18 years admitted to our single academic healthcare facility with SAB during the pre- and post-QI/NAM period were included in the evaluation (N=234). **Results:** Charts from 107 patients before the change and 126 patients after the change were reviewed for clinical information. ID consultation occurred for 85% of patients with SAB pre-intervention and 97% post-intervention ($p=.001$). The time to initiation of first line antibiotic or appropriately chosen second line antibiotic after notification of a positive blood culture decreased by a mean of 17.3 hours (95% CI, 26.3 to 8.3 hrs, $p<.001$) with implementation of NAM and the QI bundle. Time to nafcillin or ceftazolin for patients with methicillin-sensitive *S. aureus* decreased by a mean of 31.3 hours (95% CI, 46.5 to 16.1 hours, $p<.001$). In-hospital mortality was unchanged by the intervention. **Conclusions:** Institution of a streamlined and standardized response to SAB, in conjunction with microarray diagnostic technology, resulted in improved antibiotic

stewardship and increased the involvement of ID consultants in care. We have started to extend this program to other gram positive organisms as well.

Author Disclosure Block:

H.L. Cox: None. **M.M. Richey:** None. **A.J. Mathers:** None. **W.M. Novicoff:** None. **J.C. Eby:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Molecular Screening for Esbl and Carbapenemase During a Prevalence Study in Two Dutch Hospitals

Author Block:

I. O. M. Op den Buijs, R. T. J. M. Roymans, J. T. W. Nijs, L. van Dommelen, I. T. M. A. Overdeest, J. H. B. van de Bovenkamp; PAMM, Veldhoven, Netherlands

Abstract Body:

Background: Globally, the prevalence of extended spectrum β -lactamase (ESBL) and carbapenemase producing *Enterobacteriaceae* (CPE) has been rising. This study describes the comparison of selective culture and molecular testing for ESBL colonization using the BD MAX system. Furthermore, screening for CPE using qPCR is described. **Methods:** During a period of one month, rectal swabs were taken from 479 patients as part of a cross-sectional prevalence survey. These swabs were tested in ESBL and CPE qPCR using the Check-Direct ESBL and CPE screen qPCR kits on the BD MAX system. These kits can detect the most common ESBL and CPE resistance genes (CTX-M1, CTX-M2, CTX-M9, SHV, KPC, VIM, OXA-48, NDM, respectively). Also, all samples were cultured for ESBL. For CPE, only CPE qPCR positive samples were cultured. ESBL and CPE cultures were performed using a selective broth followed by a subculture on a selective agar. Bacteria suspected for ESBL or CPE were confirmed for ESBL or CPE by qPCR. Sensitivity, specificity, positive, and negative predictive value and Cohen's Kappa coefficient were calculated for qPCR. Selective culture is used as the gold standard for the analyses. **Results:** Out of 479 samples, 3% was inhibited in either the ESBL (n=6) or CPE (n=9) qPCR. These samples were excluded from the analyses. Of the remaining ESBL samples, 36 were found positive by culture, and 42 by qPCR. The additional ESBL positives found by qPCR had Cq values between 23 and 43. All discrepant samples were retested in the Check-Direct ESBL screen assay. Results after discrepant analysis show a sensitivity, specificity, positive and negative predictive value of 97.3%, 98.6%, 85.7% and 99.8%, respectively. The Kappa coefficient was calculated and resulted in an inter-rater agreement of 0.9. A total of eight CPE positives were found in qPCR with Cq values between 39 and 49. None of these CPE's could be cultured. **Conclusions:** Our study showed a very good strength of agreement between

qPCR and culture for ESBL screening (Kappa coefficient of 0.9). Molecular screening is less laborious than culture, and it has a high NPV of 99.8%. This makes the Check-Direct ESBL screen kit a promising pre-screening method for ESBL directly from clinical samples. The Check-direct CPE screen kit needs more investigation.

Author Disclosure Block:

I.O.M. Op den Buijs: None. **R.T.J.M. Roymans:** None. **J.T.W. Nijs:** None. **L. van Dommelen:** None. **I.T.M.A. Overdeest:** None. **J.H.B. van de Bovenkamp:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Emergence and Vanishment of Multidrug-Resistant *Acinetobacter baumannii* on Healthy Youngsters Concurrently Burned in a Dust Explosion in Taiwan: The Implication for Antimicrobial Stewardship

Author Block:

S-S. Shie, P-Y. Huang, J-J. Ye, Y-M. Wu, T-S. Wu, T-L. Wu, S-S. Chuang, M-H. Cheng, C-T. Huang; Chang Gung Mem. Hosp., Taoyuan, Taiwan

Abstract Body:

Background: Carbapenem has been associated with the occurrence of multidrug resistant *Acinetobacter baumannii* (MDRAB). The relationship between discontinued use of carbapenem and the clearance of MDRAB is not known. We sought to determine whether carbapenem exposure is associated with acquisition and clearance of MDRAB. **Methods:** A retrospective study was conducted at two intensive care units of a tertiary medical center exclusively for healthy youngsters concurrently burned by a dust explosion in Taiwan on June 27, 2015. More than 400 healthy youngsters were injured by the accident. Forty-two of them were admitted to Chang Gung Memorial Hospital Lin-Kou Medical Center. Cases were the patients with MDRAB detected from any sites during the first month of admission. Controls were those never with MDRAB isolated in the same period. Use of carbapenem and other antimicrobials were compared by days of therapy (DOTs) per 1,000 patient-days (DOTs/1,000PD). Antimicrobial use was also determined and correlated with the clearance of MDRAB in the case patients. Median-unbiased estimation and mid-p exact confidence intervals were used for statistical analysis.

Results: A total of 42 patients were studied. MDRAB developed in 9/42 (21%). Among these healthy youngsters, there was no difference in clinical characteristics. They were all seriously burned of comparable disease severity. While use of carbapenem in the cases was significantly higher than that in the controls (652 DOTs/1,000PD vs. 409 DOTs/1,000PD, $P < 0.001$), use of beta-lactam in the cases was significantly lower than that in the control (213 DOTs/1,000PD vs 436 DOTs/PD, $P < 0.001$). For the cases, clearance of MDRAB was associated with lower use of carbapenem (449 DOTs/1,000PD vs. 708 DOTs/1000PD, $P = 0.012$) and higher use of beta-lactam (694 DOTs/1,000PD vs. 185 DOTs/1,000PD, $P < 0.001$). **Conclusions:** Both emergence and vanishment of

MDRAB are closely related to carbapenem exposure in this homogeneous critically burn patients. Our findings imply that early-discontinued use could be an essential practice in antibiotic stewardship for control of MDRAB when carbapenem use is inevitable.

Author Disclosure Block:

S. Shie: None. **P. Huang:** None. **J. Ye:** None. **Y. Wu:** None. **T. Wu:** None. **T. Wu:** None. **S. Chuang:** None. **M. Cheng:** None. **C. Huang:** None.

Session Number:

332

Session Title:

Antimicrobial Stewardship II

Publishing Title:

Microbiology Plate Rounds: Antimicrobial Stewardship (AS) and Clinical Microbiology Laboratory (CML) Work Together to Enhance Timely, Clinically Relevant Patient Care

Author Block:

S. H. MacVane, L. L. Steed; Med. Univ. of South Carolina, Charleston, SC

Abstract Body:

Background: Collaboration between AS & CML is fundamental to achieving maximum benefits of rapid diagnostics. However, data on other AS activities in the CML are sparse. The purpose of this study was to describe AS activities during daily microbiology plate rounds during a typical month at an academic medical center. **Methods:** AS interventions occurring during plate rounds were tracked during November 2015. An AS PharmD has routinely attended daily (M-F) plate rounds to provide clinical correlation when needed & to prospectively monitor critical cultures since 2012. Rounds consists of presentation & discussion of interesting or challenging patient cases/cultures by a small group consisting of the CML director, technical staff, pharmacist, & trainees to optimize patient care & use of laboratory resources. **Results:** A total of 85 interventions were made over the course of 19 plate rounds (mean 4.5 intervention/day). The majority of interventions were on blood (29%), urine (29%), & exudate cultures (27%). Twenty four % of cultures were polymicrobial. The most common intervention was liaison services between CML & clinicians (42%), which included clarifying specimen source or providing other pertinent clinical information to aid staff in appropriate culture work-up or testing & reporting restricted antibiotics. Management of multidrug-resistant (MDR) organisms (22%) & clarification of culture reports & antimicrobial susceptibility results (18%) were also common. A change of antimicrobial therapy was made in 33% of patients (19% de-escalation & 14% escalation). Interventions were accepted 95% of the time. Most frequent outcomes of interventions were classified as: clarified culture workup/reporting (40%) to reduce time to clinically actionable results, avoided unnecessary culture workup (14%), & optimized antimicrobial therapy (31%). **Conclusions:** AS involvement during plate rounds was a welcomed & effective initiative with high rates of accepted interventions. AS interventions were diverse and included liaison services, MDR

organisms, & assistance with culture reporting. Expanding AS services into the CML offers new opportunities that positively impact utilization of antimicrobials and laboratory resources, as well as familiarizing AS members with microbiology procedures & practices.

Author Disclosure Block:

S.H. MacVane: None. **L.L. Steed:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Trends in Carbapenem Non-susceptibility by Pathogen: 2008 to 2015

Author Block:

Y. P. Tabak¹, C. A. DeRyke², V. Gupta¹, D. D. DePestel², S. S. Sen², X. Sun¹, R. S. Johannes¹, **E. McCann²**; ¹Becton, Dickinson & Company, Franklin Lakes, NJ, ²Merck & Co. Inc., Rahway, NJ

Abstract Body:

Background: Longitudinal data on pathogen-specific carbapenem non-susceptibility by source are limited. **Methods:** We analyzed electronic data in a Becton, Dickinson & Company research database from 154 U.S. hospitals. All non-duplicate Gram-negative isolates (first isolate of a species per 30 day period) from all sources (urine, skin, blood, intra-abdominal, respiratory, and ‘other’) were classified as non-susceptible (NS): if a) intermediate or resistant to imipenem or meropenem for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* OR b) intermediate or resistant to imipenem, meropenem, or ertapenem. Isolates were classified as hospital-onset (HO) if collected >3 days post-admission or ≤14 days post-discharge. All others were classified as non-HO. Isolates were also categorized into 4 groups: *P. aeruginosa*, non-*Escherichia coli* Enterobacteriaceae, *E. coli*, and *A. baumannii*. **Results:** From 2008 to 2015, 2,070,536 Gram-negative isolates were tested; 74,487 (3.6%) were NS to carbapenems. The NS rate was 9.7% for HO (36,083/372,621) and 2.3% (38,404/1,697,915) for non-HO isolates. Top NS isolates were *P. aeruginosa* (63.3%, n=47,172) and non-*E. coli* Enterobacteriaceae (25.2%, n=18,761). For pathogen trends, *P. aeruginosa* NS rates decreased slightly overall (19.1% to 18.3%, p<0.01, -4.2% [relative difference]), but increased in respiratory (27.7% to 30.1%) and ‘other’ (10.5% to 12.0%) sources (both p<0.05). Non-*E. coli* Enterobacteriaceae NS rates increased overall (2.3% to 4.1%, p<0.01, +78.3%) and for each isolate source (all p<0.01, except for blood [p=0.06]). *E. coli* NS rates remained at a low level (0.22% to 0.18%, p<0.01, -18.2%). *A. baumannii* NS rates decreased overall (40.8% to 31.0%, p<0.01, -24.0%) and for each isolate source (all p<0.01, except for respiratory [p=0.26] and skin [p=0.72]). **Conclusions:** Approximately one in ten HO Gram-negative pathogens in U.S. hospitals was NS to carbapenems. *P. aeruginosa* was the most common NS organism identified. While some

decreases in carbapenem NS isolates were observed, they fall short of stated goals for reductions in non-susceptibility. Of note was the 78.3% increase in carbapenem non-susceptibility in non-*E. coli* Enterobacteriaceae.

Author Disclosure Block:

Y.P. Tabak: D. Employee; Self; BD. **C.A. DeRyke:** D. Employee; Self; Merck & Co. Inc. **V. Gupta:** D. Employee; Self; BD. **D.D. DePestel:** D. Employee; Self; Merck & Co. Inc. **S.S. Sen:** D. Employee; Self; Merck & Co. Inc. **X. Sun:** D. Employee; Self; BD. **R.S. Johannes:** D. Employee; Self; BD. **E. McCann:** D. Employee; Self; Merck & Co. Inc..

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Hospital “*Klebsiella Pneumoniae*” Isolated In Russia: Antibacterial Resistance, Genotyping, And Virulence

Author Block:

N. Fursova¹, E. Astashkin¹, A. Lev¹, N. Kartsev¹, E. Leonova¹, O. Ershova², N. Volozhantsev¹, V. Malikov³, E. Svetoch¹, I. Dyatlov¹; ¹SRСAMB, Obolensk, Russian Federation, ²SRI Burdenko, Moscow, Russian Federation, ³ICH No.1, Moscow, Russian Federation

Abstract Body:

Background: The study of the molecular mechanisms for “*Klebsiella pneumoniae*” multi drug resistance (MDR) and virulence is important for public health because of this bacterium is one of the main nosocomial pathogens. **Methods:** The MALDI-TOF Biotyper (Bruker, Germany) was used for bacterial identification. Susceptibility to 9 functional classes of antibacterials was determined. PCR was done for detection of resistance genes *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{NDM}, *int1*, *int2*, *ompK36* and virulence genes *rmpA*, *aer*, *uge2*, *wabG*, *kfu*, *fimH*, *allS*. Genotyping was done by MLST according Pasteur Institute (Paris), and by RAPD-PCR method. DNA sequences were submitted to the GenBank database. Strain virulence (LD50) was determined on a model of outbred mice. **Results:** Antibacterial resistant hospital “*K. pneumoniae*” isolates (n=194) were collected in Moscow in 2012-2015. Major isolates (98%) were resistant to three and more antibacterial functional classes. The resistance mechanism was based on the *bla*_{CTX-M} (79%), *bla*_{TEM} (52%), *bla*_{SHV} (79%), *bla*_{OXA-48-like} (47%) genes and class 1 integrons (45%). A significant genetic heterogeneity of “*K. pneumoniae*” isolates was revealed - 52 RAPD-genotypes, 7 sequence types (ST23, ST86, ST147, ST218, ST395, ST833 and novel ST2174), and 18 virulence genotypes (Vir-genotypes). Novel ST2174 [ID 3642, Pasteur Institute] was identified for *K. pneumoniae ozaenae* strain I-3014 and characterized by a novel *gapA125* allele [GenBank KU510247]. Three virulence groups, high virulent with LD50 ≤ 10² CFU (n=5), moderate virulence with LD50 10² - 10⁴ CFU (n=5), and avirulent with LD50 > 10⁴ CFU (n=27) were identified. High virulence was associated with ST23, ST86 and ST218 which were coordinated with RAPD-genotypes R23, R8, R18, R11, Ri1, Ri2 and Vir-1,

Vir-3, Vir-13 genotypes. **Conclusions:** A novel “*K. pneumoniae*” sequence type ST2174 was identified. Correlation between virulence degree, sequence-type, RAPD-genotype and Vir-genotype was revealed for MDR hospital “*K. pneumoniae*” strains. These data are important for profound epidemiological analysis and for the choice of strategy to fight MDR pathogens.

Author Disclosure Block:

N. Fursova: None. **E. Astashkin:** None. **A. Lev:** None. **N. Kartsev:** None. **E. Leonova:** None. **O. Ershova:** None. **N. Volozhantsev:** None. **V. Malikov:** None. **E. Svetoch:** None. **I. Dyatlov:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Discrimination of *Klebsiella pneumoniae* bla-Groups by Maldi-Tof Mass Spectrometry

Author Block:

J. Liese, A. Dinkelacker; Univ. Hosp. Tübingen, Tübingen, Germany

Abstract Body:

Background: *Klebsiella (K.) pneumoniae* is a commonly encountered pathogen, which can cause a wide range of infections. Antibiotic resistant strains have emerged over the last years, which are frequent causes of nosocomial outbreaks. Rapid typing of isolates is desirable to detect potential transmission events in these situations. However, commonly used typing methods are costly and/or labor intensive. MALDI-TOF mass spectrometry (MS) is routinely used in microbiological laboratories for rapid species identification, but the potential of this method for discrimination of bacterial strains on a sub-species level is not yet fully investigated. The goal of this study was to establish markers in MALDI-TOF spectra of *K. pneumoniae* isolates that allow for distinguishing isolates on a sub-species level. **Methods:** 40 strains of *K. pneumoniae*, which were previously analyzed by whole genome sequencing, were grouped according to the presence of *bla* genes. MALDI-TOF spectra of the strains were recorded in octuplets on three days on a MALDI Biotyper system (Bruker) and spectra were imported into the BioNumerics 7 software (Applied Maths). Summary spectra were then calculated for each isolate and these spectra were again summarized according to *bla* groups. The amino acid sequence of ribosomal proteins was calculated from the corresponding genes in the annotated genome sequences using the ExPASy online tool. **Results:** *K. pneumoniae* isolates could be assigned to *bla*SHV or *bla*LEN groups. Summary MALDI-TOF spectra of bacterial isolates revealed 15 mass peaks specific for *bla*LEN strains and 13 peaks specific for *bla*SHV. Nine of these specific peaks could be found in both groups in a <100 Da range, potentially representing protein isoforms with one (or more) amino acid substitution(s). The calculated masses of the ribosomal proteins L31p, and de-methionated L28p, and S15p were found to differ between both *bla* groups and these masses matched three of the specific peak masses for the respective group. Three more peak masses could be identified as the double charged ions of these proteins. Unbiased comparison of all open

reading frames in the genomes additionally revealed that the single and double charged isoforms of the stress-response protein CsbD matched two more specific peak masses. **Conclusions:** MALDI-TOF spectra contain information that allow to distinguish *K. pneumoniae* isolates on a sub-species level.

Author Disclosure Block:

J. Liese: None. **A. Dinkelacker:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Does *Acinetobacter baumannii* Serve as a Reservoir for *bla*_{NDM} Dissemination into *Enterobacteriaceae*?

Author Block:

A. Adler, M.D., R. Glick, Y. Carmeli; Tel-Aviv Sourasky Med. Ctr., Tel-Aviv, Israel

Abstract Body:

Background: Although NDM-producing *Acinetobacter baumannii* (NDMAb) were identified in surveillance cultures in 2009 in Israel, NDM-producing *Enterobacteriaceae* (NDME) infections have emerged only recently. We aimed to describe the epidemiology of NDME and NDMAb, to analyze their molecular features and to explore the possibility of horizontal gene transfer (HGT) of the *bla*_{NDM} gene between these microbial families. **Methods:** The study was done at the Tel-Aviv Sourasky Medical Center in Israel from December 2014 until August 2015. Surveillance rectal cultures were collected per hospital policies and were analyzed for the presence of NDME and NDMAb. NDME infected patients were placed in cohort. Isolates were tested by PCR's for the *bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48} and the *bla*_{VIM} genes and typing was done by PFGE. The locations of the *bla*_{NDM} gene within the IS*Aba125* transposon and on a conjugative plasmid were studied by PCR's and transformation, respectively. A transmission event (TE) was determined if patients shared the same NDME or NDMAb PFGE type and were simultaneously in the same ward. Possible HGT-related TE was considered if the two isolates shared identical *bla*_{NDM} gene allele and transposon. **Results:** The number of carbapenem-resistant *A. baumannii*-infected patients was 311, of which 15 (4.8%) were NDMAb (clinical-9, surveillance-4, both-2). The number of carbapenemase-producing *Enterobacteriaceae* (CPE)-infected patients was 104, of which 13 (12.5%) were NDME (clinical-3, surveillance-8, both-2). All NDMAb isolate except one harbored the *bla*_{NDM-1} allele that was located within an IS*Aba125* transposon. Although only 3TE of NDMAb were identified, the majority of patients (n=9) were infected by one strain. NDME were either *E. coli* (n=4) or *K. pneumoniae* (n=9) of different PFGE types. All NDME isolates harbored the *bla*_{NDM-1} allele on a transferable plasmid but the gene was located within an IS*Aba125* in only 3 isolates. Four NDME-infected patients were positive upon admission and only 1 TE was identified. One possible HGT-related TE from NDMAb to NDME

was identified. **Conclusion:** The occurrence of NDMAb and NDME are similar, despite the use of cohorting only for NDME. Whereas NDMAb appears to disseminate by clonal spread, the source of NDME remains elusive in most cases, and thus may be related in part to HGT from NDMAb.

Author Disclosure Block:

A. Adler: None. **R. Glick:** None. **Y. Carmeli:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Polymyxin B Resistance in Kpc-Producing *Klebsiella pneumoniae* Bacteremia: A Cohort Study

Author Block:

F. Ramos¹, M. Rigatto¹, A. Zavascki²; ¹Hosp. São Lucas da PUCRS, Porto Alegre, Brazil, ²Hosp. de Clinicas de Porto Alegre, Porto Alegre, Brazil

Abstract Body:

Background: Polymyxins remain one of the last available options for the treatment of multiresistant Gram negative bacteria. KPC- producing *Klebsiella pneumoniae* resistant to polymyxins have been isolated with growing frequency in the last decade, imposing an important therapeutic challenge. This study aims to analyze the main characteristics and outcomes of KPC-producing *K.pneumoniae* bacteremia and to evaluate the impact of polymyxin resistance. **Methods:** We performed a prospective cohort from 2013-2015 in two tertiary care hospitals in Porto Alegre, Brazil. All patients ≥ 18 years old, with KPC-producing *K. pneumoniae* isolated from blood samples were included in the analysis. Demographic data, treatment and 30-day mortality were assessed and compared between polymyxin B (PMB) -resistant (R) and susceptible (S) strains. **Results:** Sixty-four patients were included for analysis: 45 (70.3%) and 19 (29.7%) with PMB-S and -R strains, respectively. The mean age was 62 ± 15.7 years and 36 (56.2%) were man. Charlson score was: 2(2-3.8), and did not significantly differ between groups ($P=0.80$). A total of 1(37.8%) and 11 (57.9%) were at ICU $P=0.29$, in the PMB-S and R groups, respectively. Meropenem MICs were 32 (4-32) and 32 (16-32) mg/L in PMB -S and R groups, respectively, $P=0.18$. In the PMB-S group, 33(73.3%) patients were treated with polymyxin B and (60.0%) patients received combination therapy, mostly with meropenem 24 (53.3%). In the PMB-R group 18 (94.7%) patients received combination therapy with meropenem. Five (26.3%) patients were treated with dual carbapenem (meropenem+ertapenem). Time from bacteria isolation to adjustment of definitive antibiotic treatment was longer in the polymyxin B resistant group: 87.2 ± 44.0 vs 30.4 ± 41.1 hours ($P < 0.001$). The overall mortality was 30(46.9%) patients: 21 (46.7%) and 9 (47.4%), $P=0.99$, in the PMB-S and R groups respectively. ICU admission was the only factor significantly associated to 30-day mortality ($P=0.042$). **Conclusions:** Blood

infections due to polymyxin B resistant KPC-producing *K. pneumonia* were associated to a delay in definitive antibiotic therapy. Nevertheless, no difference in 30-day mortality regarding polymyxin resistance was shown in this study. Other factors potentially associated with mortality in this cohort are currently under investigation.

Author Disclosure Block:

F. Ramos: None. **M. Rigatto:** None. **A. Zavascki:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Proof-of-Concept Trial of the Combination of Lactitol and *Lactobacillus* for the GI Eradication of OXA-48 Producing *Enterobacteriaceae* (OXA-48-PE)

Author Block:

J. C. Ramos, J. R. Arribas, F. Arnalich, J. Garcia, J. Mingorance, R. Herruzo, G. Ruiz, A. Borobia, F. Lázaro, J. R. Paño; Hosp. Univ. La Paz-IdiPAZ, Madrid, Spain

Abstract Body:

Background: It is unknown if gastrointestinal (GI) decontamination with probiotics and lactitol can eradicate GI colonization by carbapenemase-producing enterobacteriaceae (CPE). **Methods:** Proof-of-concept, single-arm clinical trial consisting of a combination of lactitol - 10 g tid - and probiotics (*L. bifidus* and *L. acidophilus*) - 2×10^9 CFU tid -po for 3 weeks. Non-immunocompromised adult outpatients without recent or anticipated antibiotic intake, and persistent (≥ 6 months) GI colonization by OXA-48-PE were included. Most significant exclusion criteria were: age over 75, recent GI surgery, significant electrolytic disturbances, GFR <30 ml/min and having non-removable endovascular devices. Rectal swab was taken at the screening and stool samples in the following visits. Primary endpoint was sustained GI eradication of OXA-48-PE (6 weeks after the end of therapy -EoT-). Secondary endpoints were OXA-48-PE eradication at EoT as well as assessment of safety and tolerability. Burden of blaOXA-48 was determined normalizing the number of copies blaOXA-48 to 16S gene using a specific real-time PCR in the first four patients. **Results:** 918 patients with history of OXA-48-PE GI colonization were screened, of which 24 fulfilled clinical and epidemiological inclusion criteria. The majority of persistent OXA-48-PE GI carriers were elderly and pluripathological patients, which complicated patient recruitment. Persistent GI carriage was identified in 8/24 (33.3%), all of which received the study medication and had a complete follow-up. OXA-48-PE eradication at EoT and 6 weeks after were 62.5% (95% CI: 29%-96.%) and 37.5% (95% CI: 4%-71%) respectively. Burden of blaOXA-48 gene was determined in 4 patients (1 with EoT response only, 1 with sustained eradication and 2 with no response) showing between 2 and 3 log decrease at the EoT. Tolerability of the study treatment was very good, except for flatulence (62%) and mild diarrhea (20%). **Conclusions:** GI eradication was not consistently achieved. However the combination of

lactitol and *Lactobacillus sp* was associated with a decrease of fecal burden of blaOXA-48 gene at the EoT. These results suggest that this combination should be studied in trials exploring other doses and/or duration of therapy.

Author Disclosure Block:

J.C. Ramos: None. **J.R. Arribas:** None. **F. Arnalich:** None. **J. Garcia:** None. **J. Mingorance:** None. **R. Herruzo:** None. **G. Ruiz:** None. **A. Borobia:** None. **F. Lázaro:** None. **J.R. Paño:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Epidemiology & Risk Factors of Extensively Drug-resistant (Xdr) *Pseudomonas aeruginosa* Infection

Author Block:

N. Palavutitotai, A. Jitmuang, S. Tongchai, P. Kiratisin, N. Augkasekwinai; Faculty of Med. Siriraj Hosp., Mahidol Univ., Bangkok, Thailand

Abstract Body:

Background: Extensively drug-resistant *Pseudomonas aeruginosa* (XDR-PA) has been increasing in healthcare-associated infections worldwide. This study aimed to determine the prevalence, the factors associated with the XDR-PA infections & the factors associated with the clinical outcomes & mortality in patients with XDR-PA infection. **Methods:** retrospective study of adult hospitalized patients with *Pseudomonas aeruginosa* (PA) nosocomial infections was performed between April & December 2014. **Results:** A total of 292 patients with 308 episodes of PA nosocomial infections were included. Of these, 74 (24%) were XDR-PA strains, 33 (10.7%) were non-XDR MDR-PA strains, & 201 (65.2%) were caused by susceptible strains. Prior PA colonization (adjusted odds ratio [aOR] 3.72; 95% CI 1.33-10.39) & APACHE II scores (aOR 1.12; 95% CI 1.01-1.24) were independently associated with XDR-PA infections. All XDR-PA strains remained susceptible to colistin & approximately 30% were susceptible to aminoglycoside. The independent factors related to 7-days favorable clinical outcome were appropriate empirical antibiotic (aOR 3.00; 95% CI 1.31-6.89) & 7-days favorable microbiological outcome (aOR 7.73; 95% CI 2.85-20.99). Patients with XDR-PA had a significantly higher mortality rate than those with non-XDRPA (47.1% vs. 27.7%, $p=0.004$). The factors independently associated with overall mortality were, being a medical patient (aOR 2.62; 95% CI 1.26-5.42), receipt of mechanical ventilator (aOR 3.10; 95% CI 1.49-6.47), & severe sepsis/septic shock (aOR 5.79; 95% CI 2.55-13.13). **Conclusions:** Nosocomial infection caused by XDR-PA is not uncommon. Given the high mortality rate of patients with XDR-PA infection, timely appropriate empirical antibiotic with colistin should be considered as empirical therapy for serious infections where XDR-PA infection is suspected.

Author Disclosure Block:

N. Palavutitotai: None. **A. Jitmuang:** None. **S. Tongsai:** None. **P. Kiratisin:** None. **N. Augkasekwinai:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Derivation And Validation Of A Mortality Risk Assessment Model For Vancomycin-Resistant Enterococcal (Vre) Bacteremia Based On Source Of Infection

Author Block:

N. S. Britt¹, E. M. Potter², N. Patel³, M. E. Steed⁴; ¹Barnes-Jewish Hosp., St. Louis, MO, ²Dwight D. Eisenhower VA Med. Ctr., Leavenworth, KS, ³Albany Coll. of Pharmacy and Hlth.Sci., Albany, NY, ⁴Univ. of Kansas Sch. of Pharmacy, Kansas City, KS

Abstract Body:

Background: Vancomycin-resistant enterococcal bloodstream infections (VRE-BSI) cause significant mortality. The relationship between source of infection and clinical outcomes has not been extensively evaluated in VRE-BSI. **Methods:** A retrospective cohort study of hospitalized Veterans Affairs (VA) patients (2004-2013) with ≥ 1 blood culture positive for VRE treated with daptomycin (DAP) or linezolid (LZD) was conducted. Exclusion criteria included: i) treatment < 48 hours; and ii) VRE-BSI caused by a DAP-nonsusceptible or LZD-resistant isolate. The source of VRE-BSI as documented by a treating physician was categorized into the following source categories: i) genitourinary (GU); ii) line-associated; iii) abdominal/biliary; iv) gastrointestinal (GI); v) endocarditis; and vi) wound/bone. A risk assessment model was derived based on the risk of 30-day mortality associated with each source and grouped into low-, medium-and high-risk categories. The performance of these categories was evaluated by multivariable logistic regression in a derivation and validation cohort constructed by bootstrapping (9,999 replications). **Results:** A total of 810 patients were included (DAP, $n=413$; LZD, $n=397$) with an overall mortality of 39.5% ($n=320/810$). In the derivation cohort, GU and wound/bone sources were classified as low-risk (mortality $<35\%$); abdominal/biliary and line-associated sources were medium-risk (mortality 35-40%); and endocarditis, GI, and unknown sources were high-risk (mortality $>40\%$). After adjusting for treatment (DAP or LZD), age, intensive care unit status, severe liver disease, thrombocytopenia, and APACHE II score, source risk category was independently associated with mortality ($P=0.007$). After bootstrapping, the relationship between source risk category and mortality persisted ($P=0.025$). **Conclusions:** Source of infection was independently

associated with 30-day mortality in a large derivation and validation cohort of patients with VRE-BSI. These robust mortality risk categories based on source of infection should be applied to future studies of VRE-BSI.

Author Disclosure Block:

N.S. Britt: None. **E.M. Potter:** None. **N. Patel:** None. **M.E. Steed:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Effect of Decolonization on Nasal and Skin Microbiota

Author Block:

P. Hogan, M. Wallace, D. K. Warren, C. A. Burnham, **S. A. Fritz**; Wash. Univ. Sch. Med., St. Louis, MO

Abstract Body:

Background: Topical antimicrobials are often employed for decolonization and infection prevention and may alter the endogenous microbiota of the skin. The objective of this study was to compare the microbial richness from community-dwelling subjects and intensive care unit (ICU) patients before and after the use of topical antimicrobials. **Methods:** We enrolled 15 adults at risk for *Staphylococcus aureus* infection. Community subjects underwent a 5 day decolonization protocol (twice daily intranasal mupirocin and daily dilute bleach water baths). ICU patients received daily chlorhexidine baths. Swab samples were collected from 5 anatomic sites (Table) immediately before, and again after, decolonization. A variety of culture media and incubation environments were used to recover bacteria and fungi; isolates were identified using MALDI-TOF MS. **Results:** Overall, 174 unique organisms were recovered. The number of unique organisms (richness) did not differ significantly before and after decolonization (Table). At baseline, the microbial richness was similar between community and ICU subjects; at the second sampling there was a trend for lower richness in ICU patients compared to community subjects ($p=0.06$). *S. aureus* was recovered from all 8 community subjects at both samplings. In the ICU, 3 subjects were colonized with *S. aureus* at baseline and 4 following decolonization. Three community subjects were colonized with *Enterobacteriaceae* at baseline and 4 following decolonization. *Enterobacteriaceae* were less common in the ICU, with 3 subjects colonized at baseline and 2 following decolonization. **Conclusions:** Although not statistically significant, the overall microbial richness was lower after decolonization. The long term health

implications of this alteration are an important area of future investigation.

Table. Number of Unique Microorganisms Recovered at Each Anatomic Site Before and After Decolonization in Community-Dwelling and Hospitalized Subjects						
Site	Community Population, Number unique organisms, Median (IQR)		<i>P</i> value*	ICU Patients, Number unique organisms, Median (IQR)		<i>P</i> value*
	Baseline N=8	Day 5 N=8		Baseline N=7	Second Sampling** N=6	
Axilla	5 (3.25-5)	4 (2-5.75)	0.26	5 (3-7)	4 (3-6.25)	0.36
Forearm	8 (6.25-9)	7.5 (6-10.25)	1.00	6 (5-7)	5.5 (3.5-6.25)	0.17
Inguinal fold	10 (4.75-13)	8 (5.25-11)	0.11	5 (4-13)	6.5 (5-8.5)	0.69
Nares	5.5 (4.25-8)	7 (3.75-9.25)	0.57	6 (5-7)	6 (4.75-7.25)	0.92
Shin	7 (5.25-8)	6.5 (2.25-10.25)	0.72	6 (4-6)	2.5 (1.75-5.75)	0.22
All sites	21 (13.75-26.5)	17.5 (15.25-26.75)	0.78	16 (14-24)	14.5 (11.75-17)	0.17

Abbreviations: IQR, interquartile range (25th-75th percentile); ICU, intensive care unit
 *Related samples Wilcoxon signed rank test
 **Second sampling of ICU patients occurred 1-3 days after the baseline sampling

Author Disclosure Block:

P. Hogan: None. **M. Wallace:** None. **D.K. Warren:** None. **C.A. Burnham:** None. **S.A. Fritz:** None.

Session Number:

333

Session Title:

Epidemiology and Control of Nosocomial MDRO

Publishing Title:

Contaminated Gloves Contribute to Crosstransmission of Healthcare-Associated Pathogens among Healthcare Workers

Author Block:

S. Otani¹, H. Takoi², K. Fujita², M. Gorai¹, R. Okuda¹, Y. Mano¹, K. Matsuda², Y. Saito², A. Gemma², N. Furuya¹; ¹Bunkyo Gakuin Univ. Graduate Sch., Tokyo, Japan, ²Nippon Med. Sch., Tokyo, Japan

Abstract Body:

Background: Infection control is a priority for all hospitals to reduce the spread of healthcare-associated infections. Gloving is recommended as a barrier protection for healthcare workers to reduce the risk of contamination during contact with infectious sputum, urine and body fluids. Failure to change or remove contaminated gloves carries a high-risk of healthcare-associated pathogens transmission. One critical aspect of crosstransmission of microorganisms among healthcare workers is the ability of microorganisms to transmit and survive on hospital surfaces. The aim of this study was to determine how many bacteria would transmit from gloves to hospital surfaces. **Methods:** *E. coli*, extended-spectrum beta-lactamase (ESBL) producing *E. coli*, *K. pneumoniae*, ESBL producing *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, which represent common pathogens that may contribute to healthcare-associated infections were evaluated in this study. Nitrile examination gloves were inoculated with 10⁵, 10³ and 10 CFU/ 10 microliter of the specific microorganism. Immediately after inoculation (0 second), 30 seconds and 3 minutes (gloves surface dried completely), contaminated gloves touched to a sterilized polypropylene surface. Then, the number of viable bacteria on the polypropylene surface were quantified. **Results:** All tested microorganisms transmitted 5-10% of inoculation from the gloves to the polypropylene surface at 0 second. All tested microorganisms except *K. pneumoniae* and *A. baumannii* decreased dose- and time-dependently and did not detect on the polypropylene surface at 3 minutes after inoculation of groves. In contrast, *K. pneumoniae* and *A. baumannii* remained approximately 10 CFU on the polypropylene surface at 3 minutes after inoculation of groves. Antibiotic sensitivity had no consistent effect on survival on the polypropylene surface. **Conclusions:** Contaminated gloves contribute to transmission of healthcare-

associated pathogens among healthcare workers. *K. pneumoniae* and *A. baumannii* are high risk of transmission from the gloves to hospital surfaces. This study suggests that proper glove use may decrease the risk of healthcare-associated infections.

Author Disclosure Block:

S. Otani: None. **H. Takoi:** None. **K. Fujita:** None. **M. Gorai:** None. **R. Okuda:** None. **Y. Mano:** None. **K. Matsuda:** None. **Y. Saito:** None. **A. Gemma:** None. **N. Furuya:** None.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

HIV-1 Seroconversion Across 17 International Demonstration Projects with Pre-Exposure Prophylaxis (PrEP) with Oral Emtricitabine/Tenofovir Disoproxil Fumarate (FTC/TDF)

Author Block:

S. McCallister, D. Magnuson, R. Guzman, V. Shvachko, K. Rawlings, R. Mera; Gilead Sci. Inc., Foster City, CA

Abstract Body:

Background: HIV-1 seroconversion rates in carefully managed PrEP clinical studies varied by the treatment population evaluated, from 1.2/100 person years (PY) in United Kingdom urban clinics (PROUD) to 1.9/100 PY in the US, South America and Africa (iPrEX). We collected HIV-1 seroconversion data with use of FTC/TDF for PrEP from diverse countries and populations in a real-world setting to compare to clinical study data. **Methods:** Patient time exposure from 2011 through mid-2015 was collected from 4,981 HIV-negative adults participating in 17 ongoing international PrEP demonstration projects from North and South America, Asia, and Africa; all participants received open-label FTC/TDF for daily use. All studies conducted HIV-1 and sexually transmitted infection testing. Time exposed to FTC/TDF while at risk of seroconversion was analyzed; participants in deferred FTC/TDF and other non-PrEP arms were excluded and exact Poisson statistics were used to compute rates and confidence intervals. **Results:** The 4,981 participants from the 17 studies had 4,171 PY of exposure to FTC/TDF. A total of 54 HIV-1 seroconversions occurred, a rate of 1.29/100 PY (95% CI: 0.97-1.69). Median time to seroconversion was 268 days, range 4-601 (IQR: 171-425). 9/54 (14.8%) reported signs or symptoms of acute retroviral syndrome at the time of seroconversion. Mean age of those who seroconverted: 25.1 ± 7 years; 61% were <25 years old. 96% of those seroconverting were male, contributing 3826.2 PY of FTC/TDF exposure, for a rate of 1.36/100 PY (95% CI: 1.02-1.78). Women had 278.6 PYs of FTC/TDF exposure for a rate of 0.72/100 PY (95% CI: 0.09-2.60). 25/54 seroconverters were from the USA. Race for seroconverters: black 43%, mixed 39%, white 11%, Asian 2%. Genotypic resistance data for 18 of 54 seroconverters was available; 15/18 (83%) had wild-type virus, 3/18 had M184V/I; there were no reports of K65R or K70R. **Conclusions:** This large meta-analysis

of HIV-1 seroconversion data with the use of FTC/TDF for PrEP demonstrates a low overall seroconversion rate in a real world setting with an upper confidence interval below 2 infections per 100 PY. While these data are reassuring and inform prevention programs in diverse settings, more data are needed in women and transgendered individuals.

Author Disclosure Block:

S. McCallister: D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder. **D. Magnuson:** D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder. **R. Guzman:** D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder. **V. Shvachko:** D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder. **K. Rawlings:** D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder. **R. Mera:** D. Employee; Self; Gilead employee. **K. Shareholder** (excluding diversified mutual funds); Self; Gilead shareholder.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Development Of Dolutegravir Combination Nanoparticle Fabrications for HIV Prophylaxis

Author Block:

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Abstract Body:

Background: The numbers of individuals infected with HIV could be reduced with highly effective pre-exposure prophylaxis (PrEP). Dolutegravir (DTG) is an integrase strand transfer inhibitor with potent anti-HIV activity. Cellulose acetate phthalate (CAP) is a natural polymer with HIV-1 entry inhibitory properties. Development of DTG-loaded CAP nanoparticles (DTG-CAP-NP) could serve as a novel combination for PrEP. **Methods:** An oil-in-water homogenization was used to fabricate DTG-CAP-NP and CAP-NP. Pluronic F127 (2%) and organic to aqueous phase ratio of 1:1 were used for DTG-CAP-NP and CAP-NP. Encapsulation efficiency (EE) of DTG in DTG-CAP-NP was validated by HPLC. Cytotoxicity of DTG-CAP-NP was evaluated and compared to CAP-NP and DTG solution in cell lines and human Epivaginal™ tissues. Intracellular delivery of DTG to cell lines was determined by pretreatment with DTG-CAP-NP or DTG solution and cell lysates were analyzed by HPLC. To evaluate antiviral activity, TZM-bl cells were treated with DTG-CAP-NP and DTG solution for 1 or 3 days then infected with HIV-1_{NL-4-3}. Luminescence was measured after 48 h. **Results:** Mean (+ SD) size $< 80.7 \pm 1.7$ nm and surface charge -33.67 ± 1.9 mV for DTG-CAP-NP. EE of DTG was $\geq 26.3\%$ in CAP-DTG-NP. DTG-CAP-NP were significantly less toxic to cell lines as compared to DTG solution and non-toxic to endocervical tissues. CAP-NP were not toxic to cell lines or Epivaginal™ tissues at concentrations as high as 100 $\mu\text{g/mL}$. DTG-CAP-NP showed complete inhibition of HIV-1 infection at a concentration equivalent to 5 ng of DTG whereas DTG solution and CAP-NP showed significantly less inhibition at this concentration ($p < 0.05$). These data indicate synergy between DTG and CAP as well as the advantage of nanoscale fabrication. **Conclusions:** DTG-CAP-NP would be a novel vaginal microbicide with greater potency than DTG in solution and may offer a sustained release PrEP option for HIV. This is the first report of DTG fabricated into a NP formulation for PrEP for treatment.

Author Disclosure Block:

A. Shibata: None. **P. Bruck:** None. **R. Pham:** None. **S. Mandel:** None. **C. Destache:** None.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Racial Characteristics of FTC/TDF for Pre-Exposure Prophylaxis Users in the US

Author Block:

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Abstract Body:

Background: Blacks account for an estimated 44% of all new HIV infections in the US, with Black women accounting for 29% of that number at a rate of 20 times that of White women. This study explores the racial characteristics of US pre-exposure prophylaxis (PrEP) users since the approval of FTC/TDF (Truvada®) for PrEP in 2012. **Methods:** National electronic patient level data was collected from 82% of all US retail pharmacies that dispensed FTC/TDF between January 1, 2012 and September 30, 2015. A previously described algorithm identified use of FTC/TDF for PrEP by excluding FTC/TDF use for HIV treatment, post-exposure prophylaxis, and off-label treatment of chronic hepatitis B. De-identified patient-level data including prescription refill data, medical claims, and patient demographics were analyzed via logistic regression. Data was projected to all retail pharmacies. **Results:** A total of 49,158 unique individuals have started FTC/TDF for PrEP from 2012-2015. Data on race was available for 43.7% of subjects. Blacks represented 10.1%, with Whites, Hispanics and Asians being 74.5%, 11.7% and 3.7% respectively. The proportion of Blacks who started PrEP dropped from 12.3% in 2012 to 9.7% in 2015. Women comprised 20.7% of those who initiated PrEP overall, but the percent of female new starts dropped, from 41.6% in Q3 2013 to 13.8% in Q3 2015 and Black women were over four times less likely to have initiated PrEP, compared to White women.

New FTC/TDF for PrEP starts	Unique PrEP users	Mean age in years	% Female
Q2-Q3 2013	3,158	36.7	44.2
Q4 2013 - Q1 2014	3,670	38.2	26.3
Q2-Q3 2014	8,255	38.4	16.3

Q4 2014 - Q1 2015	11,817	38.4	13.0
Q2-Q3 2015	15,456	37.6	11.0

Conclusion: The population of PrEP users in the US is significantly increasing, but is not proportionally aligned with the racial demographics of new infections. New starts of FTC/TDF for PrEP increased among Whites in 2015 while Black uptake lagged. Male uptake increased while the female uptake remained flat and disproportionately lower in Black women. HIV prevention messaging and services need to be racially focused to decrease new infections in populations with the most severe burden of HIV.

Author Disclosure Block:

S. Bush: D. Employee; Self; Gilead Sciences. **D. Magnuson:** D. Employee; Self; Gilead Sciences. **M. Rawlings:** D. Employee; Self; Gilead Sciences. **T. Hawkins:** D. Employee; Self; Gilead Sciences. **S. McCallister:** D. Employee; Self; Gilead Sciences. **R. Mera Giler:** D. Employee; Self; Gilead Sciences.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Pro140 Sc Monotherapy Provides Long-term, Full Virologic Suppression in Hiv Patients

Author Block:

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Abstract Body:

Background: In patients infected exclusively with CCR5-tropic HIV-1, PRO140 (humanized CCR5 mAb) demonstrated potent antiviral activity of $\geq 1.65 \log_{10}$ mean viral load (VL) reduction as a weekly subcutaneous injection (SC). We evaluated PRO 140 SC monotherapy (MT) in a single-arm, open-label phase 2b extension study for long-term VL suppression following initial antiretroviral therapy (ART). **Methods:** 39 adult patients (11-cohort 1, 28-cohort 2) infected exclusively with CCR5-tropic HIV-1 (Trofile[®] DNA Co-receptor Tropism Assay) and virologically suppressed on ART (VL <40 c/mL (LabCorp)) were switched to weekly PRO140 350 mg SC MT. After 13 weeks of viral suppression, 16 subjects in cohort 2 were trained to self-administer PRO140 SC and offered continuation of MT. **Results:** Of the 15 eligible subjects (86.7% male, 20% non-white; median age 55.3 yers; median CD4 T-cell count 586 cells/mm³), 11 subjects are currently on PRO140 SC MT for >1 year of treatment (56-67 weeks). Single-copy HIV-1 RNA assay reported a median VL nadir of 0.4 c/mL (n=10; <0.5-39.9). Three subjects experienced virologic failure (VF) (2 consecutive VL ≥ 400 c/mL) with a median time of 169 days. VF subjects restarted ART and virologically suppressed (VL <40 c/mL) with a median time of 29 days. One patient discontinued at week 47 (with VL <40 c/mL) due to relocation. At VF, there was no change of co-receptor tropism (Trofile[®] RNA Assay) nor significant change in post-treatment virus inhibition for PRO 140, maraviroc, and AMD3100 compared with baseline results in VF and non-VF group of subjects. (PhenoSense[®] Entry Assay). No anti-PRO140 antibodies were detected in any subject. PRO140 was well tolerated with no drug-related major adverse events or treatment discontinuation reported. **Conclusions:** In this phase 2b extension study,

PRO140 SC provided full virologic suppression, was well tolerated, and enabled the avoidance of potential toxicity of ART while preserving drug options for >1 year. PRO140 SC may offer a simple, long-acting, single-agent maintenance therapy after initial ART in selected patients.

Author Disclosure Block:

P.J. Maddon: A. Board Member; Self; Progenics Pharmaceuticals, Inc.. C. Consultant; Self; CytoDyn Inc.. K. Shareholder (excluding diversified mutual funds); Self; Progenics Pharmaceuticals, Inc. and CytoDyn Inc.. M. Independent Contractor; Self; CytoDyn Inc. **K. Dhody:** D. Employee; Self; Amarex Clinical Research, LLC. **U. Kowalczyk:** D. Employee; Self; Quest Clinical Research. **K. Kazempour:** A. Board Member; Self; Amarex Clinical Research, LLC. D. Employee; Self; Amarex Clinical Research, LLC. K. Shareholder (excluding diversified mutual funds); Self; Amarex Clinical Research, LLC. **N. Pourhassan:** A. Board Member; Self; CytoDyn Inc.. D. Employee; Self; CytoDyn Inc.. K. Shareholder (excluding diversified mutual funds); Self; CytoDyn Inc. **J. Lalezari:** D. Employee; Self; Quest Clinical Research.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Knockout of the Ccr5 Gene Using the Crispr/Cas9 System

Author Block:

J. L. Taylor, D. Balawender, M. Neudeck, L. E. Parrish-Thompson, K. Zamborsky;
Lorain County Community Coll., Elyria, OH

Abstract Body:

Some survivors of the Black Plague, caused by *Yersenia pestis*, have a selective advantage in that they lack a functional *ccr5* gene. A 32 base pair deletion mutation, delta 32, confers resistance to *Yersenia pestis* and HIV infection. In the mid-1990's, it was discovered that some people who were exposed but not infected with HIV were homozygous for delta 32 (Liu, R, Paxton, WA, *et al* Cell 86.3 (1996): 367-77). This observation suggested that a cure or prevention for HIV might be possible by down modulating the expression of the product of the *ccr5* gene. The natural ligand of this receptor has been used effectively as an HIV prevention. A gene therapy that removes the *ccr5* gene, once thought to be impossible, now is becoming obtainable because of recent developments in gene editing technology. Timothy Ray Brown, the only person to be cured of HIV, received a bone marrow transplant from a donor who was homozygous for the *ccr5* delta 32 gene (Hütter, G., Nowak, D., *et al* NEJM 12 Feb. 2009). CCR5 is a secondary receptor for entry of the virus into human T cells. It has been hypothesized that the amino-terminus of the CCR5 delta 32 protein is capable of exerting a negative regulatory effect on wild type CCR5 as well as CXCR4, an additional secondary co-receptor (Agrawal, L., Lu, X., *et al* J Virol, Mar. 2004). The goal of this study is to develop techniques to efficiently remove the *ccr5* gene in primates, humans, and *in vivo*. Gene editing was performed using the CRISPR/Cas9 system (Origene). H9 cells were cultured in RPMI supplemented with 10% FBS, 1% pen/strep. Puromycin toxicity was determined by serial diluting puromycin into culture medium and counting cells. Individual cells obtained by dilution were examined by PCR using *ccr5* primers. The CRISPR/Cas9 system was used to eliminate knockout expression of the CCR5 protein by removing a section of the sequence from both copies of the *ccr5* gene. The human T cell line H9 was co-transfected with plasmids, plasmids containing guide RNA sequences that have homology to the amino-terminus of the *ccr5* gene along with a plasmid containing

the CRISPR/Cas9 gene. Stable transformants were obtained by puromycin selection after determining of H9 puromycin toxicity. Transformed cells were biologically cloned by dilution. Two cell lines were identified as potential *ccr5* knockouts. The successful ablation of CCR5 will be confirmed and used to test expression of both CCR5 and CXCR4.

Author Disclosure Block:

J.L. Taylor: None. **D. Balawender:** None. **M. Neudeck:** None. **L.E. Parrish-Thompson:** None. **K. Zamborsky:** None.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Efficacy and Safety of Tenofovir Alafenamide in HIV-Infected Women with Renal Impairment: 96 Week Results

Author Block:

C. McDonald¹, A. Khalsa², S. Kerkar³, J. Bartczak⁴, I. Brar⁵, S. Jiang⁶, L. Zhong⁶, M. Das⁶, M. Fordyce⁶; ¹Tarrant County ID Associates, Ft. Worth, TX, ²McDowell Hlth.care Ctr, Phoenix, AZ, ³Desert Med. Group, Palm Springs, CA, ⁴Rowan Tree Med., Wilton Manors, FL, ⁵Henry Ford Hosp., Detroit, MI, ⁶Gilead Sci., Foster City, CA

Abstract Body:

Background: Women with HIV and renal disease urgently need antiretroviral regimens with high efficacy and improved tolerability. Tenofovir alafenamide (TAF) is a novel prodrug of tenofovir (TFV) that results in 90% lower plasma TFV levels as compared to tenofovir disoproxil fumarate (TDF), and has demonstrated improved renal and bone safety. Study GS-US-292-0112 assessed the safety and efficacy of a once-daily single tablet regimen of elvitegravir, cobicistat, emtricitabine, and TAF (E/C/F/TAF) in HIV-1 infected patients with mild to moderate renal impairment. **Methods:** Virologically suppressed adults (N=242) with stable renal impairment (eGFR_{CG} 30 to 69 mL/min) switched to open-label E/C/F/TAF. We present Week 96 (W96) outcomes. **Results:** At W96, 90% of women had VL <50 compared to 88% of men. 21% of participants were female. More women were non-white (black 26% vs. 16%); (Hispanic 16% vs. 12%); (Asian 18% vs. 13%); and acquired HIV heterosexually (90% vs. 28%). At baseline, women had lower bone mineral density (BMD) at hip (0.84 vs. 0.92 g/cm²) and spine (0.962 vs. 1.10 g/cm²) compared to men (p<0.001 for both); 59% of women had osteopenia or osteoporosis compared to 51% of men. At W96, women had greater percentage increases in spine BMD (3.6% vs. 1.6%; p=0.09) and similar increases in hip BMD (2.1% vs. 1.8%; p=0.96); 19% of women vs. 10% of men improved their osteopenia/osteoporosis. **Conclusions:** HIV-infected women with renal impairment who switched to E/C/F/TAF had excellent virologic suppression and improvement in BMD after two years. E/C/F/TAF may fulfill an unmet need as a potent and safe regimen for women with impaired renal function.

Author Disclosure Block:

C. McDonald: H. Research Contractor; Self; Gilead, Viiv, BMS, Janssen, Merck. L. Speaker's Bureau; Self; Gilead, Viiv, BMS. **A. Khalsa:** H. Research Contractor; Self; Gilead. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, Janssen, Viiv. L. Speaker's Bureau; Self; Gilead, Janssen, Viiv. **S. Kerkar:** H. Research Contractor; Self; Gilead. **J. Bartczak:** C. Consultant; Self; Gilead, Theratechnologies. H. Research Contractor; Self; Gilead. L. Speaker's Bureau; Self; Viiv. **I. Brar:** E. Grant Investigator; Self; Gilead, Janssen, Viiv. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead. K. Shareholder (excluding diversified mutual funds); Self; Gilead. L. Speaker's Bureau; Self; Gilead, Janssen. **S. Jiang:** D. Employee; Self; Gilead. K. Shareholder (excluding diversified mutual funds); Self; Gilead. **L. Zhong:** D. Employee; Self; Gilead. K. Shareholder (excluding diversified mutual funds); Self; Gilead. **M. Das:** D. Employee; Self; Gilead Sciences. K. Shareholder (excluding diversified mutual funds); Self; Gilead Sciences. **M. Fordyce:** D. Employee; Self; Gilead. K. Shareholder (excluding diversified mutual funds); Self; Gilead.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Tenofovir Alafenamide in Patients with Diabetes and Renal Impairment: Renal Safety Through 96 Weeks

Author Block:

D. Stein¹, A. Pozniak², S. Gupta³, F. Post⁴, J. Arribas⁵, M. Bloch⁶, P. Benson⁷, G. Crofoot⁸, S. Jiang⁹, M. Das⁹, M. Fordyce⁹; ¹Albert Einstein Coll. of Med., Bronx, NY, ²Chelsea and Westminster Hosp. NHS Fndn. Trust, London, United Kingdom, ³Indiana Univ. Sch. of Med., Indianapolis, IN, ⁴King's Coll. Hosp. NHS Fndn. Trust, London, United Kingdom, ⁵Hosp. Univ.rio La Paz, IdiPAZ, Madrid, Spain, ⁶Holdsworth House Med. Practice, Sydney, Australia, ⁷Be Well Med. Ctr., Berkley, MI, ⁸Gordon Crofoot Res., Houston, TX, ⁹Gilead Sci., Foster City, CA

Abstract Body:

Background: Efficacious and safe antiretroviral regimens are needed for HIV-infected patients with diabetes. Tenofovir alafenamide (TAF) has 90% lower plasma tenofovir levels compared to TDF, and has no effect on renal tubular function. Study 112 assessed the safety and efficacy of a single tablet regimen of elvitegravir, cobicistat, emtricitabine, and TAF (E/C/F/TAF) in HIV-1 infected adults with renal impairment from multiple causes. Outcomes in diabetics have not been reported. **Methods:** 242 virologically suppressed adults with stable renal impairment (eGFR_{CG} 30 to 69 mL/min) had their treatment switched to once-daily E/C/F/TAF. Week 96 safety results for patients with diabetes are presented. **Results:** 33/242 (14%) who switched to E/C/F/TAF had diabetes. At baseline, more diabetic patients were of black race (33% vs 16%) and had hypertension (58% vs 36%). At Week 96 week, 94% of diabetics remained virologically suppressed. Estimates of glomerular function among diabetics remained stable: median change from baseline eGFR_{CKD-EPI_{sCr}} 0.0 mL/min/1.73m² (p=0.86), and eGFR_{CKD-EPI_{cys C}} +0.5 mL/min/1.73m² (p=0.97). Tubular proteinuria improved significantly for diabetic subjects switching to E/C/F/TAF (retinol binding protein:Cr, median % change -68, p=0.048; beta-2-microglobulin:Cr, median % change, -82, p<0.001), while decreases in median total proteinuria (UPCR, -28%, p=0.077) and albuminuria (UACR, -42%, p=0.50) were not significant. One diabetic subject discontinued therapy due to eGFR decline below 30 mL/min without evidence of tubulopathy. **Conclusions:** HIV-infected

patients with renal impairment (eGFR 30 to 69 mL/min) and diabetes who switched to once daily single-tablet E/C/F/TAF had durable virologic suppression and positive renal outcomes after two years. These data support the use of E/C/F/TAF as a potent and safe regimen for diabetic adults with impaired renal function.

Author Disclosure Block:

D. Stein: F. Investigator; Self; Gilead Sciences, Sangamo Biosciences. **A. Pozniak:** F. Investigator; Self; Gilead Sciences, Merck, ViiV, BMS. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences, Merck, Janssen, ViiV, BMS. **S. Gupta:** F. Investigator; Self; Gilead Sciences, Janssen, Merck. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences, ICON Clinical Research. **F. Post:** F. Investigator; Self; Gilead Sciences, ViiV. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences, ViiV, Janssen, MSD, Abbvie. **J. Arribas:** F. Investigator; Self; Gilead Sciences. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences, ViiV, Janssen, Abbvie, BMS, MSD. **M. Bloch:** E. Grant Investigator; Self; Gilead Sciences, ViiV, Abbvie, BMS, Merck, Lilly, Novartis, Amgen. **P. Benson:** F. Investigator; Self; Gilead Sciences. **K.** Shareholder (excluding diversified mutual funds); Self; Gilead Sciences. **L.** Speaker's Bureau; Self; Gilead Sciences. **G. Crofoot:** F. Investigator; Self; Gilead Sciences, ViiV, GSK, Pfizer, Merck, Sangamo. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences, ViiV. **S. Jiang:** D. Employee; Self; Gilead Sciences. **M. Das:** D. Employee; Self; Gilead Sciences. **M. Fordyce:** D. Employee; Self; Gilead Sciences.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Darunavir and Cobicistat in Older HIV-1-Infected Patients in a 48-Week Phase 3 Trial

Author Block:

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Abstract Body:

Background: Aging of the HIV-1-infected patient population warrants examination of clinical outcomes in older patients. In the ARTEMIS trial of darunavir (DRV) + ritonavir, similar efficacy/safety profiles were observed in older (age >45 y) and younger patients. We assessed outcomes in HIV-1-infected older versus younger subjects in another clinical trial, which evaluated DRV + cobicistat (COBI) treatment. **Methods:** Efficacy and safety of DRV 800 mg and COBI 150 mg once-daily + 2 fully active nucleos(t)ide reverse transcriptase inhibitors were evaluated in HIV-1-infected adults enrolled in a 48-week, single arm, Phase 3b trial. In these post hoc analyses, efficacy and safety were assessed in patients >45 and ≤45 y. **Results:** Of 313 patients (median [range] age, 35 [18-72] y), 74 (24%) were >45 y and 239 (76%) were ≤45 y. Most patients were treatment-naïve (295/313; 94%) and received a tenofovir disoproxil fumarate (TDF)-based backbone (311/313; 99%). Baseline mean (standard error [SE]) HIV-1 RNA levels were 4.83 (0.09) and 4.74 (0.05) log₁₀ copies/mL, and mean (SE) CD4+ counts were 314.3 (21.0) and 384.3 (13.5) cells/mm³ in older and younger patients, respectively. After 48 weeks of DRV + COBI treatment, 78.4% of older patients and 81.6% of younger patients had an HIV-1 RNA level of <50 copies/mL (95% CI of the difference: -7.4%, 13.8%; *P* = 0.540), with a mean change in CD4+ count of +186.6 and +190.7 cells/mm³. The overall incidence of adverse events (AEs) was similar (87.8% vs 92.5%). Rates of AEs (grades 2-4) and laboratory abnormalities (grades 3-4) were low and generally comparable across both groups. One serious AE was considered related to study drug in the older group, versus 2 in the younger group. A total of 2 (2.7%) older patients and 7 (2.9%) younger patients discontinued the study due to an AE. **Conclusions:** In this analysis, the safety of DRV + COBI in HIV-1-infected patients >45 y was comparable to that observed in

patients ≤ 45 y. Efficacy results were generally comparable across age groups. Overall, findings were consistent with ARTEMIS.

Author Disclosure Block:

K. Brown: D. Employee; Self; Janssen Scientific Affairs. **B. Patterson:** D. Employee; Self; Janssen Scientific Affairs. **C. Liu:** D. Employee; Self; Janssen Research & Development. **M. Patel:** D. Employee; Self; Janssen Scientific Affairs. **R.E. Nettles:** D. Employee; Self; Janssen Scientific Affairs. **L. Stewart:** D. Employee; Self; Janssen Scientific Affairs. **J. Szwarcberg:** D. Employee; Self; Gilead Sciences.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Switching from TDF to TAF in Patients with High Risk for CKD

Author Block:

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Abstract Body:

Background: Due to a 91% reduction in plasma tenofovir levels, tenofovir alafenamide (TAF) compared to tenofovir disoproxil fumarate (TDF)-based regimens have significantly less impact on markers of renal and bone health in HIV patients. This analysis describes renal outcomes in patients at risk for chronic kidney disease (CKD) after switching from TDF to TAF. **Methods:** In Study GS-US-292-0109, HIV suppressed adults on TDF regimens were randomized 2:1 to switch to single-tablet regimen elvitegravir, cobicistat, emtricitabine, TAF (E/C/F/TAF) or continue the TDF regimen. Renal outcomes included discontinuations due to renal adverse events (AEs), incident of eGFR_{CG} <60 mL/min, and urine protein, albumin, retinol binding protein, and beta-2-microglobulin to creatinine ratios (UPCR, UACR, RBPCR, B2MCR) for groups with higher and lower risk for CKD. High risk was defined as ≥2 risk factors at baseline: female sex, age ≥50 years, Black race, any NSAID use, CD4 <200 cells/uL, dyslipidemia, HTN, DM, and clinical or subclinical renal AEs. Low risk was defined as ≤1 risk factor. **Results:** 784 patients switched to E/C/F/TAF and 394 continued a TDF regimen. The proportion of patients with high CKD risk was balanced between arms, 34% E/C/F/TAF vs 36% TDF regimen. Discontinuations due to renal AEs and incident of eGFR_{CG} <60 mL/min were low for both treatment arms and risk groups (Table). There were statistically significant declines from baseline in quantitative proteinuria on E/C/F/TAF versus an increase from baseline on TDF regimens across risk groups (Table). Both arms maintained high rates of virologic suppression at Week 48, 97% vs 94%. **Conclusions:** Patients with high CKD risk who switched to E/C/F/TAF had more

favorable renal outcomes compared to those who continued a TDF regimen. These data provide further support for the switch from a TDF- to TAF-based regimen.

Renal Outcomes By Treatment Arm and CKD Risk Group Through Week 48				
CKD Risk: High and Low	E/C/F/TAF (High) (N=263)	TDF Regimens (High) (N=143)	E/C/F/TAF (Low) (N=521)	TDF Regimens (Low) (N=251)
Discontinuations due to renal AEs	0.8% (No Fanconi Syndrome)	1.4% (1 Fanconi Syndrome)	0% (No Fanconi Syndrome)	0.4% (No Fanconi Syndrome)
Incident of eGFR _{CG} <60 mL/min	3%	6%	1%	4%
Median % change from baseline				
UPCR	-21%	12%	-25%	9%
UACR	-16%	6%	-21%	10%
RBPCR	-35%	13%	-36%	19%
B2MCR	-47%	11%	-56%	23%

Author Disclosure Block:

G. Huhn: C. Consultant; Self; Gilead, Viiv. H. Research Contractor; Self; Gilead, Viiv, Janssen, BMS. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, Viiv. **B. Rijnders:** E. Grant Investigator; Self; Gilead, MSD. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, MSD, ViiV, Pfizer, Jansen-cilag, BMS, Abbvie. **M. Thompson:** F. Investigator; Self; BMS, Gilead, Pfizer, Merck, ViiV. **P. Tebas:** C. Consultant; Self; Merck. F. Investigator; Self; Gilead. M. Independent Contractor; Self; Glaxo (Adjudication committee). **P. Shalit:** C. Consultant; Self; Gilead Sciences, Bristol Meyers-Squibb, Merck, Janssen. I. Research Relationship; Self; Gilead Sciences, Janssen, Glaxo Smithkline. L. Speaker's Bureau; Self; Gilead Sciences, Janssen, Bristol Meyers-Squibb, Merck. **Y. Liu:** D. Employee; Self; Gilead Sciences. **T. Nguyen-Cleary:** D. Employee; Self; Gilead Sciences. **M. Das:** D. Employee; Self; Gilead Sciences. **S. McCallister:** D. Employee; Self; Gilead Sciences.

Session Number:

371

Session Title:

Antiretrovirals for Prevention and Treatment

Publishing Title:

Integrase Resistant HIV-1 and Resistance to Recommended Regimens

Author Block:

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Abstract Body:

Background:HIV-1 integrase is crucial in the viral life cycle. The NIH Guidelines for treatment include integrase (IN) inhibitors (INSTI) in four of the five recommended regimen options for drug-naïve patients. Of the three approved INSTIs, raltegravir (RAL) and elvitegravir (EVG) have low-intermediate genetic barriers to the development of drug resistance, while dolutegravir (DTG) has a higher barrier.**Methods:**All specimens submitted for resistance testing containing any level of IN resistance at or above “Low” (as interpreted by the Stanford HIVdb) were also analyzed for resistance to all protease, RT, and IN inhibitors.**Results:**Of 210 specimens with \geq Low resistance to any INSTI, 15.7, 93.3, and 99.0% of specimens had \geq Low resistance to DTG, EVG, and RAL, respectively. Notably, 25.2% had High resistance to both EVG and RAL, and 2.4% had High resistance to all three INSTIs. A significant percent had at least Low resistance to two of the three drugs in INSTI-based combinations (Table). For the commonly used drugs paired with INSTIs, there were 9.0% with \geq Low resistance to both TDF and FTC, and 32.9% to both ABC and 3TC. The most common IN resistance mutations were E157Q, N155Y/S/H, L74I/M, T97A, E138K/A/, Q148H/R/K, G140S/A, E92Q, Y143R/S/C/H, and S147G. Notably, only 8.6% of these specimens had any \geq Low resistance to any PI. 34.3% had \geq Low resistance to any NNRTI. Although the treatment history is not available, it would appear that the majority were on protease-sparing regimens.**Conclusions:**Despite the recent approval of DTG and its high genetic barrier to resistance, the most highly recommended regimens containing INSTIs may not always be highly effective ART, due in part to resistance to the most common pairs of NRTIs used

(ABC + 3TC or TDF + FTC), and to the high levels of resistance to EVG and RAL.

Multi-drug resistance specimens with any \geq Low INSTI resistance for NIH DHHS regimens

NIH Guidelines	Basis of combination	Evidence/ Rating	Antivirals in combination	% \geq Low resistance for 2 drugs	% \geq Low resistance for 3 drugs	Total % \geq Low resistance for \geq 2 drugs
Recommended Regimens	INSTI-based	AI	DTG+ABC+3TC	26.2	8.6	34.8
	INSTI-based	AI	DTG+TDF+FTC	11.4	4.3	15.7
	INSTI-based	AI	EVG+TDF+FTC	28.1	8.1	36.2
	INSTI-based	AI	RAL+TDF+FTC	28.6	9.0	37.6
	PI-based ¹	AI	DRV+TDF+FTC	11.0	0.48	11.4
Alternative regimens	NNRTI-based ²	BI	EFV+TDF+FTC	14.8	4.3	19.0
	NNRTI-based	BI	RPV+TDF+FTC	13.8	3.8	17.6
	PI-based	BI/III	ATV+TDF+FTC	11.0	1.4	12.4
	PI-based	BI	DRV+ABC+3TC	33.3	0.95	34.3

¹Protease inhibitor
²Nonnucleoside RT inhibitor

Author Disclosure Block:

M.S. Smith: D. Employee; Self; Viracor-IBT Laboratories. **J. Nutt:** D. Employee; Self; Viracor-IBT Laboratories. **B. Schindel:** D. Employee; Self; Viracor-IBT Laboratories. **M. Altrich:** D. Employee; Self; Viracor-IBT Laboratories.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Natural Evolution of Hiv Infection to Cure and Endogenisation

Author Block:

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Abstract Body:

Background: All vertebrate genomes harbor endogenous retrovirus sequences that are footprints of ancient retroviral epidemics and are vertically transferred to progeny. Such sequences represent $\approx 8\%$ of the human genome. As vertical transmission of integrated endogenous retroviral sequences, mostly highly degraded, is a general biological phenomenon currently on-going in koalas, we speculated that it may also occur in some cases for HIV in humans. **Methods:** We sought persons spontaneously cured of HIV with integrated degraded HIV DNA. We define them as patients with western blot confirmed seropositivity since >5 years, never treated with antiretrovirals, symptomless with normal CD4 cell counts during all follow up; virologically, no living HIV is retrieved by stimulated PBMC coculture, plasma HIV RNA is not detected, and PBMC HIV DNA can only be found by performing highly sensitive techniques including hundreds of nested PCR targeting all genome regions and next generation sequencing. **Results:** We identified 3 patients spontaneously cured of HIV in a cohort of 1,700 HIV-infected patients ($\sim 2\%$). They included an injection drug user man infected for ≥ 30 years; a man having sex with men infected 5 years ago; a woman infected 10 years ago through heterosexual contact with evidence of HIV DNA sequences transferred to her child. PCR success rate was very low ($< 21\%$) as compared to positive controls, and obtained HIV DNA sequences contained many tryptophan-to-stop codons. Looking for HIV endogenization, we investigated the case of the 4-year-old daughter of the HIV cured woman who was never viremic. This girl was seronegative, without plasma HIV RNA nor living HIV. However, 18/468 nested PCR were positive on PBMC that yielded 2 original HIV sequences best matching with the mother's sequences. These results were obtained in two different assays performed in different buildings 5 months apart on two different samples. **Conclusions:** We believe that in rare cases spontaneous HIV cure may happen following infection. Our data show that HIV sequences may be transferred vertically

without transfer of living HIV. Our approach using ultra-sensitive techniques might allow re-evaluating HIV infection natural history.

Author Disclosure Block:

P. Colson: None. **C. Tamalet:** None. **C. Dhiver:** None. **I. Ravaux:** None. **A. Stein:** None. **R. Didier:** None.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Dynamics and Networks of HIV Transmission Through a Virological Surveillance of Very Recent Infections Within the Mandatory Anonymous HIV Case Reporting System, France, 2012-2014

Author Block:

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Abstract Body:

Background: HIV viruses isolated at the early stage of infection are indicators of the variants spreading in a population. The aim of the study was to combine an immunoassay for very recent infection to a phylogenetic analysis of HIV viruses and epidemiological information to analyze the diversity of HIV strains currently transmitted at a country level. **Methods:** The French reporting system for new HIV diagnoses is linked to a virological surveillance using an assay for recent infection performed on dried serum spots (DSS). A threshold defining very recent infection (VRI) was calibrated for a mean duration of 31 days. All samples corresponding to a VRI collected between 2012 and 2014 were included. RNA extracted from DSS. Network analysis was performed to infer genetic relationships, i.e. clusters, between HIV partial *env* sequences. A multivariate analysis using logistic regression was performed to identify factors associated with clustering. **Results:** Of 17,010 DSS collected during the 3-year period, 1,868 corresponded to a VRI (11.0%) of which 723 *env* fragments (38.7%) were amplified and sequenced. The final study population included 549 (75.9%) cases for which both epidemiological and virological data were available. 98% were males and 74% MSM. Non-B viruses were found in 196 cases (35.7%). CRF02_AG was the most prevalent non-B clade (96 cases). 43 clusters were identified (range 2-11 cases), including 107 individuals (19.5%) among whom 70 MSM, mostly located in Paris area and South-Eastern France. Within 8 clusters involving ≥ 3 individuals, 96% were MSM. The largest cluster involved MSM infected by a CRF02_AG variant. The only factor associated with clustering was being MSM (OR=5.5, 95IC=[2.1-14.1], p=0.0004). **Conclusions:** The

study shows the feasibility of the surveillance of the HIV epidemic at a country level using DSS for network analysis combined with epidemiological data. MSM represent the main driving force of the current French epidemic. Transmission cluster observation allows identification of specific sexual networks that may be targets for intervention.

Author Disclosure Block:

J. Capsec: None. **D. Brand:** None. **A. Chaillon:** None. **F. Cazein:** None. **S. Levu:** None. **A. Moreau:** None. **J. Pillonel:** None. **A. Millon:** None. **S. Brunet:** None. **D. Thierry:** None. **D. Smith:** None. **F. Lot:** None. **F. Barin:** None. **L. Grammatico-Guillon:** None.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Targeted Next Generation Rna Sequencing of Respiratory Syncytial Virus Produces Whole Genomes and Provides Additional Critical Genetic Virulence and Epidemiological Information

Author Block:

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Abstract Body:

Background: Respiratory syncytial virus (RSV) is a major cause of childhood morbidity. Significant annual fluctuations in incidence and severity occur, yet the specific genetic variation that influences transmission, virulence, and pathogenesis for RSV is poorly understood. **Methods:** We developed a hybridization-based method to target and enrich complete coding sequences of respiratory syncytial virus from clinical samples. The enriched samples undergo deep sequencing in a high-throughput, multiplexed, rapid manner on the Illumina MiSeq. A custom bioinformatic pipeline is used to determine specific viruses, construct nearly complete genome sequences, assess viral gene expression, detect genetic variation and conduct phylogenetic analysis. **Results:** We have completed deep, next generation sequencing of 102 RSV positive samples across four infection seasons. Of the samples sequenced, we have generated complete or nearly complete genomes from 69 of 102 (67.6%) samples and have covered >50% of the genome from 29 of 102 samples (28.4%). Together, 96% of samples sequenced have >50% of their genomes sequenced. Alignment of sequencing reads to their appropriate prototype RSV A (NC_001803) and RSV B (AY353550) reference sequences produced an average of 410 variants (range 257-474) and 466 variants (range 418-510), respectively. The average number of non-synonymous variants was 79 for both RSV A and RSV B samples. Strain and phylogenetic analysis reveal the presence of at least 6 distinct strains of RSV co-circulating during the same infection season. **Conclusions:** Taken together, our data reveal that current clinical RSV isolates differ from significantly for their reference sequences and suggest that genetic diversity of co-circulating RSV strains during the same infection season may be underappreciated. Evaluation of RSV by targeted, deep, next generation RNA sequencing provides important information about

clinical viral isolates currently not detected by clinical testing that may reveal genetic factors that impact clinical severity of illness and inform clinical management.

Author Disclosure Block:

D.L. Dinwiddie: None. **K. Schwalm:** None. **W.N. Dehority:** None. **S.A. Young:** None.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Availability of 24/7 Hsv Pcr Test Directly Impacts Acyclovir Exposure

Author Block:

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Abstract Body:

Background: Herpes simplex virus (HSV) infection of the central nervous system (CNS) is associated with significant morbidity and mortality. Current guidelines recommend early treatment of all suspected HSV CNS infection with acyclovir. Ruling out HSV CNS infection allows for discontinuation of acyclovir and avoidance of unnecessary adverse reactions and costs. This study assessed the impact of a Direct HSV (dHSV) PCR assay on acyclovir therapy in children suspicious for HSV CNS infection. **Methods:** A pre- and post-implementation study was conducted on pediatric patients presenting to the Emergency Department with signs and symptoms of meningitis or encephalitis. A total of 200 patients with HSV PCR ordered on CSF were included in the retrospective analysis; 100 pre-implementation and 100 post-implementation. In the pre-implementation period, an indirect HSV (iHSV) PCR assay was performed six days a week, 9am to 5pm versus a dHSV PCR assay offered 24 hours a day, 7 days a week post-implementation. Medical chart review was performed to determine duration of acyclovir therapy. **Results:** The patients ranged from 1 day to 21 years of age (mean: 3.8 years) with no significant differences in baseline demographics noted between the two groups. The vast majority of patients had no significant comorbidities (70%, pre-implementation; 72%, post-implementation). 1/200 patients were positive for HSV PCR from CSF, although HSV was detected from alternate sources in 4 patients. An additional 11 patients (5.6%) were diagnosed with meningitis due to Enterovirus (n=9), *Cryptococcus* (n=1) and VZV (n=1). Implementation of dHSV PCR decreased average time to reporting from 23 h to 10 h ($p < 0.001$). Acyclovir therapy was initiated in 63 patients pre-implementation and 71 post-implementation and mean time to initiation was not significantly different between the two groups (pre: 6 h; post: 5 h; $p = 0.18$). In patients with negative HSV PCR, the mean time from collection to acyclovir discontinuation was 18 hours longer pre-implementation (35 h vs 17 h; $p < 0.001$). Two patients post-implementation avoided

acyclovir therapy entirely due to availability of results prior to initiation. **Conclusions:** Timely exclusion of HSV infection, through the availability of a 24/7 dHSV PCR test, allows for prompt discontinuation of acyclovir therapy, avoiding unnecessary costs and potential adverse reactions.

Author Disclosure Block:

B. Bowland: None. **T. Van:** None. **M. Lustestica:** None. **J. Dien Bard:** None.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Clinical Utility of On-Demand Multiplex Respiratory Virus Testing among Outpatients

Author Block:

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Abstract Body:

Background: Commercial multiplex PCR tests for respiratory tract infections include up to 20 targets for common respiratory pathogens, predominantly viruses. While there is a specific therapeutic intervention available for outpatients who test positive for influenza viruses (oseltamivir), the implications of testing positive for a non-influenza virus are unclear. We therefore sought to evaluate therapeutic and other measures associated with testing positive for influenza and non-influenza respiratory viruses among outpatients at a large Veterans Administration medical center. **Methods:** Results of the FilmArray Respiratory Panel (Biofire, Salt Lake City, UT) from December 15, 2014 to April 15, 2015 were evaluated among 271 outpatients at the West Haven Veterans Administration Hospital, and patient medical records were subsequently reviewed. Differences in antibiotic and oseltamivir prescription rates were analyzed. **Results:** Among 271 patients tested in outpatient centers (emergency department, off-site urgent care, and outpatient clinics), a total 205 patients were managed as outpatients (Table 1). Among these 205 outpatients, 80 tested positive for influenza, 70 tested positive for a non-influenza pathogen, and 55 tested negative. Rates of oseltamivir and antibiotic prescriptions were significantly different among the three test groups (Chi-squared = 123.5, $p < 0.0001$ and Chi-squared = 8.2, $p = 0.017$, respectively). **Conclusion:** Outpatients who tested positive for influenza viruses received fewer antibiotic prescriptions and more oseltamivir prescriptions than those who tested positive for another pathogen or those who tested negative. There was no difference in antibiotic prescription rates among those who tested positive for a non-influenza pathogen compared to those who tested negative, suggesting that testing for influenza viruses alone may be sufficient and more cost-effective than multiplex pathogen testing for outpatients with respiratory tract infections.

Table 1: Demographics and anti-microbials for all non-admitted patients

	Influenza Detected	Non-Influenza Pathogen	No Pathogen Detected	Totals
Total Included	80	70	55	205
Age at Testing (Mean +/- SD)	57.2 (+/- 18.9)	59.1 (+/- 16.0)	58.2 (+/- 17.0)	58.1 (+/- 17.4)
Test Turn-Around-Time (Mean, Range)	2.2 (1.2 -13.6 hrs)	2.3 (1.2 - 48.6 hrs)	1.8 (1.2 - 4.2 hrs)	2.1 (1.2 - 48.6 hrs)
Order Location (%ER)	57.5%	60.0%	54.5%	57.6%
Underlying Lung Ds (% with Lung Ds)	21.3%	17.1%	30.9%	22.4%
% Immunosuppressed	6.3%	11.4%	12.7%	9.8%
Oseltamivir (% Prescribed)	77.5%	4.3%	1.8%	32.2%
Anti-Bacterial (% Prescribed)	28.8%	50.0%	47.3%	41.0%

Author Disclosure Block:

D.A. Green: None. **L. Hitoaliuj:** None. **S. Campbell:** None. **D.R. Peaper:** L. Speaker's Bureau; Self; Nanosphere.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

CMX001 Treatment of BK Virus-Associated Nephropathy in Recipients of Allogeneic Hematopoietic Stem Cell Transplants

Author Block:

G. Schrank, J. Gandhi, I. E. Stillman, M. McMasters, C. S. Tan; Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract Body:

Background: Recipients of umbilical cord blood transplants (UCBT) and patients with severe acute graft versus host disease (aGVHD) are at highest risk of BK disease, including polyomavirus associated nephropathy (PVAN)¹. There is currently no effective treatment for BKV associated PVAN. We report using the novel anti-viral CMX001, a lipophilic inhibitor of DNA polymerase, in two cases of PVAN in recipients of allogeneic HSCT. **Methods:** We obtained CMX001 with Emergency INDs approved by the FDA. Patient clinical symptoms, side effects, creatinine, and BK viral loads in serum were monitored. BKV-specific lymphocyte counts were measured in one patient by detection of IFN- γ release of CD8⁺ and CD4⁺ T lymphocytes after stimulation of a pool of overlapping BKV VP1 capsid peptides. **Results:** Patient One: 45 year old man with acute myelogenous leukemia underwent double UCBT, complicated by aGVHD. On day 90, the patient presented with acute kidney injury and BK viremia 1.06E+7 copies/mL by PCR. Renal biopsy revealed BKV nephropathy. CMX001 was given at 100mg two times a week. After the 1st dose, BKV serum viral load decreased to 4.59E+4 copies/mL. After the 3rd dose, the patient reported nausea and vomiting, and requested discontinuation of CMX001. We detected BKV-specific CD4⁺ (2.07%) and CD8⁺ T cells (5.07%). Patient Two: 68 year old man with multiple myeloma underwent nonmyeloablative match related donor SCT, complicated by post-transplant lymphoproliferative disorder treated with rituximab and donor lymphocyte infusion (DLI). Three years post-transplant, the patient had hematuria, dysuria, and oliguric renal failure, with serum BKV 1.58E+5 copies/mL. Two 100mg doses of CMX001 were given 3 days apart. Serum creatinine decreased from 7.0 to 1.9. BKV serum viral load decreased to below the limit of detection at 3 weeks. The patient endorsed nausea and watery diarrhea with mild abdominal cramping. CMX001 was discontinued. **Conclusion:** CMX001 treatment significantly decreased BKV

in serum of our patients. Larger studies are needed to investigate the role of CMX001 in treatment of PVAN in HSCT patients.

Author Disclosure Block:

G. Schrank: None. **J. Gandhi:** None. **I.E. Stillman:** None. **M. McMasters:** None. **C.S. Tan:** None.

Session Number:

372

Session Title:

Clinical Virology

Publishing Title:

Correlation Between Human West Nile Virus Disease and Infected Mosquitoes

Author Block:

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Abstract Body:

Background: States may use considerable resources to determine the incidence of mosquitoes infected by West Nile virus (WNV). In this study, WNV incidence reported in humans was compared with that in mosquitoes in 7 states that consistently determined mosquito infections from 2003-2014 in most or all of their counties. Reported WNV infection in humans and mosquitoes is highly correlated in several states but not in others. Even within states such as California, where overall correlation is high, only some of the counties, primarily in the Central Valley, show a strong correlation over many years, regardless of precipitation and humidity in those regions during the recent extended drought. Strength of correlation in some counties varied over time as the initial wave of WNV spread westward over the country, in many areas peaking around 2006/2007. Data will be presented from the time the virus appeared across the country until the present as well as data from only the last 7 years, likely to be more relevant to predicting locations of human infections. The aim is to determine usefulness of mosquito monitoring in predicting areas of human infection.**Methods:** Reported incidence of human and mosquito infections with WNV from the United States Geological Survey were correlated yearly from 2003-2014. Seven states with the most complete data were examined on a county-by-county basis. Additionally, weekly time frames in which infections began to be reported in humans and mosquitoes were examined.**Results:** In 4 states (Connecticut, Arizona, California, Nevada), correlation coefficients of reported incidence of human and mosquito infections were consistently higher than 0.6 in some, but not all, counties. Mosquito infections preceded human infections by weeks. States with low statewide correlation include Indiana, Massachusetts, and New Jersey. Even in these states, however, the correlation coefficient in some counties exceeded 0.9.**Conclusions:** Trapping and analyzing mosquito pools for the presence of WNV is expensive and labor-intensive. This study demonstrates a strong correlation between human and mosquito infections in some states and counties, including some major

population centers. In these areas, mosquito testing may be useful in predicting human incidence in order to permit timely preventive measures. In areas with low correlation, however, resources may be more profitably used for other public health concerns.

Author Disclosure Block:

L.A. Beltz: None.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

WCK 4234, a Novel Diazabicyclooctane, Overcoming OXA-Carbapenemases in Enterobacteriaceae, Pseudomonas and Acinetobacter

Author Block:

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Abstract Body:

Background. Diazabicyclooctanes (DBOs) are being developed as inhibitors of Class A and C β -lactamases. Inhibition of OXA (Class D) carbapenemases is variable, and most *Acinetobacter* spp. with OXA carbapenemases are resistant to developmental DBO combinations. We describe a novel DBO, WCK 4234, addressing this limitation. **Methods.** MICs were determined by CLSI agar dilution for clinical Enterobacteriaceae (n=191), *A. baumannii* (60) and *P. aeruginosa* (97), selected to represent carbapenemase producers and those with carbapenem resistance via porin loss together with AmpC or ESBL activity. **Results.** The panel included 31 Enterobacteriaceae with OXA-48/181 enzymes; half of them with imipenem or meropenem MICs ≥ 16 mg/L. Median MIC reductions for both carbapenems were 64- and 128-fold with WCK 4234 at 4 and 8 mg/L, respectively; 29/31 OXA-48/181-positive Enterobacteriaceae were inhibited by imipenem+WCK 4234 1+4 mg/L and all 31 by meropenem-WCK 4234 0.5+4 mg/L. Carbapenems were also potentiated for *P. aeruginosa* isolates (n=2) with OXA-48 enzyme, with meropenem MICs reduced from 64-128 mg/L to 2-4 mg/L by WCK 4234 at 4 or 8 mg/L. Forty *A. baumannii* with OXA carbapenemases were tested: all with MICs ≥ 16 mg/l for one or both carbapenems; geometric mean MICs were 36 mg/L imipenem and 42 mg/L meropenem. WCK4234 gave median 16-fold potentiation of both carbapenems at 4 mg/L and 32-fold at 8 mg/L; 35/40 OXA-positive *A. baumannii* were inhibited by imipenem+WCK 4234 4+4 mg/L, and 32/40 by meropenem+WCK 4234 4+4 mg/L. These proportion rose to 36/40 and 35/40 respectively with WCK 4234 at 8 mg/L. Like other DBOs, WCK 4234 reversed KPC-mediated resistance in Enterobacteriaceae, reducing meropenem MICs to ≤ 1 mg/L in 90% of cases; it also gave 2-64-fold reductions in carbapenem MICs for Enterobacteriaceae and *P. aeruginosa* with combinations of AmpC or ESBL and impermeability; potentiation was greater for

meropenem than imipenem and for isolates with copious AmpC. WCK 4234 did not potentiate carbapenems against strains with metallo-carbapenemases and lacked direct antibacterial activity. **Conclusion.** WCK 4234 distinctively overcame resistance mediated by OXA carbapenemases, including in *A. baumannii* and behaved similarly to other DBOs against strains with Class A and C carbapenemases.

Author Disclosure Block:

S. Mushtaq: None. **A. Vickers:** None. **N. Woodford:** H. Research Contractor; Self; Achaogen, Accelerate, Allecra, Amplex, AstraZeneca, Becton Dickinson, Meiji, Roche, Wockhardt. **D.M. Livermore:** C. Consultant; Self; Accelerate, Achaogen, Adenium, Alere, Allecra, Altermune, Astellas, AstraZeneca, Auspherix, Basilea, Bayer, BioVersys, Cubist, Cycle, Discuva, GSK, Nordic, Meiji, Pfizer, Roche, Shionogi, Tet. H. Research Contractor; Self; Roche, Melinta, Tetrphase, Wockhardt, Meiji,. **K.** Shareholder (excluding diversified mutual funds); Self; Merck, Dechra, Pfizer, Perkin Elmer, GSK. **L.** Speaker's Bureau; Self; AOP Orphan, Astellas, AstraZeneca, Curetis, Merck, Pfizer,.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

Antibacterial Activity of WCK 5999 Tested against Contemporary *Enterobacteriaceae* (ENT) Isolates from a Worldwide Surveillance Program (2015)

Author Block:

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Abstract Body:

Background: WCK 5999 is a new carbapenem/ β -lactamase inhibitor combination containing meropenem (MEM) and a novel broader spectrum β -lactamase inhibitor, WCK 4234, with enhanced activity against Class D carbapenemases. The *in vitro* activity of MEM-WCK 4234 at both fixed 4 (F4) and 8 (F8) $\mu\text{g/mL}$ and comparators was evaluated against 1,404 ENT isolates collected in a worldwide surveillance program during 2015. **Methods:** MEM-WCK 4234 and comparator compound MIC values were determined using a reference broth microdilution method. **Results:** MEM-WCK 4234 (F4) MIC₅₀/MIC₉₀ values against 1,404 ENT were $\leq 0.015/0.06 \mu\text{g/mL}$ (Table). The highest MEM-WCK 4234 (F4) MIC was 2 $\mu\text{g/mL}$ against a single *Klebsiella pneumoniae* isolate (1 $\mu\text{g/mL}$ for MEM-WCK 4234 [F8]) compared to $>32 \mu\text{g/mL}$ for MEM alone. MEM-WCK 4234 (F8) MIC values were very similar to those of MEM-WCK4234 (F4) against each of the organism groups tested. Applying CLSI breakpoint interpretive criteria for MEM to MEM-WCK 4234 (F4) resulted in $>99.9\%$ of the ENT being categorized as susceptible (S). Corresponding S rates for MEM, ceftazidime (CAZ), gentamicin (GEN) and piperacillin/tazobactam (P/T) against ENT were 97.4, 78.6, 85.9 and 87.2%, respectively. Reduced S was observed for CAZ and P/T against *Enterobacter* spp. (66.2 and 77.6% S, respectively) and *Klebsiella pneumoniae* (65.5, and 77.1% S, respectively) compared to MEM-WCK 4234 (F4) (MIC_{50/90}, 0.03/0.03 $\mu\text{g/mL}$; 100% S). MEM-WCK 4234 (F4) MIC₉₀ values were four-fold lower than MEM against *Enterobacter* spp. and *K. pneumoniae* and eight to >2048 -fold lower than CAZ, GEN, and P/T against *Citrobacter* spp., *E. coli*, *Proteus mirabilis* and *Serratia marcescens*. **Conclusions:** The potent *in vitro* activity of MEM-WCK 4234 (WCK 5999) against ENT including activity against isolates with reduced S to MEM, CAZ, GEN and/or P/T support the continued development of this antibacterial combination.

Organism (#)	MIC ₅₀ /MIC ₉₀ µg/mL (%Susceptible ^a)				
	MEM-WCK 4234 (F4)	MEM	CAZ	GEN	P/T
Enterobacteriaceae (1,404)	≤0.015/0.06 (>99.9) ^b	0.03/0.06 (97.4%)	0.25/>32 (78.6%)	0.5->8 (85.9%)	2/32 (87.2%)
<i>Citrobacter</i> spp. (42)	0.03/0.03 (100%) ^b	≤0.015/0.06 (100.0%)	0.25/>32 (81.0%)	0.5/0.5 (95.2%)	2/64 (85.7%)
<i>Enterobacter</i> spp. (219)	0.03/0.03 (100%) ^b	0.03/0.12 (96.3%)	0.25/>32 (66.2%)	0.25/4 (90.4%)	2/64 (77.6%)
<i>E. coli</i> (561)	≤0.015/≤0.015 (100%)	≤0.015/0.03 (99.6%)	0.25/32 (82.5%)	0.5/>8 (83.8%)	2/8 (92.7%)
<i>K. pneumoniae</i> (316)	0.03/0.03 (99.7%) ^b	0.03/0.12 (92.4%)	0.25/>32 (65.5%)	0.25/>8 (79.4%)	4/>128 (77.1%)
<i>P. mirabilis</i> (85)	0.06/0.12 (100%) ^b	0.06/0.12 (100.0%)	0.06/1 (96.5%)	0.5/>8 (87.1%)	0.25/1 (98.8%)
<i>S. marcescens</i> (66)	0.03/0.06 (100%) ^b	0.06/0.06 (97.0%)	0.12/0.5 (93.9%)	0.5/1 (93.9%)	2/8 (95.5%)

a. According to CLSI breakpoints; b. % inhibited at ≤1 µg/mL MEM.

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

WCK 5999 (Carbapenem-WCK 4234): Eradication of KPC-Expressing *K. pneumoniae* (KP-KPC), Derepressed AmpC *P. aeruginosa* (PA-AmpC) and OXA Carbapenemase-Expressing *A. baumannii* (Ab-OXA) in Neutropenic Mouse Lung and Thigh Infection Models

Author Block:

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Abstract Body:

Background: WCK 4234 is an inhibitor of ESBLs, Class C, KPCs and Class D β -lactamases. WCK 4234 is being developed in combination with carbapenem to treat infections caused by resistant Enteric, *Pseudomonas* and *Acinetobacter* pathogens. In this study, lung and thigh eradication efficacy of WCK 4234 in combination with imipenem (IPM) was explored. **Methods:** MICs were determined using CLSI Broth micro-dilution method. Neutropenic lung infection studies were conducted employing KP-KPC H521 and PA-AmpC 1405 (initial inoculum: 6.38 and 6.84 \log_{10} CFU/lung, treatment: initiated 2 and 4h post-infection, respectively). Neutropenic thigh infection model employing AB-OXA NCTC 13301 strain with bacterial load of 6.43 \log_{10} CFU/thigh at initiation of therapy (2h post-infection). IPM-WCK 4234 (1:1) was administered subcutaneously for 2 days in both lung and thigh infections [Lung infection: KP-KPC (six times, 2h apart), PA-AmpC (four times, 3h apart); Thigh infection: AB-OXA (four times, 3h apart)]. Viable bacterial load in lung and thigh was enumerated 18h post-treatment and compared with that of load at initiation of treatment. **Results:** Addition of WCK 4234 (4 $\mu\text{g}/\text{mL}$) to imipenem (IPM) lowered the standalone IPM MICs of KP-KPC, PA-AmpC and AB-OXA strains from >16, 16 and >16 $\mu\text{g}/\text{mL}$ to 0.25, 4 and 2 $\mu\text{g}/\text{mL}$, respectively. In KP-KPC strain, combination of WCK 4234 and IPM (both 25 mg/kg) reduced lung burden by 3.45 \log_{10} CFU/lung, while in PA-AmpC, IPM-WCK 4234 at same doses caused bacteriostatic effect. In thigh infection study involving AB-OXA, IPM-WCK 4234 (both at 50 mg/kg) reduced bacterial load by 1.70 \log_{10} CFU/thigh. Standalone IPM was ineffective for all the strains. **Conclusions:** Despite infrequent dosing, IPM-WCK 4234

provided useful antibacterial eradication effects for KPC *K. pneumoniae*, derepressed AmpC *P. aeruginosa*, and OXA carbapenemases- expressing *A. baumannii*.

Author Disclosure Block:

S.S. Takalkar: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversified mutual funds); Self; Wockhardt Ltd.,. **K.V. Umalkar:** D. Employee; Self; Wockhardt Research Center. **J.S. Satav:** D. Employee; Self; Wockhardt Research Center. **A.P. Udaykar:** D. Employee; Self; Wockhardt Research Center. **A.M. Kulkarni :** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversified mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversified mutual funds); Self; Wockhardt Ltd.,.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

Novel Bridged Diazabicyclooctanes (DBOS) are Effective Inhibitors of Representative Class A, C, and D β -Lactamases Expressed by Multidrug-Resistant (MDR) Pathogens

Author Block:

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Abstract Body:

Background: Presently, very limited treatment options exist to combat infections caused by MDR Gram negative bacteria. Contributing significantly to the MDR phenotypes are carbapenemases such as OXA-23, OXA-24/40, OXA-48, and KPC-2, which are becoming highly prevalent around the world. Complicating matters, AmpCs are often also present. Thus, a dire need exists to design novel β -lactamase inhibitors to target these β -lactamases. Here, we examined three novel DBOs (WCK 4234, WCK 5107 (INN: Zidebactam), and WCK 5153) against OXA-23, OXA-24/40, OXA-48, KPC-2, PDC-3, and ADC-7. **Methods:** All β -lactamases were purified. Steady-state inhibitor kinetic parameters $K_{i\text{ app}}$, k_2/K , and k_{off} as well as the turnover number (t_n) at 24 hr were determined for WCK DBOs. Mass spectrometry was employed to map reaction intermediates using β -lactamases reacted with WCK DBOs for 5 min and 24 hr. Avibactam and relebactam were used as comparator DBOs. **Results:** WCK 4234 demonstrated activity against all three classes of β -lactamases with $K_{i\text{ app}}$ values of ≤ 8 μM (Table). Importantly, WCK 4234 displayed ~ 70 -200-fold increased k_2/K values against the OXA carbapenemases compared to avibactam; relebactam did not possess activity against the OXAs tested (Table). WCK 5107 and WCK 5153 acylated PDC-3 very rapidly as well as KPC-2 and ADC-7, but did not inhibit the class D enzymes (Table). The k_{off} values were in the 0.001-0.0003 range for the WCK DBOs. The t_n value for the WCK 4234 for the OXA β -lactamases was 1. ADC-7 and PDC-3 possessed t_n values of 10 and 3 for WCK 4234, respectively. In the case of WCK 4234, mass spectrometry revealed that the covalent complex that was initially formed was not present with ADC-7 and PDC-3 with WCK 4234 at 24 hr; loss of the sulfate group of the DBOs

was also observed with the OXAs, ADC-7, and PDC-3. **Conclusions:** WCK 4234 extends the inhibitory profile of DBOs to OXA carbapenemases, class D enzymes previously impervious to inhibition, while maintaining class A and C activity.

Kinetic parameters of inhibition						
	$K_{i\text{ app}}$ (μM)	k_2/K ($\text{M}^{-1}\text{s}^{-1}$)	k_2/K ($\text{M}^{-1}\text{s}^{-1}$)	k_2/K ($\text{M}^{-1}\text{s}^{-1}$)	k_2/K ($\text{M}^{-1}\text{s}^{-1}$)	k_2/K ($\text{M}^{-1}\text{s}^{-1}$)
	WCK 4234	WCK 4234	WCK 5107	WCK 5153	Avibactam	Relebactam
OXA-23	8.0	12,510	not determinable (ND)	ND	ND	ND
OXA-24/40	5.0	3,630	ND	ND	ND	ND
OXA-48	0.3	312,600	ND	ND	1,400	ND
KPC-2	0.3	91,360	8,954	9,240	13,000	29,260
PDC-3	3.8	18,000	553,920	609,960	29,000	19,400
ADC-7	8.0	8,880	30,720	32,040	4,344	7,020

Author Disclosure Block:

C.R. Bethel: None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; Wockhardt, Merck, AstraZeneca, Actavis. **M.D. Barnes:** None. **R.A. Bonomo:** E. Grant Investigator; Self; Wockhardt, Merck, AstraZeneca, Actavis, GSK.

Session Number:

373

Session Title:**New Beta-lactamase and Carbapenemase Inhibitors****Publishing Title:*****In Vitro* Activity of Ceftolozane-Tazobactam (C/T) against Gram-Negative Pathogens Isolated from Patients in Canadian Hospitals in 2011-2015****Author Block:**

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Abstract Body:

Background: C/T is a novel cephalosporin-beta-lactamase inhibitor combination with demonstrated in vitro activity against Gram-negative pathogens involved in complicated intra-abdominal and urinary tract infections. We determined the in vitro activity of ceftolozane with tazobactam (fixed 4 µg/mL concentration) and comparators versus Gram-negative pathogens, including extended-spectrum β-lactamase producing (ESBL) *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates recovered from January 2011 to December 2015 from patients in medical and surgical wards, intensive care units, clinics, and emergency rooms at 15 Canadian hospitals. **Methods:** Antimicrobial susceptibility testing was performed using broth microdilution panels following CLSI recommendations (M07-A10). Susceptibility was defined in accordance with CLSI, except for C/T, where the FDA breakpoints were used. Cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp. isolates were genetically characterized for ESBL-production using PCR and sequence analysis. **Results:**

<u>MIC₉₀/% susceptible</u>				
	<u>C/T</u>	<u>Ceftazidime</u>	<u>Meropenem</u>	<u>TZP</u>
<i>Escherichia coli</i> (4757)	0.5/99.5	1/93.8	≤0.03/100	4/97.8
<i>E. coli</i> CRO-R (400)	1/95.8	>32/30.8	≤0.03/99.8	16/91.8
<i>E. coli</i> ESBL (295)	1/96.3	>32/34.2	≤0.03/99.7	16/93.2
<i>Pseudomonas aeruginosa</i> (2337)	1/98.2 8/87.8	32/81.8 >32/0	8/79.5 32/44.3	64/84.3 512/10.3

<i>P. aeruginosa</i> (CAZ-R) (271)	4/91.5 4/93.0	>32/1.8 >32/40.0	32/39.4 >32/0	512/0 256/36.0
<i>P. aeruginosa</i> (TZP-R) (170)				
<i>P. aeruginosa</i> (MER-R) (300)				
<i>Klebsiella pneumoniae</i> (1541)	0.5/98.6 >64/77.6	1/96.0 >32/18.3	≤0.03/99.7 0.25/93	8/97.3 >512/63.4
<i>K. pneumoniae</i> CRO-R (71)	32/82.5	>32/25.8	0.12/96.8	>512/64.5
<i>K. pneumoniae</i> ESBL (62)				
<i>Enterobacter cloacae</i> (626)	8/84.1	>32/77.3	0.12/99.2	64/85.6
<i>E. cloacae</i> CRO-R (154)	16/41.6	>32/9.7	0.25/96.8	128/41.6
<i>E. cloacae</i> ERT-R (22)	16/37.5	>32/4.6	2/77.3	256/27.3
<i>Serratia marcescens</i> (391)	1/100	1/99.5	0.06/99.5	4/95.9
<i>Klebsiella oxytoca</i> (402)	0.5/100	0.5/98.5	≤0.03/100	128/88.1
<i>Proteus mirabilis</i> (381)	0.5/99.5	≤0.25/99.0	0.12/100	<1/100
<i>Enterobacter aerogenes</i> (176)	2/93.1	>32/76.1	0.12/99.4	32/88.6
<i>Acinetobacter baumannii</i> (108)	2/NA‡	32/79.6	1/95.4	64/85.2
<i>Stenotrophomonas maltophilia</i> (402)	>64/NA*	>32/23.6	>32/NA	>512/NA
C/T: ceftolozane-tazobactam, CRO-R: ceftriaxone-resistant; CAZ-R: ceftazidime-resistant; TZP: piperacillin-tazobactam, MER-R: meropenem-resistant, ERT-R: ertapenem-resistant, ESBL: extended-spectrum β-lactamase producing. ‡94.6% of isolates had MIC ≤4 μg/mL *28.6% of isolates had MIC ≤4μg/mL				

Conclusions: *Enterobacteriaceae* susceptibility to C/T, including isolates with resistance to oximinocephalosporins by a variety of mechanisms, was comparable or modestly better than piperacillin- tazobactam. *P. aeruginosa* isolates, including those resistant to piperacillin-tazobactam, meropenem or ceftazidime were highly susceptible to C/T. C/T was also active against *Acinetobacter baumannii*. Ceftriaxone-and ertapenem-resistant *Enterobacter cloacae* were generally resistant to C/T. In-vitro activity suggests that C/T may be useful for the treatment of infections caused by *P. aeruginosa* as well as highly resistant strains and many β-lactam-resistant *Enterobacteriaceae*.

Author Disclosure Block:

P. Lagacé-Wiens: None. **H. Adam:** None. **M. Baxter:** None. **J. Karlowsky:** None. **A. Walkty:** None. **G.G. Zhanel:** I. Research Relationship; Self; Cubist, Astellas, Merck, Basilea, Pharmascience, The Medicines Company, Tetrphase, Sunovion.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

Aztreonam-Avibactam: A Novel Combination Effective against Clinical Isolates of *Stenotrophomonas maltophilia*

Author Block:

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Abstract Body:

Background: *Stenotrophomonas maltophilia* is a gram-negative, non-fermentative bacillus that has emerged as an important cause of nosocomial infections. Increasing resistance to antibiotics with historically good susceptibility rates like trimethoprim / sulfamethoxazole (TMP/SMX), ceftazidime (CAZ), ticarcillin-clavulanate (TIC/CLV) and fluoroquinolones, warrants the discovery of novel compounds and/or novel combination therapies. Multiple intrinsic antibiotic resistance mechanisms are present in *S. maltophilia* with the inducible L1 (class B) and L2 (class A) β -lactamases being highly notable. In this work, the aztreonam-avibactam (AZT/AVI) combination was tested against a panel of clinical isolates. We hypothesized that AZT/AVI possesses activity against *S. maltophilia* as avibactam will inhibit the class A β -lactamase, L2, while aztreonam will be resistant to hydrolysis by L1. **Methods:** Clinical isolates of *S. maltophilia* were subjected to agar dilution MIC testing with TMP/SMX, CAZ, TIC/CLV, ciprofloxacin (CIP), minocycline (MIN), colistin (COL), aztreonam (AZT) and AZT/AVI. If break points for *S. maltophilia* were not available, those for *Pseudomonas aeruginosa* were used. Isolates were evaluated for presence of *bla*_{L1} and *bla*_{L2} by PCR. Double-disk diffusion testing for the detection of a synergistic effect between AZT and AVI was also performed for selected isolates. **Results:** Using a combination of in-house and previously published primers, *bla*_{L1} and *bla*_{L2} were detected in 25/29 isolates; whereas only *bla*_{L1} or *bla*_{L2} were detected in 4 and 2 isolates, respectively. Resistance towards TMP/SMX and CAZ was detected in 27/29 and 26/29 isolates, respectively. In contrast, only 7/29 and 1/29 isolates were resistant to CIP and MIN, respectively. Finally, resistance to COL and TIC/CLV was detected in 15/29 and 12/29 isolates, respectively. Notably, AZT/AVI combination restored susceptibility in 25/29 isolates, and synergy was observed in all the selected isolates. **Conclusions:** Results shown herein suggest that AZT/AVI is an effective combination against multidrug

resistant strains of *S. maltophilia*. The AZT/AVI combination would address an important unmet medical need towards an uncommon difficult-to-treat pathogen.

Author Disclosure Block:

M.F. Mojica: None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; AstraZeneca, Actavis, Merck, Wockhardt. **M.R. Jacobs:** E. Grant Investigator; Self; Wockhardt, AstraZeneca, Actavis. **J.J. LiPuma:** E. Grant Investigator; Self; AstraZeneca, Actavis. **R.A. Bonomo:** E. Grant Investigator; Self; AstraZeneca, Actavis, Merck, Wockhardt, GSK.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

FOX-4 Cephamicinase Interaction with Boronic Acid Transition State Inhibitors (BATSI)s)

Author Block:

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Abstract Body:

Background: Bacterial resistance to β -lactams underscores a pressing need for novel antibacterials. BATSIs bind efficiently to the serine nucleophile of class A and C β -lactamases and the structures of these complexes provide mechanistic information about the enzymes. The FOX1-10 family of β -lactamases hydrolyzes cephamycins with a 7 α -methoxy side chain and shows extended-spectrum activity toward ceftazidime and cefepime. To understand the mechanistic basis for this unique hydrolytic profile in FOX enzymes, we investigated the steady-state inhibition of FOX-4 by novel BATSIs and determined the crystal structure of FOX-4 in complex with two of them. **Methods:** FOX-4 was purified using *m*-aminophenyl boronic acid agarose. FOX-4/BATSI co-crystals grew in 0.05 M Zn acetate and 20% PEG 3350. For inhibition studies, BATSIs were pre-incubated with FOX-4. **Results:** The FOX-4 was probed with four novel BATSIs; the variable groups mimicked the R1 side chains of cephalothin ($K_{i\text{ app}} = 0.032 \pm 0.003 \mu\text{M}$, SM23 or $0.42 \pm 0.02 \mu\text{M}$, EC04), cefotaxime ($K_{i\text{ app}} = 0.26 \pm 0.02 \mu\text{M}$), and ceftazidime ($K_{i\text{ app}} = 0.11 \pm 0.02 \mu\text{M}$, LP06). Residue replacements in the active site explained differences in affinity of BATSIs between FOX-4 and other class C enzymes. The structures of FOX-4 in complex with sulfate, SM23 and LP06 were determined to $<1.5 \text{ \AA}$ resolution. The BATSIs bind to FOX-4 comparably to other AmpCs. The *m*-carboxyphenyl ring of SM23 is less hydrogen-bonded in FOX-4 than in AmpC from *E. coli*, allowing the ring to impinge on Leu119, changing its conformation. The oxyimino side chain of LP06 is disordered, and may interact with Phe293, a residue unique to the FOX family. Ordered waters that interact with the BATSI, Asn343, Asn346, and Arg349 are repositioned in FOX-4 compared to AmpC. **Conclusions:** The interaction between

FOX-4 and the BATSI reveals important electronic and structural features of this active site. The conserved ordered water molecules implicated in catalysis near residues 343, 346, and 349 are repositioned in FOX-4 and a hydrophobic pocket near Phe293 and Leu119 is rearranged by the presence of the BATSI side chain. These structures provide insight into how the transition state of the cephamycinase differs from other kinds of AmpCs.

Author Disclosure Block:

S.T. Lefurgy: None. **B. Biju:** None. **R. Toro:** None. **V.N. Malashkevich:** None. **S.C. Almo:** None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; AstraZeneca, Merck, Wockhardt, Actavis. **E. Caselli:** D. Employee; Self; Therabor. **F. Prati:** A. Board Member; Self; Therabor. D. Employee; Self; Therabor. **R.A. Bonomo:** E. Grant Investigator; Self; AstraZeneca, Merck, Actavis, Wockhardt, GSK.

Session Number:

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Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

Crystallographic Analyses of Inhibition of *Klebsiella* β -Lactamase KPC-2 by Novel Bridged Diazabicyclooctane (DBO) β -Lactamase Inhibitors

Author Block:

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Abstract Body:

Background: The inhibition of serine β -lactamases by DBOs such as avibactam is an attractive new strategy in our therapeutic armamentarium. Three novel DBOs (WCK 4234, 5107, and 5153) with different sidechains at the C2 position are currently in development. In this study, we examined the structural properties and the contributions of the C2 substituents to the inhibitory profile of these novel DBO inhibitors against KPC-2, a class A carbapenemase. **Methods:** The DBOs WCK 4234, WCK 5107 (INN: Zidebactam), and WCK 5153 complexed to KPC-2 were probed using protein crystallography involving both co-crystallization and inhibitor soaking experiments. X-ray diffraction data were collected at the SSRL synchrotron facility and the resolution of diffraction datasets ranged between 1.8 and 1.44Å. The six KPC-2 inhibitor complex structures were refined using REFMAC. **Results:** The 3 inhibitor soaked and inhibitor co-crystallized KPC-2 complex structures reveal that the DBOs bonded covalently to the catalytic S70. The position of the DBO scaffold in each of the structures is very similar to that of avibactam when bound to KPC-2, a structure previously determined (PLOS One, 10(9), 2015) using crystal soaking. A notable difference is that in two of the co-crystallized structures (WCK 5107 and WCK 5153), the sulfate moiety is no longer covalently connected to the DBO scaffold whereas this desulfation is not observed in their respective inhibitor soaked structure. Unlike WCK 5107 and WCK 5153, WCK 4234 has a nitrile moiety at the C2 position which is rigid and interacts with the N132 sidechain. This nitrile moiety at the C2 position of WCK 4234 could enhance inhibitory activity and protect this DBO inhibitor from desulfation when covalently attached to KPC-2 (K_i of WCK 4234 vs. KPC-2 is 0.3 μ M). **Conclusions:** The DBO KPC-2 complex crystal structures reveal that the three DBO inhibitors examined herein inhibit KPC-2 by forming similar covalent complexes. Interestingly, two DBOs (WCK 5107 and WCK

5153) undergo desulfation under co-crystallization conditions, whereas WCK 4234 does not (this is unique compared to avibactam and KPC-2). The details observed here and in other comparable structures with DBOs are offering new mechanistic insights into secondary active site chemistry followed by this novel class.

Author Disclosure Block:

N.Q. Nguyen: None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; Wockhardt, Merck, AstraZeneca, Actavis. **R.A. Bonomo:** E. Grant Investigator; Self; Wockhardt, Merck, AstraZeneca, Actavis, GSK. **F. van den Akker:** E. Grant Investigator; Self; Wockhardt.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

“Size Matters”: Probing the Mechanisms of Inactivation of Class C β -Lactamases with Avibactam (AVI)

Author Block:

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Abstract Body:

Background: AVI is a diazabicyclooctane (DBO) inhibitor that is paired with ceftazidime for the treatment of serious Gram negative infections. The chemical structure of AVI closely resembles portions of the cephem bicyclic ring system, and AVI was shown to bond covalently and reversibly to many serine β -lactamases. In rare cases, AVI is hydrolyzed. Knowledge of the interactions of AVI with different target enzymes is required to anticipate future resistance threats. Here, we probed the atomic structure of AVI with P99, GC1 and FOX-4, three representative class C β -lactamases with unique hydrolytic properties, in order to understand how diverse active site topologies affected AVI inhibition. **Methods:** P99 and GC1 β -lactamases from *Enterobacter cloacae* were crystallized in space group $P2_12_12$ with one molecule in the asymmetric unit by vapor diffusion method and FOX-4 was $P2_12_12_1$ with two molecules. Refinements were performed by SHELX (GC1 and P99) or PHENIX (FOX-4). **Results:** AVI complexes were obtained by soaking with 1 mM AVI for 60 min. Diffraction data of P99, GC1 and FOX-4 β -lactamases complexes with AVI were collected and resolved to 1.15, 1.15 and 1.5 Å resolution, respectively. From the current models (~ 1.5 Å), the binding of P99 and FOX-4 seems to be similar to AmpC (*Pseudomonas aeruginosa*):AVI complex reported previously. On the other hand, the GC1:AVI complex appears more flexible: two alternate conformations of sulfonate group and at least two conformations for Ω loop region. **Conclusions:** AVI bound to P99, FOX-4, and GC1 reveals similar features compared to the previous *P. aeruginosa* AmpC structure. Again, the movement of the Tyr150 recapitulates a key finding important in acylation/deacylation chemistry. Interestingly, the extended spectrum class C β -lactamase, GC1, discloses alternate conformations of AVI. Taken together, these findings suggest that differences in the

active-site volume/flexibility can be overcome by the steric properties of small molecule DBO inhibitors, specifically AVI. This key feature may explain in part activity of AVI against many class A and C enzymes.

Author Disclosure Block:

M. Nukaga: E. Grant Investigator; Self; AstraZeneca, Actavis. **S.T. Lefurgy:** None. **M.D. Barnes:** None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; Actavis, AstraZeneca, Merck, Wockhardt. **R.A. Bonomo:** E. Grant Investigator; Self; Actavis, AstraZeneca, Merck, Wockhardt, GSK.

Session Number:

373

Session Title:

New Beta-lactamase and Carbapenemase Inhibitors

Publishing Title:

Inhibition of KPC-2 by Vaborbactam (VAB; Formerly Rpx7009) Does Not Involve Ser130 (S130) That Is Important for Its Inhibition by Avibactam (AVI)

Author Block:

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Abstract Body:

Background: The S130 residue plays important role in the catalytic mechanism of beta-lactamases, including KPC-2. Mutations in this position significantly affect interactions with beta-lactams and some inhibitors (BLIs), including AVI. In molecular modelling of VAB into KPC-2, we postulated that S130 is not involved in interactions with this BLI. The objective of these studies was to evaluate the effect of the S130G substitution on KPC-2 inhibition by VAB. **Methods:** S130G was introduced in bla_{KPC-2} by site-directed mutagenesis (KPC-2-S130G). Susceptibility of the strains of *P. aeruginosa* (PAM1154 background, lacks major efflux pumps) producing either wt KPC-2 or KPC-2-S130G to various antibiotics and BLI combinations were tested. The expression level of KPC-2 and KPC-2-S130G in PAM1154 was assessed by western blotting with anti-KPC-2 antibodies. Kinetic parameters (k_{cat} , K_m) of purified KPC-2 and KPC-2-S130G and K_i for VAB and AVI were determined spectrophotometrically using nitrocefin (NCF) as substrate. **Results:** S130G mutation in KPC-2 resulted in the loss of resistance to cephalosporins and carbapenems but not penicillins. The KPC-2-S130G mutant was also significantly more resistant to potentiation of penicillins by AVI than the wild type (wt) protein; AVI at 4 µg/ml reduced carbenicillin (CAR) and piperacillin (PIP) MIC 16 and 256-fold, respectively, against the strain producing wt KPC-2; no potentiation was observed against KPC-2-S130G. Notably, the S130G mutation had no effect on potentiation of CAR and PIP by VAB. In biochemical experiments with NCF as substrate, catalytic efficiency k_{cat}/K_m for KPC-2-S130G was increased 4-fold compared to wt KPC-2. K_i for AVI with KPC-2 and KPC-2-S130G was 0.012 µM and 70 µM, respectively, representing a ~6000-fold decrease due to S130G. In contrast, K_i values of VAB with KPC-2 and KPC-2-S130G did not differ

significantly (0.034 and 0.011 μM , respectively). **Conclusions:** Both microbiological and biochemical data demonstrate that S130 that plays significant role in inhibition of KPC-2 by AVI, but this position appears to have no role in inhibition of KPC-2 by VAB. These data indicate that modification of the S130 side-chain does not disrupt key interactions between VAB and KPC-2, and confirms the distinctive binding mode of VAB in this enzyme.

Author Disclosure Block:

R. Tsivkovski: D. Employee; Self; The Medicines Company. **M. Totrov:** D. Employee; Self; Molsoft. **O. Lomovskaya:** D. Employee; Self; The Medicines Company.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

The Impact of *ramR* Sequence Variations on Tigecycline Susceptibility in *Klebsiella pneumoniae*

Author Block:

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Abstract Body:

Background: Mutations in *ramR* encoding defective RamR repressors lead to overexpression of *ramA* and multidrug-resistance (MDR) in *Klebsiella pneumoniae* (KP). Since MDR strains containing *ramR* sequence variants (*ramR-v*) often have other resistance determinants, (TGC) susceptibility, a variety of *ramR-v* were cloned and recombinantly expressed in two unrelated KP hosts containing inactivated RamR.

Methods: The *ramR* genes of 18 KP strains were sequenced to confirm *ramR-v* vs. the reference strain, GenBank # AHF70994.1. *ramR-v* genes were cloned into pBAD-myc-hisB containing a gentamicin resistance gene and transformed into KP488 (RamR⁻, TGC MIC=4 µg/mL) and KP1914 (RamR⁻, TGC MIC=8 µg/mL). MIC assays were run as per CLSI guidelines using arabinose to overproduce RamR variant repressors (RamR-v); controls contained either empty vector or vector expressing wild-type (WT) *ramR*. Total RNA was isolated from a subset of KP488 strains grown to log phase, and cDNA stocks were prepared; *ramA* and *acrA* transcription was quantified by a TaqMan qPCR assay.

Results: Strains producing RamR-v T18I, A19V, Y59C, K63M, and I141T all showed reversal of TGC-resistance (TGC-R) in both KP hosts, similar to strains producing WT RamR, whereas production of RamR-v A40T, A40P, G42E, T43R, L44Q, F45L, A105E, Q122-stop, and A153P RamR-v did not reverse TGC-R in either KP host. Production of RamR-v A22T and G96D showed partial reversal of TGC-R in KP488, and full reversal in KP1914. RamR-v E41K did not reverse TGC-R in KP488, but showed full reversal in KP1914, whereas T119P partially reversed TGC-R in KP488 but not in KP1914. Production of RamR-v A19V in KP488 showed WT levels of *ramA* and *acrA* RNA while *ramA* and *acrA* RNA levels were elevated 9- and 3-fold in RamR-v G42E and Q122-stop strains, respectively.

Conclusions: Complementation studies revealed that not all amino acid variants of the RamR repressor were defective in RamR⁻ hosts. Some

RamR-v behaved differently in KP488 vs. KP1914, suggesting that RamR-mediated TGC susceptibility can involve other factors. For RamR-v A19V, G42E and Q122-stop, *ramA* and *acrA* transcript levels correlated with TGC susceptibility in KP488 and KP1914.

Author Disclosure Block:

C. Fyfe: D. Employee; Self; C. Fyfe. **K. Taquechel:** D. Employee; Self; Kiara Taquechel. **Y. Gao:** D. Employee; Self; Y. Gao. **J.A. Sutcliffe:** D. Employee; Self; J. Sutcliffe. **T.H. Grossman:** D. Employee; Self; T. Grossman.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Investigation of AcrA Interactions with Substrates and Inhibitors of Efflux Pump

Author Block:

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Abstract Body:

Background: Resistance to antibiotics is a major global threat to public health. In Gram-negative bacteria, efflux pumps of the RND superfamily make a major contribution to multidrug resistance. To date, AcrAB-TolC from *E. coli* is the best-characterized multidrug efflux complex. AcrA, the periplasmic membrane fusion protein (MFP) is a critical component of efflux pumps that is responsible for the recruitment of the outer-membrane channel and the activation of the efflux transporter. In this study we analyzed interactions of AcrA with substrates and inhibitors of the efflux pump. **Methods:** Surface Plasmon Resonance (SPR) was used to determine direct physical interaction with purified AcrA immobilized onto a CM5 chip. *In vivo* minimal potentiating concentration (MPC) and minimal inhibitory concentrations (MICs) were determined. A real-time fluorescence based assay using Hoechst 33342 (HT) was used to determine correlations between interaction, binding and efflux inhibition. **Results:** We analyzed the affinities of the efflux pump inhibitors Pa β N, MBX2319 and 1-[1-naphthylmethyl]-piperazine (NMP), as well as those of the substrates novobiocin and erythromycin, to the purified AcrA protein. Sensorgrams were collected with immobilized AcrA and increasing concentrations of ligands. We found that MBX2319, NMP and erythromycin do not bind AcrA with detectable affinities. In contrast, Pa β N and novobiocin bind AcrA with low-to-mid micromolar affinities, suggesting that these two compounds may interact with AcrA. The checkerboard assay showed that Pa β N interacts synergistically with novobiocin in *E. coli*. The MPC4 of Pa β N was found to be 16 μ M. Treatment of cells with increasing concentrations of Pa β N and NMP from 0- 50 μ M did not inhibit efflux of HT. In contrast, MBX2319, effectively inhibited the HT efflux. **Conclusions:** Our studies show that some substrates and inhibitors of efflux pumps can interact not only with the transporter AcrB but also with the periplasmic fusion protein AcrA. This result suggest that the periplasmic MFPs may directly contribute to substrate binding and activation of efflux. We have

developed an effective platform that can be used to analyze the mechanism of efflux pump inhibition through interactions with AcrA.

Author Disclosure Block:

N. Abdali: None. **H.I. Zgurskaya:** None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Sensitization of Quinolone Resistance by Targeting SOS System: A Genetic and Microbiological Approach

Author Block:

E. Recacha¹, J. Machuca¹, P. Diaz de Alba¹, M. Ramos Güelfo¹, F. Docobo Perez², J. Rodriguez Beltran³, J. Blazquez³, A. Pascual², **J-M. Rodriguez-Martinez²**; ¹Univ. Hosp. Virgen Macarena, Seville, Spain, ²Univ. of Seville, Seville, Spain, ³Inst. of Biomedicine of Seville, Seville, Spain

Abstract Body:

Background: SOS system has been postulated as target with potential impact in both quinolone resistance level and mutant development. The aim of this study was to evaluate the impact of SOS system suppression in a collection of quinolone resistant isogenic *Escherichia coli* strains using a microbiological approach. **Materials/Methods:** Six isogenic strains were used: ATCC 25922 (wild-type), EC02 (*E. coli* ATCC 25922 plus Ser83Leu substitution in GyrA), EC04 (EC02 plus Ser80Arg substitution in ParC), EC08 (EC04 plus Asp87Asn substitution in GyrA), EC09 (EC08 plus deletion in *marR*) and EC59 (EC09 expressing QnrS1). To switch-off the SOS system, *recA* gene was inactivated (all strains) and *lexA* was replaced by *lexA1* (wild-type, EC04 and EC09). Susceptibility testing to fluoroquinolones was determined by broth microdilution. Growth curves (using Infinite 200Pro, Tecan) and killing curves were determined using ciprofloxacin at 1 mg/L (breakpoint for reduced susceptibility), 2.5 mg/L (Cmax in serum) and 0.5xMIC of the wild-type for SOS system for each isogenic group. **Results:** For all the strains, MICs of ciprofloxacin decreased (2-8-folds) when SOS system was inactivated (*RecA*⁻ or *lexA1*) compared to wild-type strains. EC08 with MIC of 2 mg/L decreased to 0.5 mg/L in *recA* deficient bacteria (from intermediated-susceptibility to susceptible) and EC09 with MIC of 4 mg/L decreased to 1 mg/L (resistant to susceptible). In killing curve assays, SOS inactivation produced a clear reduction in CFU/ml (4-9 Log) after 24h-incubation at concentrations corresponding to 0.5xMIC of the wild-type strains. Similar differences were observed for EC08 and EC09 at 1 mg/L and 2.5 mg/L, respectively. In growth curve assays, SOS activation produced an increase in optical density (OD: 0.4 at 8 hours and OD: 0.6 at 24 hours), while no growth was

observed for those strains deficient in SOS system. **Conclusions:** This genetic and microbiological approach shows the sensitization of quinolone resistant *E. coli* (even involving clinical category changes) by targeting *recA* gene. SOS system inactivation might be a potential adjuvant strategy for quinolones use.

Author Disclosure Block:

E. Recacha: None. **J. Machuca:** None. **P. Diaz de Alba:** None. **M. Ramos Güelfo:** None. **F. Docobo Perez:** None. **J. Rodriguez Beltran:** None. **J. Blazquez:** None. **A. Pascual:** None. **J. Rodriguez-Martinez:** None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Zinc Prevents Emergence Of Antibiotic Resistance Via Inhibition Of The Sos Response In *escherichia Coli*

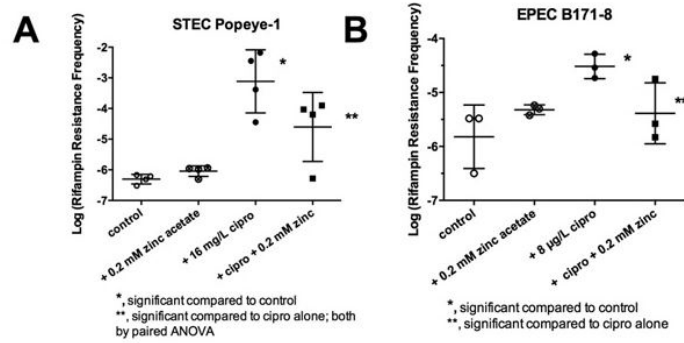
Author Block:

J. K. CRANE, B. E. Bunnell, K. L. Bair, M. D. Sutton; Univ. at Buffalo, BUFFALO, NY

Abstract Body:

Our laboratory became interested in the *E. coli* SOS response to DNA damage because of the SOS responses's role in inducing Shiga toxin (Stx) from Shiga-toxigenic *E. coli* (STEC). In addition to Stx production, the SOS response also triggers a myriad of bacterial cell responses, including DNA repair, elongation of bacterial cells, induction of error-prone DNA polymerases, induction of latent bacteriophage, and inhibition of cell division. Induction of error-prone DNA polymerases for trans-lesion synthesis of damaged DNA leads to an increased mutation rate, known as hypermutation or adaptative mutation. Zinc inhibits most of the other facets of the SOS response. Our hypothesis was that zinc would inhibit hypermutation as well. We developed procedures for inducing the SOS response in *E. coli* using ciprofloxacin (cipro). Cipro causes double-stranded breaks in bacterial DNA, strongly triggering the SOS response. First, we measured the SOS response by measuring the abundance of *recA* RNA by qRT-PCR and by use of a *recA-lacZ* reporter strain, JLM281. Next, we measured the frequency of mutation to rifampin resistance in *E. coli* exposed to cipro vs. untreated control and found that hypermutation was observed. 0.2 mM zinc acetate inhibited the SOS response and also blocked the hypermutation response in STEC, in enteropathogenic *E. coli* (EPEC), and extra-intestinal *E. coli* strains (ExPEc). Zinc also blocked hypermutation in *Klebsiella pneumoniae*. No hypermutation was observed in a *recA* mutant of STEC EDL933, called EDL933R. Conclusions: Hypermutation can be induced in *E. coli* using drugs that damage DNA, including cipro and nucleoside analogs. Functioning RecA is required for

hypermutation, and zinc blocks both the SOS response and hypermutation in *E. coli* and



Klebsiella.

Author Disclosure Block:

J.K. Crane: None. **B.E. Bunnell:** None. **K.L. Bair:** None. **M.D. Sutton:** None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

qnrD* Regulation is Sos-mediated and Tobramycin Increases *qnrD* Expression Under the Control of Sos in *Escherichia coli

Author Block:

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²Massachusetts Eye and Ear Infirmary, Harvard Med. Sch., Boston, MA, ³Brigham and Women's Hosp., Harvard Med. Sch., Boston, MA

Abstract Body:

Background: SOS regulation of the transcription of *qnrB* has been clearly described, but little is known about the effect on *qnrD*. We investigated SOS transcriptional regulation of *qnrD*, carried by plasmid pDIJ09-518a after treatment with sub-inhibitory concentrations of ciprofloxacin (CIP), mitomycin C (MMC) and tobramycin (TM), using real-time quantitative PCR. **Methods:** The expression of *qnrD* transcripts was measured in *E. coli* MG1656 (WT) and derivatives $\Delta recA$, $\Delta lexA$ and *lexA*ind3 (non-cleavable LexA) carrying pDIJ09-518a after exposure to CIP 0.06 $\mu\text{g/mL}$, MMC 0.1 $\mu\text{g/mL}$ and TM 0.1 $\mu\text{g/mL}$. The fold change in *qnrD* gene expression was calculated using the $2^{-\Delta\Delta CT}$ method, in triplicate, and from 3 independent experiments. **Results:** In the WT strain, *qnrD* transcript expression increased 3 fold with SOS inducers CIP and MMC. With the $\Delta lexA$ derivative, a 2-fold increase in *qnrD* transcript expression was found, but not in the $\Delta recA$ or *lexA*ind3 derivatives. These results indicate that *qnrD* expression is mediated by the SOS response in a RecA-LexA dependent manner. Strikingly, TM induced a 3.5, 2.5, 2.5 and zero- fold changes of *qnrD* transcript expression in the WT, $\Delta lexA$, $\Delta recA$ and *lexA*ind3 respectively, showing that TM also induces the SOS response in *E. coli*. **Conclusion:** It is known that in *E. coli*, fluoroquinolones (FQ), but not aminoglycosides (AG), as described for *V. cholerae*, induce SOS responses. In this work, it was shown that SOS not only regulates transcript expression of *qnrD* but also that TM increases *qnrD* expression under the control of SOS in *E. coli* when *qnrD* is carried by pDIJ09-518a. Overall, our findings reveal an unexpected antibiotic resistance co-selection with AG that may promote emergence of FQ resistance. This could be a worst-

case scenario, enhancing the risk for emergence of high-level resistance to FQ in Qnr-producing *E. coli*.

Author Disclosure Block:

A. Babosan: None. **F. Lebreton:** None. **C. de Champs:** None. **G. Pier:** None. **D. Skurnik:** None. **T. Guillard:** None.

Session Number:

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Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Transcriptomic Analyses of an HNS-Mediated, Colistin-Resistant, Clinical Isolate of *Acinetobacter baumannii*

Author Block:

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Abstract Body:

Background: Colistin is a last-line treatment option for infections caused by multi-drug resistant strains of *Acinetobacter baumannii*. The known mechanisms of colistin resistance in *A. baumannii* involve changes to the lipopolysaccharide (LPS), via the addition of phosphoethanolamine (PEtn) or complete inactivation of lipid A biosynthesis. Previously, we characterized a colistin-susceptible *A. baumannii* parent strain and a colistin-resistant mutant isolated from a patient pre- and post-colistin treatment, and found that colistin resistance was conferred by the insertion of IS*Aba125* within *hns*, a gene encoding an HNS-family transcriptional regulator. **Method:** To understand this mechanism of colistin resistance and identify the HNS regulon in *A. baumannii*, RNA-seq analysis was performed on purified mRNA from both the pre- and post-treatment isolates, grown under normal laboratory conditions. **Results:** 171 differentially expressed genes were identified (143 genes were up regulated, 28 genes were down regulated in the colistin-resistant strain). The genes include those involved in motility (type I pili), biofilm formation, chemotaxis and the type VI secretion system. Importantly, the PEtn transferase gene, *eptA* (a *pmrC* homolog) showed increased expression (5.2 Fold change) in the *hns* mutant whereas the expression of the Pmr operon (*pmrA*, *pmrB* and *pmrC*), known to be involved in colistin-resistance via PEtn modification of lipid A, remained unchanged. Expression of *eptA* on pWH1266 in the colistin-susceptible isolate conferred colistin-resistance compared to a colistin-susceptible isolate harbouring pWH1266 vector alone. **Conclusions:** Colistin-resistance in the *hns* mutant is mediated via increased expression of *eptA* which is predicted to result in additional PEtn modification of lipid A. This is the first time an HNS-family transcriptional regulator has been associated with the regulation of a PEtn transferase and associated colistin resistance.

Author Disclosure Block:

D. Deveson Lucas: None. **D. Powell:** None. **D. Bulach:** None. **A. Wright:** None. **J. Li:** None. **M. Harper:** None. **J.D. Boyce:** None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

β -Lactamase (*bla*) Gene Expression in *Burkholderia cepacia* Complex (Bcc) Is Induced by β -Lactams

Author Block:

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Abstract Body:

Background: The Bcc is a group of 20 related species of nosocomial pathogens of which *Burkholderia multivorans* (Bm) and *Burkholderia cenocepacia* (Bcn) are most prevalent currently. β -Lactams are recommended as therapies for infections due to Bcc. The Bm and Bcn genomes possess *bla*_{penA} and *bla*_{penB}, respectively as well as *bla*_{ampC}. Upstream of *bla*_{pen}, a divergent gene for a LysR-type transcriptional regulator (LTTR) is present. In Enterobacteriaceae, LTTRs were shown to regulate expression of *bla* genes upon exposure to β -lactams by complex processes tied to peptidoglycan synthesis and recycling. Thus, LTTRs are critical components in the regulation of expression of *bla* genes and contribute to β -lactam resistance. The goal of this work is to understand the regulation of transcription of β -lactamases in Bm and Bcn to provide insights into future therapies. **Methods:** Analytic isoelectric focusing (aIEF) and/or immunoblotting (IMB) were used to measure β -lactamase protein expression upon exposure to sub-MIC concentrations of β -lactams in Bm strain 249, Bcn strain J2315, and clinical Bm isolates. We also generated homology models of the LTTRs present in Bm and Bcn. **Results:** aIEF and IMB revealed that PenA, PenB, and AmpC expression occurred upon exposure to various β -lactams. Imipenem (1 mg/L) was the strongest inducer of PenA and AmpC in Bm and ampicillin (50 mg/L) of PenB and AmpC in Bcn. Moreover, ampicillin also induced β -lactamase expression in a panel of 18 clinical isolates of Bm. The induction patterns for the clinical isolates were different in β -lactamase banding patterns (suggesting the presence of other β -lactamases or Pen variants) as well as in intensity of expression from Bm 249. Importantly, we also found that PenA was expressed without induction in one clinical strain. The putative Burkholderial LTTRs were structurally comparable to LTTRs from Enterobacteriaceae and an amino acid sequence alignment

showed ~90% similarity. **Conclusions:** Expression of β -lactamases in Bm and Bcn was inducible via growth in the presence of β -lactams. Clinical isolates of Bm demonstrated different β -lactamase induction patterns in response to β -lactams. These findings may explain the changes in resistance phenotype observed when patients are treated with β -lactams.

Author Disclosure Block:

S.A. Becka: None. **J.A. Gatta:** None. **E.T. Zeiser:** None. **J.J. LiPuma:** E. Grant Investigator; Self; AstraZeneca, Actavis. **K.M. Papp-Wallace:** E. Grant Investigator; Self; AstraZeneca, Actavis, Merck, Wockhardt.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

A Sensor Histidine Kinase is a β -Lactam Receptor and Induces Resistance to β -Lactam Antibiotics

Author Block:

X. Zhou; Univ. of Connecticut, Storrs, CT

Abstract Body:

Beta-lactams disrupt bacterial cell wall synthesis and these agents are the most widely used antibiotics. One of the principle mechanisms by which bacteria resist the action of beta-lactams is by producing beta-lactamases, enzymes which degrade beta-lactams. In Gram-negative bacteria, production of beta-lactamases is often induced in response to the antibiotic associated damage to the cell wall. Here, we have identified a novel mechanism that governs beta-lactamase production. In the Gram-negative enteric pathogen *Vibrio parahaemolyticus*, we found a histidine kinase/response regulator pair (VbrK/VbrR) that controls expression of a beta-lactamase. Mutants lacking either VbrK or VbrR do not produce the beta-lactamase and are no longer resistant to beta-lactam antibiotics. Notably, VbrK autophosphorylation is activated by beta-lactam antibiotics, but not by other lactams. However, single amino acid substitutions in the putative periplasmic binding pocket of VbrK leads its phosphorylation in response to both beta-lactam and other lactams, suggesting that this kinase is a beta-lactam receptor that can directly detect beta-lactam antibiotics instead of detecting the damage to cell wall resulting from beta-lactams. In strong support of this idea, we found that purified periplasmic sensor domain of VbrK binds penicillin and that such binding is critical for VbrK autophosphorylation and beta-lactamase production. Direct recognition of beta-lactam antibiotics by a histidine kinase receptor may enable sufficiently rapid induction of beta-lactamase to degrade beta-lactam antibiotics before the integrity of the cell wall is disturbed. Thus, it represents an evolutionarily favorable mechanism to defend against beta-lactam antibiotics.

Author Disclosure Block:

X. Zhou: None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Adaptive Laboratory Evolution of *E. coli* Reveals Slow Resistance Development to a Combination of Three Novel Antimicrobial Compounds and to the Short Amp P9-4

Author Block:

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Abstract Body:

Antimicrobial peptides (AMPs) have for long been considered as promising new antimicrobials since resistance was not expected. However, adaptive evolution experiments (ALE) have demonstrated that bacteria may indeed develop resistance also to AMPs. We and others hypothesize that the risk of resistance development decreases when two or more compounds are combined as compared to single-drug treatments. The purpose of this study was to investigate this hypothesis. We exposed *E. coli* ATCC 25922 to peptidomimetic HF-1002-2 and the AMPs novicidin and P9-4 alone and in combination in an ALE. All lineages exposed to HF-1002-2 and three out of four lineages exposed to novicidin adapted to 32× MIC, after passaging through approx. 350 generations. In contrast, only one out of four lineages exposed to the combination reached 32× MIC. Thus, resistance to novicidin and HF-1002-2, administered alone, developed more readily than in lineages exposed to the combination. Surprisingly, none of the lineages exposed to P9-4 adapted to 32× MIC. We sequenced the whole genomes of both lineages and individual clones and analyzed the sequences using CLC Genomics Workbench 8. Typically, one to two mutations (Single Nucleotide Polymorphisms or Deletion Insertion Polymorphisms) were detected in coding regions of the control lineages, while one to four mutations were found in the peptide-adapted lineages. A different pattern was seen in the adapted lineages as compared to the control ones, which were re-cultured in absence of peptides. A deletion in the gene encoding for the tetratricopeptide repeat family protein was present in seven out of eight adapted lineages. This may suggest that a common evolutionary trajectory has ensured development of resistance both to the single compounds and the combination of three. We are currently analyzing the other detected mutations and their biological role. This may explain the impact of these mutations on the different rate of resistance development. In fact, the short antimicrobial peptide P9-4 with slow resistance rate may be considered a promising candidate for further optimization and future application in clinical settings.

Author Disclosure Block:

L. Citterio: None. **H. Franzyk:** None. **L. Gram:** None.

Session Number:

374

Session Title:

RNA Modulation and Cellular Signals in Antimicrobial Resistance

Publishing Title:

Interplay between RNA Expression and Protein Production of OmpC and OmpF Porins in Ctx-M-Producing *Escherichia coli*

Author Block:

C. Suelter, N. D. Hanson; Creighton Univ., Omaha, NE

Abstract Body:

Background: Porins are the primary route of entry for β -lactam antibiotics in Gram-negative organisms. Alterations in porin production can decrease permeability across the outer membrane, limiting antibiotic entry and resulting in a carbapenem-resistant phenotype when complemented with an extended-spectrum β -lactamase (ESBL). CTX-M β -lactamases are the most prevalent ESBLs worldwide and confer resistance to cephalosporins but not carbapenems. The objectives of this study were to evaluate RNA expression and protein production of porins, OmpC and OmpF, in 9 clinical isolates expressing either CTX-M-14 or CTX-M-15 and evaluate carbapenem and ceftazidime susceptibility. **Methods:** RNA expression was analyzed by qRT-PCR, and relative fold change was calculated using the $2^{-\Delta(\Delta CT)}$ method. Porin production was evaluated by Western blot, and relative fold change was quantified using Stain-Free™ technology. Isolate XQ13 expressed CTX-M-14 at low levels and was the comparator strain in all studies. Carbapenem and ceftazidime minimum inhibitory concentrations (MICs) were measured by Etest® and susceptibilities interpreted by CLSI guidelines. **Results:** Increased *ompC* expression was observed in all isolates (2 to >15,000-fold), but surprisingly, OmpC production was decreased 3-12-fold. 3/8 isolates showed decreased *ompF* expression (2-5-fold) with similar levels of protein production. 1 isolate had 20- and 27-fold increases in OmpF RNA and protein production, respectively, with a concomitant 12-fold decrease in OmpC production. The same isolate had a 6-fold increase in ceftazidime susceptibility. All isolates were susceptible to carbapenems, but a 2- and 11-fold decrease in doripenem and ertapenem MICs were observed for the OmpF overproducing isolate, respectively. **Conclusions:** Decreased porin production in the presence of ESBL production has been associated with carbapenem resistance. However, these data suggest that an interplay between the production of OmpC and OmpF may contribute to ceftazidime and carbapenem susceptibility in ESBL-producing isolates. Our

data also suggests that the level of porin production is critical and requires a specific threshold not met in these isolates for the emergence of carbapenem resistance. The discordant production of OmpC RNA and protein indicates the complex regulation of OmpC production and its role in β -lactam resistance.

Author Disclosure Block:

C. Suelter: F. Investigator; Self; STRECK. **N.D. Hanson:** E. Grant Investigator; Self; STRECK.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

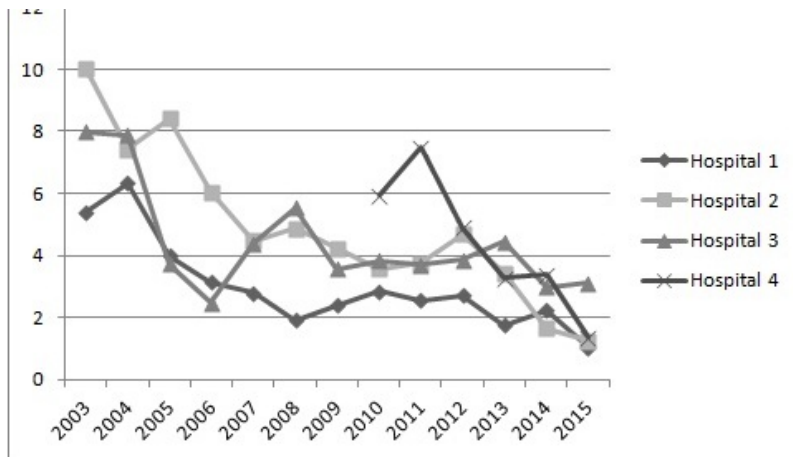
The Impact of MRSA Admission Nasal Surveillance on Nosocomial Infection: Ten Years of Testing

Author Block:

D. M. Schora, B. Smith, A. Robicsek, R. B. Thomson, Jr, L. R. Peterson; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Background: In August 2005 we were the first US system to begin testing all admitted patients for MRSA nasal colonization. Patients found colonized with MRSA were placed in contact isolation and decolonized. In 2012 the program was modified to only test patients with a high risk of MRSA colonization. To determine the effectiveness of the program, MRSA nosocomial infection rates were examined for the last 10 years. An increase in the MRSA nosocomial infection rate would warrant a new approach to surveillance.**Methods:** Data was reviewed from 8/1/2003 through 7/31/2015. Samples were tested with the BD GeneOhm™ MRSA PCR assay within 24 hours of admission. Patients with a positive test were identified as colonized, placed in contact isolation for the duration of their stay, given 5 days of nasal mupirocin and bathed with chlorhexidine on days 1, 3, 5 of mupirocin treatment. Testing compliance was monitored monthly during year 1, then every 6 months to maintain 90% compliance. A 4th hospital was added to the system where testing began in 2010. In January 2012 we modified the testing to a risk based approach and began testing approximately 50% of admissions (Robicsek, ICHE Vol 32). In 2013, mupirocin and chlorhexidine regimens were no longer given. MRSA nosocomial infection rates were determined with the Carefusion MedMined® Nosocomial Infection Marker for blood, respiratory, urine and wound infections (Peterson, ICHE Vol 33).**Results:** Rate of MRSA Nosocomial Infection (per 10,000 patient days; $p < 0.01$ for each hospital).



Conclusion: The MRSA nosocomial infection rate decreased over time and remains below pre-surveillance levels; Hospital 4 declined after implementing the program. MRSA nasal surveillance was effective and continues as part of our infection prevention program.

Author Disclosure Block:

D.M. Schora: None. **B. Smith:** None. **A. Robicsek:** None. **R.B. Thomson:** None. **L.R. Peterson:** C. Consultant; Self; Consultant with Cepheid and Roche. I. Research Relationship; Self; Research Grants with BD, Cepheid and Roche.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

Multi-Year Comparison of Panton-Valentine Leukocidin (PVL) Carrying *Staphylococcus aureus* Prevalence in Inpatient and Outpatient Populations

Author Block:

K. Mendoza, R. B. Thomson, Jr., D. Schora, L. M. Sabatini, L. R. Peterson; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Introduction: In April of 2007 we began testing methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from nasal surveillance for the presence of Panton-Valentine Leukocidin (PVL), a cytotoxin typical of community-associated MRSA (CA-MRSA). We used PVL identification as a surrogate marker for determination of CA-MRSA proportion in our patient population. Data on PVL prevalence rates were compared among inpatient and outpatient populations over 9 years. **Methods:** Data was reviewed from 04/14/2007-12/31/2015. Inpatient specimens were those patients tested upon admission to the hospital or in the ICU. Outpatient specimens were from patients tested within 30 days prior to planned surgery. The presence of PVL was detected from colonies of MRSA with an in-house developed real-time PCR (Robicsek, ICHE Vol 32). Results of the test were documented in the laboratory information system (SoftLab®, SCC Soft Computer) and yearly reports were generated with the epidemiology reporting function for data review. **Results:** Prevalence of PVL positive MRSA Isolates is in the

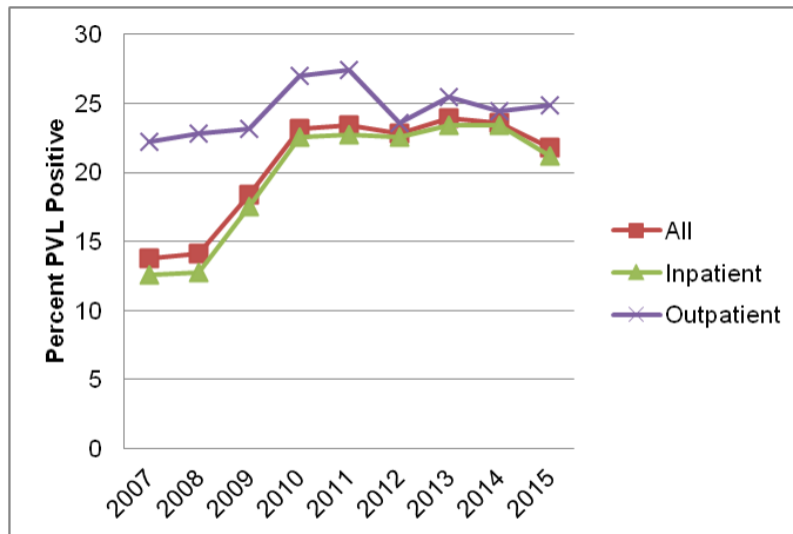


Figure.

Conclusion: At the beginning of our surveillance PVL strains dominated in the outpatient setting ($p < .001$ for 2007-9), and by the end PVL positivity in the hospital had risen to mirror that in the community ($p = .1$ for 2013-15). To reduce PVL positive (community) prevalence it is likely that both hospital and community interventions will be needed as the hospital rate increasingly followed the community rate over time. It also suggests that PVL is not better suited to expand in the hospital than are other MRSA strains, and the prevalence reflects that in the community.

Author Disclosure Block:

K. Mendoza: None. **R.B. Thomson:** None. **D. Schora:** None. **L.M. Sabatini:** None. **L.R. Peterson:** None.

Session Number:

436

Session Title:**Epidemiology and Control of MRSA****Publishing Title:****Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Canadian Hospitals from 2007-2015****Author Block:**

K. A. Nichol¹, H. J. Adam¹, G. R. Golding², M. McCracken², M. R. Baxter³, J. A. Karlowsky¹, D. J. Hoban¹, G. G. Zhanel³, Canadian Antimicrobial Resistance Alliance (CARA); ¹Diagnostic Services Manitoba, Winnipeg, MB, Canada, ²Natl. Microbiol. Lab., Winnipeg, MB, Canada, ³Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: As part of the CANWARD surveillance study, we compared the epidemiology of community-associated (CA) and healthcare-associated (HA)-MRSA genotypes in Canadian hospitals. **Methods:** Between 2007 and 2015, 1850 MRSA were collected from patients attending tertiary-care medical centres across Canada. Susceptibility testing was performed by broth microdilution in accordance with CLSI guidelines. Isolates were characterized by *spa* typing and PCR of the Panton-Valentine leukocidin (PVL) gene. **Results:** The annual proportion of MRSA genotypes is shown below:

MRSA Type	Study Year									P-value*
	2007	2008	2009	2010	2011	2012	2013	2014	2015	
All MRSA(% of all <i>S. aureus</i>)	26.1	27.0	21.0	21.2	19.3	18.2	20.1	20.2	19.2	0.0003
HA-MRSA (% of all MRSA)	79.2	69.1	65.5	58.7	59.7	54.4	57.2	56.1	48.3	<0.0001
CMRSA1 [USA600]	2.3	1.1	0.0	1.8	0.6	4.0	0.6	3.2	0.7	0.3004
CMRSA2 [USA100/800]	64.9	56.3	58.6	49.8	55.8	43.2	48.4	46.5	44.8	<0.0001
CMRSA3/6	10.6	8.8	4.7	3.1	0.6	0.0	3.8	1.9	0.0	<0.0001

CMRSA4 [USA200]	0.0	0.4	0.0	0.9	0.0	0.8	0.6	0.0	0.7	0.2708
CMRSA5 [USA500]	1.0	1.5	0.0	1.3	1.3	2.4	0.0	1.3	0.7	1
CMRSA8	0.0	0.7	1.7	1.8	1.3	4.0	3.8	3.2	1.4	0.073
CMRSA9	0.3	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	1
CA-MRSA (% of all MRSA)	19.7	27.6	31.9	38.1	36.4	39.2	35.8	36.9	39.2	<0.0001
CMRSA7 [USA400]	6.5	5.5	8.2	6.7	7.8	12.0	8.2	5.1	10.5	0.139
CMRSA10 [USA300]	13.2	22.1	23.7	31.4	28.6	27.2	27.7	31.8	28.7	<0.0001
*P-value determined by Fisher's exact test comparing 2007 vs. 2015 data.										

PVL was detected in 86.2% of CA-MRSA and 1.5% of HA-MRSA. Resistance rates (CA vs HA) were 67.1 vs 95.7% to ciprofloxacin, 73.2 vs 93.5% to clarithromycin, 12.7 vs 65.4% to clindamycin and 0 vs 10.3% to trimethoprim-sulfamethoxazole. MRSA were 100% susceptible to linezolid and 99.9% susceptible to daptomycin and vancomycin.

Conclusions: The most frequent CA-MRSA genotype was USA300 (CMRSA10) while USA100/800 (CMRSA2) was the predominant HA-MRSA genotype. Despite an overall decrease in the numbers of MRSA, the proportion of CA-MRSA in Canadian hospitals has risen significantly between 2007 and 2015.

Author Disclosure Block:

K.A. Nichol: None. **H.J. Adam:** None. **G.R. Golding:** None. **M. McCracken:** None. **M.R. Baxter:** None. **J.A. Karlowsky:** None. **D.J. Hoban:** I. Research Relationship; Self; Astellas, Basilea, Merck, Abbott, Pharmascience, Sunovion, Tetrphase, The Medicines Company, Cubist. **G.G. Zhanel:** I. Research Relationship; Self; Astellas, Basilea, Merck, Pharmascience, Sunovion, Tetrphase, The Medicines Company, Cubist.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

Predictors of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* (Hvisa) among Patients with Methicillin-Resistant *Staphylococcus aureus* (Mrsa) Infective Endocarditis (Ie)

Author Block:

E. Zasowski¹, T. Trinh¹, K. Claeys¹, M. Rybak¹, A. Casapao²; ¹Wayne State Univ., Detroit, MI, ²Husson Univ., Bangor, ME

Abstract Body:

Vancomycin (VAN) is the treatment of choice for MRSA IE but reduced VAN susceptibility phenotypes such as hVISA are associated with poor outcomes in this population. Detection of hVISA is labor intensive and not done routinely in practice. Thus clinicians must rely on risk factors and response to therapy to determine treatment but data describing risk factors for hVISA are limited. This study examines clinical factors associated with hVISA in patients with MRSA IE. Single center retrospective cohort study from 2002 - 2013. Inclusion: age ≥ 18 yr; ≥ 1 positive blood culture for MRSA with IE source available for phenotypic testing; ≥ 72 h VAN. Exclusion: VAN minimum inhibitory concentration (MIC) ≥ 4 mg/L. VAN MICs were done by broth microdilution. Modified population analysis for each strain was compared to hVISA reference strain Mu3 strain to identify hVISA as previously validated. Patient and strain characteristics were compared between hVISA and non-hVISA patients using χ^2 /Fisher's exact test or Student's t-test/Mann-Whitney U test. Variables associated with hVISA at a p-value < 0.20 were entered into multivariable logistic regression to determine independent association with hVISA. 205 patients included; 18% were hVISA. Patient characteristics: mean (SD) age 53.2 (13.8) yr; 63.9% male; median (IQR) APACHE II and Charlson Comorbidity Score 12 (8,18) and 2 (1,4) respectively. Chronic kidney disease (CKD), chronic hemodialysis (HD) and increasing VAN MIC were associated with hVISA in bivariate analysis. The proportion of hVISA increased with each log-dilution increase in MIC from 0.5, 1, and 2 mg/L (6.1%, 15.9%, 38.2% respectively, p 0.002). The results of the final multivariable model are shown below.

Variable	hVISA (N=40)	Non-hVISA (N=169)	OR (95% CI)	aOR (95%CI)
VAN MIC BMD, median (IQR)	1 (1,1)	1 (1,2)	3.67 (1.75-7.64)	3.37 (1.6-7.1)
Chronic HD, n (%)	16 (43.2)	36 (21.4)	2.79 (1.32-5.9)	2.51 (1.15-5.44)
CKD, n (%)	16 (43.2)	48 (28.6)	1.91 (0.917-3.96)	-

Receipt of chronic HD and elevated VAN MIC were independently associated with hVISA. These factors can be considered along with response to treatment can be used to guide therapy of MRSA IE although further study is warranted.

Author Disclosure Block:

E. Zasowski: None. **T. Trinh:** None. **K. Claeys:** None. **M. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company, Theravance. **E. Grant:** Investigator; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company, Theravance, NIAID. **L. Speaker's Bureau:** Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company, Theravance. **A. Casapao:** L. Speaker's Bureau; Self; Theravance.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

New Insights into the First Case of Vancomycin-Resistant *Staphylococcus aureus* (Vrsa) in New York State Using Whole Genome Sequencing

Author Block:

W. Lainhart, L. Mingle, M. Dickinson, D. Schoonmaker-Bopp, D. Kohlerschmidt, E. Nazarian, N. Dumas, K. Musser; Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY

Abstract Body:

The first case of vancomycin-resistant *Staphylococcus aureus* (VRSA) in New York State, and third in the United States, occurred in 2004. VRSA was isolated from the urine of a long-term care facility resident, suffering from multiple comorbidities, in April 2004, and was previously exposed to vancomycin in November 2003. Additional samples were isolated from this patient over the next eight months, totaling 26 isolates [12 VRSA, 6 vancomycin-intermediate *S. aureus* (VISA), 5 methicillin-resistant *S. aureus* (MRSA), and 3 vancomycin-resistant enterococci (VRE)]. Phenotypic characterization of these isolates identified differences in colony morphology and pulsed-field gel electrophoresis (PFGE) analysis yielded eight different patterns. Molecular characterization of the *S. aureus* isolates using real-time PCR detected the presence of the *mecA* gene in all but 3 samples (all VISA). Additionally, all VISA and VRSA isolates, as determined by vancomycin E-test, were positive for the *vanA* gene by real-time PCR. All 26 isolates underwent whole genome sequencing (WGS) using the Illumina MiSeq resulting in at least 24X coverage over the entire genome. Subsequent *in silico* PCR analyses found that all *S. aureus* isolates shared the same *spa* (t002) and MLST (ST-5) type, and all were Panton-Valentine Leukocidin (PVL) and Arginine Catabolic Mobile Element (ACME) negative. In addition, *mecA*-positive *S. aureus* isolates were determined to be SCC*mec* type II by *in silico* PCR. WGS single nucleotide polymorphism (SNP) analyses suggests that five vancomycin-resistance acquisition events occurred within this patient. Although vancomycin-resistance was conferred by the pF586 plasmid in all isolates, there were at least three variants of this plasmid in the patient. SNP-based phylogenetic trees of the *S. aureus* chromosome could not explain the noted differences in colony morphology and PFGE patterns. These findings show very high levels of genetic diversity among VRSA

strains within one patient, as well as differences in susceptibility to vancomycin, both of which can drastically affect infection control and epidemiological investigations.

Author Disclosure Block:

W. Lainhart: None. **L. Mingle:** None. **M. Dickinson:** None. **D. Schoonmaker-Bopp:** None. **D. Kohlerschmidt:** None. **E. Nazarian:** None. **N. Dumas:** None. **K. Musser:** None.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

Prevalence of Antiseptic Resistance Genes and Phenotypic Resistance of Topical Agents against Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Public Hospital in Singapore

Author Block:

S. H. Tan¹, W. S. Pang², Q. Y. Quek², K. L. Chan¹, L. C. Eng¹, L. S. Y. Ng¹, T. Y. Tan¹;
¹Changi Gen. Hosp., Singapore, Singapore, ²Ngee Ann Polytechnic, Singapore, Singapore

Abstract Body:

Background: MRSA colonization is a risk factor for MRSA infection. To reduce this risk, intranasal mupirocin (MUP) is most commonly used with chlorhexidine (CHX) antiseptic washes as decolonization therapy. However, we have adopted octenidine dihydrochloride (OCL) as a universal body wash on our MRSA cohort wards since June 2012; with added MUP as decolonizing therapy if required. We were concerned with resistance from this increased use, but have no baseline data and sought to determine susceptibilities for these and alternative topical agents. **Methods:** 200 non-duplicate MRSA isolates from adult screening samples were tested. Isolates were identified by MALDI-TOF with confirmatory *mecA* PCR and cefoxitin susceptibility. OCL was tested by broth microdilution; MUP high level resistance (HLR), retapamulin, bacitracin and neomycin susceptibilities were tested by disk diffusion (CLSI guidelines using CLSI and FDA breakpoints). The breakpoint for susceptibility to OCL was taken as MIC ≤ 2 $\mu\text{g/mL}$. Retapamulin breakpoints used were those described by Traczewski in 2008. The potential for decreased CHX susceptibility was determined by presence of *qac A/B* and *smr* genes, performed by multiplex PCR. **Results:** No resistance was detected against OCL and retapamulin. *qacA/B* and *smr* alone was detected in 48 and 46 of the 200 isolates, respectively; none demonstrated *qacA/B* and *smr* together. 46 isolates were MUP HLR; 36 of these strains were also *smr* or *qac A/B* positive (detailed results in table).

	Sensitive (%)		Resistant (%)	
	<i>smr</i>	<i>qac A/B</i>	<i>smr</i>	<i>qac A/B</i>

		positive	positive		positive	positive
MUP HLR	153 (77%)	15 (10%)	43 (28%)	46 (23%)	31 (67%)	5 (11%)
Neomycin	188	45 (24%)	42 (22%)	9	1	3
Bacitracin	194	45 (23%)	47 (24%)	6	1	1
Retapamulin	200	46 (23%)	48 (24%)	0	N/A	N/A
Octenidine	200	46 (23%)	48 (24%)	0	N/A	N/A

Conclusions: 23% of our study isolates was MUP HLR. If decolonization is required, susceptibility testing should be performed and OCL intranasal ointment used in patients with MUP HLR. Prevalence of CHX resistance genes in our MUP HLR isolates is considerably high (78%), suggesting a potential for decreased CHX susceptibility in addition to MUP HLR. No OCL resistance was detected and we will continue to use it on our MRSA cohort wards.

Author Disclosure Block:

S.H. Tan: None. **W.S. Pang:** None. **Q.Y. Quek:** None. **K.L. Chan:** None. **L.C. Eng:** None. **L.S.Y. Ng:** None. **T.Y. Tan:** None.

Session Number:

436

Session Title:

Epidemiology and Control of MRSA

Publishing Title:

Characteristics of *qacA/B*-Positive Methicillin-Resistant *Staphylococcus aureus* (Mrsa) Isolated from Patients in a Surgical Intensive Care Unit (Sicu)

Author Block:

O-H. Cho¹, K-H. Park², E. Baek¹, M. Bak¹, O. Jang¹, I-G. Bae¹; ¹Gyeongsang Natl. Univ. Hosp., Jinju, Korea, Republic of, ²Kyung Hee Univ. Hosp., Seoul, Korea, Republic of

Abstract Body:

Background: The widespread use of chlorhexidine for MRSA decolonization has raised the concern about developing resistance or tolerance to this agent. This study aimed to describe clinical and molecular characteristics of *qacA/B*-positive MRSA isolates in the setting of targeted MRSA decolonization. **Methods:** The setting for this study was a 14-bed SICU, where MRSA carriers received decolonization therapy using chlorhexidine bathing and intranasal mupirocin, in a tertiary hospital from December 2012 to December 2014. MRSA isolates from patients who stayed in the SICU for >48h were tested for the MIC of chlorhexidine, mupirocin resistance, the presence of *qacA/B* genes, and MLST. Clinical data on the characteristics and outcomes of MRSA carriers were reviewed. **Results:** A total of 108 MRSA isolates, including 80 (74.1%) from nasal swab cultures and 28 from clinical cultures, were available for this study. Of the 108 isolates, 39 (36.1%) carried *qacA/B* genes and 23 (21.2%) were resistant to mupirocin with 2 (8.7%) having high-level resistance. The *qacA/B*-positive MRSA isolates were classified into three MLST types: ST5 (n = 27, 69.2%), ST239 (n = 9, 23.7%), ST72 (n=2, 5.3%); meanwhile, 42 (61.8%) *qacA/B*-negative MRSA isolates were identified as ST72. The *qacA/B*-positive MRSA isolates were more likely to be associated with ICU-acquisition (71.8% vs. 39.1%; P = .001), chlorhexidine MIC ≥ 4 (97.4% vs. 81.2%; P = .016), mupirocin resistance (35.9% vs. 13.0%; P = .005), and healthcare-associated (HA) MRSA strains (94.9% vs. 39.1%; P = < .001), compared with *qacA/B*-negative isolates. However, duration of SICU stay (median, 17 days vs. 15 days; P = .144) and in-ICU mortality (15.4% vs. 20.3%; P = .528) were not significantly different between isolates with *qacA/B* positive and negative MRSA. Multivariate analysis showed that HA-MRSA strains were independently associated with *qacA/B* genes carriage (aOR, 18.64; P <

.001). **Conclusions:** Carriage of *qacA/B* in MRSA was not uncommon in the ICU where chlorhexidine-based bathing was performed and probably associated with HA-MRSA strains.

Author Disclosure Block:

O. Cho: None. **K. Park:** None. **E. Baek:** None. **M. Bak:** None. **O. Jang:** None. **I. Bae:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Neutralizing Monoclonal Antibodies Specific to the N-Terminal Region of *S. aureus* Pro-Coagulant Factors Staphylocoagulase and Von Willebrand Factor Binding Protein

Author Block:

K. Begins¹, A. Maddur², W. Church¹, P. Panizzi³, P. Bock²; ¹Green Mountain Antibodies, Burlington, VT, ²Vanderbilt Univ. Med. Ctr., Nashville, TN, ³Auburn Univ., Auburn, AL

Abstract Body:

S. aureus secretes virulence factors that facilitate colonization and evasion of host defense responses, and increase pathogenicity. The ability of *S. aureus* to clot plasma is due to the expression of staphylocoagulase (SC) and von Willebrand factor binding protein (vWbp). These proteins activate prothrombin independently and cause expression of the thrombin active site through stabilization of the active conformation via a salt-bridge between the SC N-terminus and the carboxylate group of Asp 194 on prothrombin. The prothrombin-SC or vWbp complex cleaves fibrinogen to form a fibrin clot. Murine monoclonal antibodies (mAb) were produced to peptides representing the N-termini of SC and vWbp. Antibody specificity was confirmed by ELISA and Western blotting using recombinant proteins. The ability of the antibodies to block blood coagulation was assessed by a spectrophotometric turbidity assay. The anti-SC mAb (designated GMA-2105) inhibited *S. aureus*-dependent coagulation of rabbit plasma, but the anti-vWbp mAb (designated GMA-2500) had no effect. A chimeric form of GMA-2105 (mouse variable region, human backbone) behaved similarly. Competitive inhibition assays using peptides, and blotting with truncated recombinant SC fragments, showed that GMA-2105 bound SC residues Ile1 and Val2 while binding did not require residues 8 or 9, regions of high variability in different strains of *S. aureus*. In a mouse acute septicemia model, injection of 120 ug GMA-2105 prior to injection of *S. aureus* Tager prolonged survival compared to the control group (p <0.005). Antibodies such as GMA-2105 and GMA-2500 should lead to greater understanding of the differential expression and roles of virulence factors important for fibrin deposition during *S. aureus* infection. These

antibodies might also have therapeutic effects by blocking the action of prothrombin activators secreted by *S. aureus*.

Author Disclosure Block:

K. Begins: D. Employee; Self; Green Mountain Antibodies. **A. Maddur:** None. **W. Church:** D. Employee; Self; Green Mountain Antibodies. **P. Panizzi:** None. **P. Bock:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Hamamelitannin Analogs as Potentiators for Antibiotics in the Treatment of *Mrsa* Biofilm Infections

Author Block:

G. Brackman¹, A. Vermote¹, K. Breyne², E. Meyer¹, S. Van Calenbergh¹, T. Coenye¹;
¹Ghent Univ., Gent, Belgium, ²Ghent Univ., Merelbeke, Belgium

Abstract Body:

Background: The quorum sensing (QS) modulator hamamelitannin (HAM) specifically affects *S. aureus* biofilm susceptibility through the TraP receptor by affecting cell wall synthesis and eDNA release of *S. aureus*. However, from a medicinal chemistry perspective the structure of HAM has several disadvantages and it is envisioned that more potent and druglike HAM derivatives could be synthesized. We investigated the structure-activity relationship (SAR) of novel derivatives and investigated the mechanism of action of HAM and selected analogs as well as their *in vivo* activity. **Methods:** Over 150 HAM-analogs were synthesized by applying three modifications to the HAM structure: replacement of the ester groups, modification of the galloyl moiety and removal of the anomeric hydroxyl group. Their mechanism of action was investigated using two parallel strategies. First, we evaluated the effect of the analogs on susceptibility of biofilms of *S. aureus* strains with mutations in various QS systems (e.g. *agrBCDA* and *traP*). Secondly, we evaluated the effect of the analogs on peptidoglycan structure and eDNA production. Finally, the most active analogs were evaluated in a vertebrate murine model of mastitis infection. **Results:** Analogs were developed with 100-fold increased activity compared to HAM. These analogs potentiated the effect of different classes of antibiotics towards *S. aureus* biofilms. Although this increased susceptibility was not observed in *S. aureus* strains with mutations in *traP* gene, they affected susceptibility of a *traP* complemented strain. Treatment with the HAM-analogs affected susceptibility towards lysostaphin and affected eDNA production of *S. aureus* biofilm cells. This indicates that the mechanism of action of the HAM-analogs is similar to that of the native HAM molecule. The most potent analog also increased susceptibility towards antibiotics *in vivo*. **Conclusions:** We determined the SAR of >150 HAM analogs and identified analogs with improved activity and physico-chemical properties. These compounds affect

cell-wall thickness and eDNA release, both leading to the increased susceptibility of *S. aureus* biofilm cells towards antibiotics. Our results indicate that HAM-analogs also increase susceptibility *in vivo* and that this *in vivo* effect is superior to that of HAM.

Author Disclosure Block:

G. Brackman: None. **A. Vermote:** None. **K. Breyne:** None. **E. Meyer:** None. **S. Van Calenbergh:** None. **T. Coenye:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Biaryl Hydroxyketone Compounds Inhibit Virulence Production and Biofilm Formation in Mrsa and *S. epidermidis*

Author Block:

M. Greenberg, D. Kuo, G. Yu, M. Shoham; Case Western Reserve Univ., Cleveland, OH

Abstract Body:

Antibiotic resistant bacteria, including Methicillin Resistant *Staphylococcus Aureus*, MRSA, pose a global public health concern. Therefore it is important to develop alternate strategies to combat these bacterial pathogens. We successfully used small molecules (biaryl hydroxyketones) that bind the accessory gene regulator (AgrA) to inhibit production of the proteins responsible for MRSA virulence. While the role of AgrA is less clear in biofilm formation, this a chronic problem affecting medical equipment in hospitals. We measured the efficacy of our small molecule compounds to inhibit virulence in MRSA and in other gram positive species by red blood cell lysis and lactate dehydrogenase (LDH) leakage from white blood cells, and in-vivo using an insect larvae infection model (*Galleria Mellonella*). By qPCR, we examined the transcription levels of virulence genes to confirm the inhibitory effect of the compounds on AgrA regulation of these factors. We also tested the compounds for their ability to inhibit bacterial biofilm formation. We show that the lead compounds F12 and F19 inhibit MRSA and *Staphylococcus epidermidis* induced hemolysis by up to 95%, inhibit macrophage LDH leakage 4-5 fold compared to untreated control, and significantly extend the survival of infected insect larvae. Unlike conventional antibiotics, prolonged exposure to sub MIC concentrations of the compounds did not elicit resistance as measured by hemolysis over a 21 day period. Additionally, treating the bacteria with these compounds lead to a 5-fold reduction in biofilm formation. Quantitative PCR show that the compounds significantly downregulate known MRSA virulence genes α -hemolysin (hla), phenol soluble modulins (psm α) and RNAlII compared with control housekeeping genes. Initially shown for MRSA that biaryl hydroxyketones inhibit virulence and biofilm formation, we have now extended our studies to another gram positive species, *S. epidermidis*. These compounds downregulate the MRSA virulence factors hla, psm α , RNAlII without affecting bacterial

growth confirming that they are bona fide quorum sensing inhibitors. This approach shows promise as an alternate strategy for treating these dangerous pathogens.

Author Disclosure Block:

M. Greenberg: None. **D. Kuo:** None. **G. Yu:** None. **M. Shoham:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Killing Mrsa in Wound and Surgical Infections Using Topically Applied Bacteriophage Endolysins

Author Block:

C. W. Euler¹, N. Frank², V. A. Fischetti²; ¹Hunter Coll., New York, NY, ²Rockefeller Univ., New York, NY

Abstract Body:

Staphylococcus aureus, is an opportunistic pathogen that frequently colonizes the human skin and is present in the nose of about 25-30% of U.S. adults. *S. aureus* can exist in this form without harming its host or causing symptoms. However, if there is a break in the patient's skin from a wound or surgery, or if there is a depression in the person's immune system, then colonizing *S. aureus* can cause an infection. These infections may quickly result in more serious complications such as abscesses, cellulitis, and bacteremia. Currently, 40-60% of nosocomial infections of *S. aureus* are resistant to methicillin, MRSA, these infections may be difficult to treat and result in high mortality. Thus methods must be devised to treat them quickly and effectively. Purified bacteriophage lytic enzymes "lysins" are well suited for this purpose. To examine if topical treatment with lysins could prevent wound or surgical infections we modified a MRSA deep wound infection model in rats, and applied staphylococcal specific lysins or saline as an irrigation to remove MRSA organisms (10^7 CFU) from contaminated incisions, which were aseptically closed with surgical staples. After 5-10 days wounds were opened and examined for disease and tissue analyzed for MRSA CFU. Additionally, we tested whether lysin irrigation during surgical debridement of established staphylococcal abscesses could improve infection and reduce bacterial load after 10 days, compared to saline irrigation w/wo continued vancomycin treatment. The results showed that our lysins drastically reduced abscess formation compared to controls and significantly decreased MRSA bacterial burden, when applied to contaminated surgical wounds. Lysin reduced staphylococcal CFU/gram of tissue by 3-6 logs, and 8/11 of the lysin-treated animals had CFU below our limit of detection, compared to 1/11 of the saline controls. Furthermore, one application of our lysins during debridement of the established staphylococcal abscesses in rats reduced MRSA CFU 3 logs / gram of tissue compared to

a saline alone, and had an equivalent CFU drop to twice-daily IP vancomycin treatment over 5 days (n=16 per group). These results support the further development and novel use of staphylococcal specific lysins as topical agents on wounds and surgical sites, to prevent or treat MRSA and other staphylococcal infections.

Author Disclosure Block:

C.W. Euler: None. **N. Frank:** None. **V.A. Fischetti:** I. Research Relationship; Self; Contrafect Corporation. **J.** Scientific Advisor (Review Panel or Advisory Committee); Self; Contrafect Corporation.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

***Staphylococcus aureus* Resistance to Lysin Cf-301 Does Not Arise in Human Serum**

Author Block:

R. Schuch, J. Oh, M. Wittekind; ContraFect Corp., Yonkers, NY

Abstract Body:

Background: Lysins are a new class of antimicrobials consisting of bacteriophage-derived cell-wall hydrolases. CF-301, the first lysin to enter US clinical trials, has concluded a First-in-Man Phase 1 trial and is being developed for the treatment of *S. aureus* bacteremia. CF-301 exhibits rapid *S. aureus*-specific bacteriolysis, potent anti-biofilm activity, synergy with antibiotics, and low propensity for resistance during serial passage in Mueller Hinton Broth (MHB). To extend this work, we undertook a more rigorous analysis of CF-301 resistance using a serial passage method in MHB and human serum (HuS). The study analyzed multiple lineages, a range of comparator lysins, analyses of phenotypic and genotypic resistance, and whole-genome sequencing (WGS) of clones with decreased lysin susceptibility. **Methods:** Resistance assays were based on the CLSI method for determining MICs by broth-microdilution. MRSA strain MW2 was exposed to 1.1-fold dilutions of each drug for 3 weeks. Intermediaries were recovered daily and subcultured without drug prior to final MIC determinations and analysis of phenotypes, such as oxacillin [OXA] resistance. We used three native lysins (CF-301, lysostaphin [Lst], and SAL-1), two chimeric lysins (SAL-1^{CHAP}-SAL-1^{CBD} [Ply778], Sal-1^{CHAP}-CF-301^{CBD} [Ply773]), and the antibiotic daptomycin (DAP). **Results:** After serial subculture in MHB, final fold increases in MIC were: CF-301, 1-2; Lst, 20-120; Ply778, 2-8; and daptomycin, 128. In HuS, resistance was suppressed compared to MHB and final fold increases were: CF-301, 1-2; Lst, 1-2; Ply778, 1; Ply773, 1-2; Sal-1, 1-2; and DAP, 1-2. Increases in lysin MICs were associated with decreases in OXA MICs ranging from 4-fold (CF-301, Ply778) to >512-fold (Lst). Mutations associated with increased lysin MICs were limited to loci encoding putative effectors of bacterial apoptosis, peptidoglycan and protein biosynthesis, and teichoic acid modification. **Conclusions:** CF-301 resistance, defined as a >2-fold increase in MIC, does not occur in MHB or human serum. Other lysins and DAP show resistance in MHB, but not in serum. These findings suggest that lysins, alone and in with DAP, would not be expected to develop resistance

when treating bacteremia in patients. Genetic analyses suggest that modifications to the cell envelope and/or bacterial growth rate will not significantly alter susceptibility to CF-301.

Author Disclosure Block:

R. Schuch: D. Employee; Self; ContraFect Corporation. **J. Oh:** D. Employee; Self; ContraFect Corporation. **M. Wittekind:** D. Employee; Self; ContraFect Corporation.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Identification and Mechanistic Study of a Novel Compound Suppressing the Expression of Multiple Virulence Genes in *Staphylococcus aureus*

Author Block:

R. Y. Kao, P. Gao; The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Background: The indiscriminate use of antibiotics worldwide has been one of the major driving forces for the rapid development of resistance in major bacterial pathogens such as methicillin resistant *S. aureus* (MRSA). Numerous antibiotics, natural or synthetic, have been developed and applied clinically but the rapid emergence of drug-resistant “super bugs” has rendered most of those commonly used antibiotics useless. The identification of novel compounds suppressing the virulence of bacteria without exerting bactericidal effects may offer an alternative way to combat bacterial infections. **Methods:** A *gfp-luxABCD* dual-reporter plasmid system was used for screening compounds suppressing the expression of virulence genes in *S. aureus*. A total of 20 promoters of virulence or virulence-associated genes were cloned individually into this dual-reporter system and screened with chemical libraries for the identification of anti-virulence compounds exerting no inhibitory effects on bacterial growth. Identified hits were further validated by looking at their ability to reduce virulence factors production at protein expression level using western blotting. Several compounds were confirmed to be potent suppressors of multiple virulence genes and their anti-virulence activities were assayed in mammalian cell-based adhesion/invasion assays. **Results:** Compounds suppressing the expression of multiple virulence genes in *S. aureus* at low micromolar concentrations were identified. Reductions in expression of virulence factors at protein level were also observed using western blotting. One of the selected compounds M21 has shown potent anti-virulence activities by reducing bacterial adhesion and invasion in mammalian cells using confocal microscopy. Interestingly, the elevated expression of virulence genes induced by ampicillin, a known inducer of the expression of *S. aureus* was overridden by the anti-virulence activities of compound M21. **Conclusion:** We have successfully employed a *gfp-luxABCD* dual-reporter plasmid system to screen for bioactive compounds modulating the expression of virulence genes in *S. aureus* and identified

compound M21 as a potent anti-virulence agent. The addition of M21 greatly reduces the capability of *S. aureus* to produce major virulence factors leading to diminished adhesion and invasion to host cells.

Author Disclosure Block:

R.Y. Kao: None. **P. Gao:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Post-Antibiotic Effects of Lysin Cf-301 Against *Staphylococcus aureus*

Author Block:

R. Schuch, J. Oh, M. Wittekind; ContraFect Corp., Yonkers, NY

Abstract Body:

Background: Lysins are a new class of antimicrobials consisting of bacteriophage-derived cell-wall hydrolases. CF-301, the first lysin to enter US clinical trials, has concluded a First-in-Man Phase 1 trial and is being developed for the treatment of *S. aureus* bacteremia. CF-301 exhibits rapid bacteriolysis, potent anti-biofilm activity, synergy with antibiotics, and low propensity for resistance. We examined the ability of CF-301 to suppress bacterial growth after in vitro exposure to: 1) suprainhibitory levels (i.e., the post-antibiotic effect, PAE); 2) subinhibitory levels during the PAE period (i.e., the post-antibiotic sub-MIC effect, or PA-SME), and 3) subinhibitory levels by cultures not previously exposed to drug (i.e., the sub-MIC effect, or SME). **Methods:** The PAE, PA-SME, and SME for CF-301 were determined according to standard methodologies using either 4X MIC values (for the PAE) or 0.05X-0.5X MIC values (for the SME). Variations were included to either examine the effect of daptomycin (DAP) alone and in combination with CF-301 or examine the post-antibiotic effect of CF-301 on biofilm formation. Thirteen *S. aureus* strains were analyzed here, including 5 methicillin-resistant, 2 methicillin-sensitive, 2 linezolid non-susceptible, 2 daptomycin non-susceptible, and 2 vancomycin-resistant. **Results:** We found that the CF-301 mean staphylococcal PAE is 4.8 hr. Thus, brief exposure to CF-301 resulted in a 4.8 hr delay in the resumption of vegetative growth compared to the buffer control. Depending on the sub-MIC values used, mean PA-SME and SME values were up to 5.7 and 5.8 hr, respectively. In combination experiments, brief exposure to both DAP (1.5X MIC) and CF-301 (0.01-0.1X MIC) together resulted in a PAE of 4 hr. Interestingly, brief exposure to DAP first, followed by a sub-MIC exposure to CF-301, resulted in a PA-SME of up to 4.3 hr. CF-301 at a concentration down to 0.1X MIC also showed a minimum PAE of >2 hours on biofilm formation. **Conclusions:** Post-antibiotic growth effects lasted up to 4->6 hours after exposure to CF-301 in either the presence or absence of DAP. In addition to suppression of vegetative growth, the CF-301 PAE also delayed biofilm formation.

Together, these findings suggest that the PAE will be an important factor on decreasing bacterial viability and reducing the colonization of secondary sites during treatment.

Author Disclosure Block:

R. Schuch: D. Employee; Self; ContraFect Corporation. **J. Oh:** D. Employee; Self; ContraFect Corporation. **M. Wittekind:** D. Employee; Self; ContraFect Corporation.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Fluorescence Based High Throughput Screening Strategy For Identifying Antimicrobial Agents Effective Against Methicillin-Resistant *staphylococcus Aureus* Persisters

Author Block:

W. Kim¹, A. L. Conery², B. B. Fuchs¹, F. M. Ausubel², E. Mylonakis¹; ¹Rhode Island Hosp., Brown Med. Sch., Providence, RI, ²Massachusetts Gen. Hosp., Harvard Med. Sch., Boston, MA

Abstract Body:

Background: *Staphylococcus aureus* is a Gram-positive human pathogen that colonizes about one-third of healthy individuals and causes a wide range of infections. A significant challenge in treating *S. aureus* infections is its ability to develop antibiotic-resistance and to form persisters, which are dormant, non-growing cells that exhibit a high level of tolerance to most conventional antibiotics. Vancomycin is currently the antibiotic of last resort for *S. aureus*, but vancomycin-intermediate or resistant strains are arising, and moreover, vancomycin is ineffective against methicillin-resistant *S. aureus* (MRSA) persisters. Therefore, new antibiotics effective against both antibiotic-resistant and-tolerant *S. aureus* are urgently needed. **Methods:** Using the fluorescent nucleic acid dye SYTOX Green that only stains cells with compromised membranes, we developed a high-throughput screening strategy for identifying compounds that kill MRSA persisters. The assay robustness was evaluated by calculating a statistical parameter, Z'-factor. This strategy was employed in screening a library consisting of hits from our previous screening of small molecules that rescue the nematode *Caenorhabditis elegans* from MRSA infection. A system of giant unilamellar vesicles (GUVs) that model the cell membrane was used for elucidating the mode of action of NH125. **Results:** The assay proved robust and suitable for use in high-throughput screening. We discovered that NH125, formerly known as a bacterial histidine kinase inhibitor, kills MRSA persisters by inducing cell membrane permeabilization. We found that NH125 kills MRSA persisters by directly interacting with and disrupting membrane lipid bilayers rather than by inhibiting essential bacterial kinases. **Conclusions:** Our results suggest that the SYTOX Green screening assay is amendable for large-scale screens for identifying

potential antibiotics to treat MRSA persisters as well as persisters formed by other important pathogens. NH125 could be a lead compound for treating MRSA persisters and should be further evaluated.

Author Disclosure Block:

W. Kim: None. **A.L. Conery:** None. **B.B. Fuchs:** None. **F.M. Ausubel:** None. **E. Mylonakis:** None.

Session Number:

437

Session Title:

***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies**

Publishing Title:

Efficacy of the Fabi Inhibitor Debio 1450 for the Treatment of *Staphylococcus aureus*-induced Acute Osteomyelitis in Rabbit

Author Block:

M. Barbier¹, A. Menetrey¹, A. Haouala¹, J. Bravo¹, F. Wittke¹, C. Jacqueline², **G. Vuagniaux¹**; ¹Debiopharm Intl. SA, Lausanne, Switzerland, ²Atlangram, Nantes, France

Abstract Body:

The treatment of osteomyelitis (OM) remains a major clinical challenge. *Staphylococcus aureus* and CoNS are the most common causative organisms responsible for more than 50% of OM cases. Debio 1452 is the active moiety of the prodrug Debio 1450. Debio 1452 is an inhibitor of staphylococcal FabI, a critical enzyme for bacterial fatty acid synthesis. Debio 1450 may represent a new potential therapeutic option for the treatment of bone infections due to its unique mode of action and high activity against staphylococcal species. The aim of the study was to evaluate the *in vivo* efficacy of Debio 1450 using a rabbit model of acute experimental OM. MIC assays were performed by broth microdilution using CLSI guidelines. OM was induced in rabbit through inoculation of the knee with 10⁸ CFU of a MRSA clinical strain after bone trepanation. On day 3 post-inoculation, surgical debridement was performed to mimic a surgical procedure. Samples of femoral red bone marrow (BM) and epiphyseal spongy bone were removed and treatment was started for 4 days with human equivalent doses: Debio 1450 12.5 mg/kg BID iv or its vehicle BID iv, or vancomycin 100 mg/kg once daily iv. On day 7 post-inoculation, the bacterial counts were determined in BM and bone samples. Infected and non-infected BM, bone and plasma samples were collected for quantification of Debio 1452 by LC-MS/MS. MICs for the MRSA isolate were 0.004 µg/ml for Debio 1452 and 1 µg/ml for vancomycin. The *in vivo* outcomes are shown in the table below. Debio 1450 demonstrated significant activity in BM and bone. No significant difference was observed between vancomycin and vehicle in bacterial counts. No development of resistance against Debio 1450 was observed after the 4-day treatment period. Debio 1452 presented high bone-to-plasma ratios. In conclusion, experimental OM efficacy and drug bone penetration results suggest that Debio 1450 may have a therapeutic potential in the treatment of staphylococcal OM.

	Mean \pm SD $\Delta\log_{10}$ CFU / g of tissue (day 3 - day 7)	
Treatment (number of animals)	Bone marrow	Epiphyseal bone
Vehicle (5)	0.43 \pm 0.58	0.27 \pm 0.60
Debio 1450 (10)	-3.62 \pm 0.67 *	-2.52 \pm 1.25 *
Vancomycin (6)	-1.05 \pm 1.30	-0.49 \pm 0.65

p<0.001 vs vehicle and vancomycin

Author Disclosure Block:

M. Barbier: None. **A. Menetrey:** None. **A. Haouala:** None. **J. Bravo:** None. **F. Wittke:** None. **C. Jacqueline:** None. **G. Vuagniaux:** None.

Session Number:

437

Session Title:***Staphylococcus aureus* Infection Treatments: New Compounds, New Strategies****Publishing Title:****Copper-Biased Antimicrobial Screen Discovers Novel Copper-Dependent Staphylococcal Inhibitors****Author Block:**

A. Dalecki, F. Wolschendorf; Univ. of Alabama Birmingham, Birmingham, AL

Abstract Body:

Despite a technological revolution, advanced high throughput antimicrobial screening methods have failed to fulfill their promise. Of sixteen clinically used antibiotic classes, all but two were discovered prior to the 1970s. A growing chorus advocates returning to natural inspirations, from which almost all antibiotics previously arose. Among these inspirations lies metal-mediated innate immunity, by which macrophages unleash a copper burst to destroy pathogens. Although historically medicinally unattractive, the recently described phenomenon of copper-dependent inhibitors has galvanized research exploring the use of ligand complexes to harness copper's antibacterial properties. Here, we report the outcome of the first combinatorial screening campaign to identify copper-dependent inhibitors of *Staphylococcus aureus*. 10,000 compounds were assayed in parallel for anti-staphylococcal activity, both with and without added copper. Hits were defined as those compounds exhibiting inhibition in the presence of copper, but impotent in its absence. 53 copper-dependent hit molecules were uncovered, nearly doubling the overall hit rate of the traditional campaign conducted in parallel. A hit family with an extended thiourea core structure, termed the NNSN motif, was well featured. Following a structure-activity relationship analysis, the NNSN motif was confirmed to be both copper-dependent and copper-specific, with MICs ranging from low micromolar to sub-micromolar, while also being non-toxic in eukaryotic cell culture. Finally, the ChEMBL chemical database was used for a chemoinformatic analysis of the motif, establishing NNSNs as an unrecognized family of Staphylococcal inhibitors, in spite their common presence in screening libraries. Therefore, this novel copper-biased screening assay revealed potent molecular inhibitors even in previously exhausted chemical libraries, potentially offering an economically attractive solution for future drug discovery efforts.

Author Disclosure Block:

A. Dalecki: None. **F. Wolschendorf:** None.

Session Number:

094

Session Title:

Building Better Starter Cultures for the Production of Improved Fermented Foods

Publishing Title:

Elucidation of Gouda Cheese Associated Microbiota Via Metagenomic Analysis

Author Block:

C. K. Carstens¹, J. K. Salazar², Y. Wang², K. Schill², M. L. Tortorello²; ¹Illinois Inst. of Technology, Bedford Park, IL, ²U. S. Food and Drug Admin., Bedford Park, IL

Abstract Body:

Background: During 1998 to 2011, 90 cheese-associated human outbreaks occurred in the U. S., of which approximately 49% were associated with pasteurized cheeses. In this study, metagenomics analysis of pasteurized Gouda cheeses was conducted in order to determine the commonly associated microbiota of commercially available varieties. A better understanding of how taxonomic diversity varies between cheeses will help further functional knowledge of cheese and aid in assessments of microbiological quality.

Methods: Three brands of pasteurized Gouda cheese (A- wax packaging, B- and C- plastic packaging) were purchased from local retailers. Two gram replicate samples were homogenized with 20% sodium citrate. After centrifugation, pellets were used for DNA extraction using the MoBio PowerFood Microbial DNA Isolation kit. PCR was conducted using 16s v3 and v4 variable region primers. Resulting amplicons were indexed, pooled, and a library was prepared using the Illumina Nextera XT kit. The library was sequenced on the MiSeq and data analyzed using MiSeq Reporter. **Results:** Sequence reads passing quality filtering for Brands A, B, and C were 97.4, 96.9, and 96.7%, respectively. Firmicutes dominated the populations of all samples, followed by Proteobacteria. Brand A had significantly more microbial diversity than Brands B and C at the genus level. Forty genus-level classifications were identified in Brand A, although populations consisted mainly of *Lactococcus*, *Arsenophonus*, and *Salinivibrio* at 79.3, 9.6, and 2.2%, respectively. Brands B and C had similar microbial populations, consisting mostly of *Lactococcus*, *Lactobacillus*, and *Streptococcus*, however a significantly higher *Lactococcus* population was seen in Brand C. **Conclusion:** This study determined the microbial diversity in three brands of Gouda cheese. Data suggest that Brands B and C may have used similar starter cultures consisting of *Lactococcus*, *Lactobacillus*, and *Streptococcus*. The starter culture used for Brand A likely consisted of *Lactococcus*. It is possible that *Arsenophonus*, an insect endosymbiont, derived from cheese mites, which

are associated with cheesemaking procedures as flavor enhancers. The type of packaging used to store Gouda cheeses or other cheesemaking practices may also play a role in the ultimate microbial diversity.

Author Disclosure Block:

C.K. Carstens: None. **J.K. Salazar:** None. **Y. Wang:** None. **K. Schill:** None. **M.L. Tortorello:** None.

Session Number:

094

Session Title:

Building Better Starter Cultures for the Production of Improved Fermented Foods

Publishing Title:

Use of Ems Mutagenesis and Next Generation Sequencing for Mechanistic Study of High Molecular Weight Yeast Glucans Utilization by *Bifidobacterium Breve*

Author Block:

H. Y. Keung, H. S. Kwan; The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Yeast glucans are shown to have beneficial effects such as antitumor and immunomodulation. Recently, high molecular weight glucans are considered as potential prebiotics. This project aims to explore this potential and to elucidate the underlying mechanism using the probiotic, *Bifidobacterium breve*. Random mutagenesis using ethyl methanesulfonate (EMS) was employed to induce low mutation frequency per bacterial genome. Commercial yeast cell wall glucans were used to develop mutant screening plates. Mutants with transportation or utilization deficiency were isolated for genome sequencing on Ion Torrent PGM platform. Mutation sites were identified and studied for each mutant to elucidate plausible key players involved along the pathways. Wild type and 13 mutants of 6 phenotypes related to yeast glucans utilization were genome sequenced. By mapping sequenced reads to the reference *B. breve* genome (accession no. NZ_AP012324.1), 36 significant ($p < 0.05$) single nucleotide variations (SNVs) were identified, which involved 24 different genes. Phenotype association study of the SNVs showed that potential candidate genes include *LacI* transcriptional regulators (TF) and ABC transporter components. Reverse transcription real-time PCR result indicated there are differential expressions of the ABC transporter components ($p < 0.05$) and pullulanase ($p < 0.01$) genes adjacent to the *LacI* TF in wild type grown on yeast glucans. A SNV caused stop codon gained in an ABC transporter ATP-binding protein led to growth failure of the mutant on yeast glucans, which implied its role in transportation of yeast glucans or the broken down products. Characterization of carbohydrates utilization of the mutant using API CH50 system indicated that maltose, D-raffinose, starch, glycogen and D-turanose fermentation did not occur in the mutant. It is proposed that in *B. breve*, high molecular glucans were catabolized by pullulanase extracellularly into oligo-glucosides, which are then transported into the bacteria through ABC transporter system composed of the mentioned ATP-binding protein.

Author Disclosure Block:

H.Y. Keung: None. **H.S. Kwan:** None.

Session Number:

094

Session Title:

Building Better Starter Cultures for the Production of Improved Fermented Foods

Publishing Title:

Functional Properties of Lactic Acid Bacteria Isolated from Raw Beef in Nigeria

Author Block:

O. Ajao¹, k. Banwo¹, A. Sanni¹, O. Ogunremi²; ¹Univ. of Ibadan, Ibadan, Nigeria, ²Samuel Adegboyega Univ., Edo, Nigeria

Abstract Body:

Background: Lactic acid bacteria (LAB) are of major importance in food industry due to their ability to improve nutritional quality, shelf-life and confer health promoting effect. They are part of the microflora of many food substrates including cereal, milk, legume and meat. Meat (beef) is a major source of nutrient in African region. In Africa meat processing remains traditional and uncontrolled; hence there is concern about the safety, stability and consistency in quality of meat product. LAB are known to produce metabolite and exhibit activities which can improve stability of meat and confer health benefits. Thus, this study aimed at determining the functional properties of LAB isolated from raw beef samples. **Methods:** Raw beef was obtained immediately after slaughtering, from cows in University of Ibadan meat shop and Bodija abattoir, Oyo state twice. A total of twenty three presumptive LAB strains were isolated from beef samples and morphologically and physiologically characterized. The LAB strains were assessed for some functional properties which include, acid production, tolerance to low pH and different concentrations of bile determined by spectrophotometric method, cell surface hydrophobicity using the microbial adhesion to hydrocarbon (MATH) and antimicrobial activity using the agar well diffusion assay. **Results:** The most frequently occurring LAB was *L. lactis* (65%), followed by *Lb. pentosus* (19%), *Lb. acidophilus* (1%), *Lb. plantarum* (1%), *Lb. paracasei* (1%) and *Lb. brevis* (1%). The best acid producer after 24hrs was *Lb. acidophilus* Css12 with pH 3.04, while the least was *L. lactis* Csb24 with pH 4.90. At pH 2.5, the most tolerant strain was *Lb. acidophilus* Css12 at 90.0% while the least was *Lb. pentosus* Css2 at 37.5% after 6 hrs. *Lb. pentosus* Css2 had the highest tolerance to 0.5 and 1% bile after 4hrs while *L. lactis* Csb24 had the least tolerance. *Lb. acidophilus* Css12 exhibited the highest adherence to xylene at 75.0%, while *L. lactis* Csb2 was least at 17.8%. The LAB strains had inhibitory activities *E. coli*, *S. aureus*, *S. typhi*, *L. monocytogene* except *L. lactis* Csb2 and *Lb. brevis* Csb12 which had no

inhibitory effect on *S. typhi*. **Conclusion:** LAB strains isolated from meat samples show functional starter culture potential with the ability to improve the microbiological safety and stability of meat and meat products and confer health benefits on consumers.

Author Disclosure Block:

O. Ajao: None. **K. Banwo:** None. **A. Sanni:** None. **O. Ogunremi:** None.

Session Number:

095

Session Title:

Building the Great Wall: Diverse Bacterial Growth Strategies

Publishing Title:

Transcriptional Snapshots of a Cell Wall Defect of *Staphylococcus aureus*

Author Block:

R. P. Portela¹, A. M. Ludovice¹, F. R. Pinto², R. G. Sobral¹; ¹UCIBIO-REQUIMTE, Caparica, Portugal, ²Biosystems and Integrative Sci. Inst. (BioISI), Lisboa, Portugal

Abstract Body:

The transcriptomic profile of a *Staphylococcus aureus* conditional cell wall mutant (COLspacmurF) suggested that a wide range of physiological alterations occur in the path from wall damage to cellular death, involving shift of energy producing pathways away from O₂ respiration and TCA cycle by stimulating fermentation (1). In order to identify the metabolic steps which link the primary damage to cell death, we provided a time scale of expression events by taking consecutive snapshots of COLspacmurF transcriptome. To choose the time points to be analysed, the peptidoglycan of COLspacmurF grown without inducer was extracted along time, purified and analysed by RP-HPLC. The incorporation of the abnormal muropeptide was quantified and, unexpectedly, found to accumulate constantly along the growth curve. COLspacmurF was grown with optimal inducer to early-exponential phase and the culture was divided in two batches of fresh medium, one with and another without inducer. Total RNA was extracted for 3 biological replicates, at 6 consecutive time points until stationary phase was reached. The RNA samples were tested for integrity and hybridized to Affymetrix genechips. The transcriptome profile of each time-point was associated to its respective HPLC pattern allowing to link each cell response to a specific degree of cell wall damage. The experimental data is presently being integrated through an *in silico* reconstructed metabolic network model for *S. aureus* strain COL. The results indicate that two main biosynthetic pathways are affected in the mutant along time, the wall teichoic acids and the lipids biosynthetic pathways.

Author Disclosure Block:

R.P. Portela: None. **A.M. Ludovice:** None. **F.R. Pinto:** None. **R.G. Sobral:** None.

Session Number:

095

Session Title:

Building the Great Wall: Diverse Bacterial Growth Strategies

Publishing Title:

Relative Rates of Surface and Volume Synthesis Set Bacterial Cell Size

Author Block:

L. K. Harris, J. A. Theriot; Stanford Univ., Stanford, CA

Abstract Body:

Many studies have focused on the mechanisms underlying length and width determination in rod-shaped bacteria. Here, by instead focusing on cell surface area to volume ratio (SA/V), we demonstrate that SA/V homeostasis underlies size determination. We propose a model whereby the instantaneous rates of surface and volume synthesis both scale with volume. This model predicts that these relative rates dictate SA/V and that cells approach a new steady-state SA/V exponentially, with a decay constant equal to the volume growth rate. To test this, we exposed diverse bacterial species to sublethal concentrations of a cell wall biosynthesis inhibitor and observed dose-dependent decreases in SA/V. Furthermore, this decrease was exponential and had the expected decay constant. The model also quantitatively describes SA/V alterations induced by other chemical, nutritional, and genetic perturbations. We additionally present evidence for a surface material accumulation threshold underlying division, sensitizing cell length to changes in SA/V requirements.

Author Disclosure Block:

L.K. Harris: None. **J.A. Theriot:** None.

Session Number:

095

Session Title:

Building the Great Wall: Diverse Bacterial Growth Strategies

Publishing Title:

Global Regulators of Cell Shape in *Caulobacter*

Author Block:

K. Sundararajan, S. Woldemeskel, **E. D. Goley**; Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract Body:

Bacterial cell shape emerges as a dynamic product of physical and chemical elements in the cell. Shape is maintained when growth conditions are stable, yet can change in response to intrinsic or extrinsic signals. Spatially regulated peptidoglycan (PG) metabolism is required for shape determination and maintenance. However, how distinct cell shapes can emerge from the same set of chemical and physical elements in different organisms or in the same organism under different growth conditions is largely unclear. We study morphogenetic mechanisms upstream and downstream of the bacterial tubulin, FtsZ, a polymerizing GTPase required for cytokinesis in most bacteria, as a paradigm for cell shape regulation in bacteria. FtsZ polymerizes to form the cytokinetic ring, recruits the division machinery, and orchestrates envelope invagination. FtsZ comprises three regions: the polymerizing GTPase domain; a poorly conserved, disordered C-terminal linker (CTL); and a C-terminal conserved (CTC) peptide that binds membrane-anchoring proteins. The CTL of FtsZ is notably long in α -proteobacteria, prompting us to investigate its role in *Caulobacter crescentus*. *C. crescentus* is well-suited to studies of morphogenesis, as it undergoes morphological transitions both during normal growth and in response to environmental stress. To do this, we generated an FtsZ variant with the CTL deleted (Δ CTL), expressed it in *C. crescentus*, and assessed its effects on morphology and viability. Surprisingly, production of Δ CTL was dominant lethal: cells became filamentous and exhibited envelope bulging and lysis in a manner reminiscent of treatment with β -lactam antibiotics, indicating defects in PG metabolism. To understand how Δ CTL exerts its effects, we screened for extragenic mutations that suppress its lethality. Mutations in conserved stress response pathways, including the (p)ppGpp and phosphate starvation pathways, suppress the filamentation, bulging and lysis associated with Δ CTL production. Moreover, Δ CTL-suppressing mutations in these pathways and in the conserved transcriptional regulator, CdnL, cause large-scale changes

to cell shape and development in a wild-type background, identifying these factors as global regulators of morphogenesis under normal growth and stress conditions. The mechanisms by which these conserved pathways impinge on PG metabolism and cell shape are the target of current studies.

Author Disclosure Block:

K. Sundararajan: None. **S. Woldemeskel:** None. **E.D. Goley:** None.

Session Number:

095

Session Title:

Building the Great Wall: Diverse Bacterial Growth Strategies

Publishing Title:

DDI3: A Novel Player in the Mycobacterial Replisome

Author Block:

K. M. Mann, D. Huang, C. L. Stallings; Washington Univ. in St. Louis, Saint Louis, MO

Abstract Body:

The ability of *Mycobacterium tuberculosis* (*Mtb*) to survive in a harsh host environment is key to its success as a pathogen. Host-derived stresses and antibiotics damage bacterial DNA, which *Mtb* must repair in order to survive. To investigate DNA damage repair in mycobacteria, we identified genes upregulated in response to double-stranded DNA (dsDNA) breaks in *Mycobacterium smegmatis* (*Msm*). One of the DNA damage inducible genes, *ddi3*, was upregulated in response to dsDNA breaks in log but not stationary phase of growth. *ddi3* is an uncharacterized gene with no known domains that is conserved throughout the class *Actinobacteria*. We have determined that *ddi3* is essential in *Msm*. Therefore, to study Ddi3, we engineered *Msm* to conditionally deplete *ddi3* transcripts. *Msm* depleted of Ddi3 exhibits slower growth, increased sensitivity to DNA damaging agents, and a filamentous cellular morphology with cell lengths 1-2 times that of replete controls. Elongated cell length indicates a block in cell cycle progression. To further examine this block, we used DAPI staining and fluorescence microscopy to monitor DNA distribution during Ddi3 depletion. We observed abnormal nucleoid morphology in Ddi3-depleted *Msm*, including anucleate cells. Ddi3 depletion also results in decreased rates of DNA synthesis as measured by ³H-thymidine incorporation and decreased DNA content per cell as measured by flow cytometry. Abnormal nucleoid morphology and decreased rates of DNA synthesis in *ddi3* mutants suggest a role for Ddi3 in DNA replication. To investigate the function of Ddi3, we engineered *Msm* to express an HA-tagged version of Ddi3. Using this strain, we immunoprecipitated cell lysates and analyzed eluates by mass spectrometry to identify Ddi3-interacting proteins. This approach detected a number of both replicative and DNA damage repair helicases, as well as other DNA replication proteins. Current work is focusing on determining the exact role of Ddi3 within the replisome. We have identified a novel gene that is essential for mycobacterial growth, has a role in DNA replication, and is conserved in all actinobacteria. There is recent appreciation for the distinctiveness of mycobacterial

growth relative to the model organisms *E. coli* and *B. subtilis*, and this study further emphasizes the diversity of bacterial strategies in even the most conserved biological processes, highlighting the importance of studying non-model organisms.

Author Disclosure Block:

K.M. Mann: None. **D. Huang:** None. **C.L. Stallings:** None.

Session Number:

101

Session Title:**Expanding Host Capabilities through the Microbiome****Publishing Title:****Calvin Cycle 2.0****Author Block:**

O. Dmytrenko¹, F. J. Stewart², D. R. Utter¹, C. M. Cavanaugh¹; ¹Harvard Univ., Cambridge, MA, ²Georgia Tech, Atlanta, GA

Abstract Body:

The majority of organic carbon on Earth comes from autotrophic CO₂ fixation. The Calvin-Benson-Bassham (CBB) cycle is the primary pathway for fixing CO₂ into biomass. This cycle is conserved from bacteria to plants, with the exception of chemosynthetic bacterial symbionts and a few of their closest relatives. These bacteria lack the *fbp* gene for fructose biphosphatase (FBPase), an essential enzyme in the CBB cycle. It has been hypothesized that these organisms alternatively use a pyrophosphate-dependent phosphofructokinase (PfkA) to dephosphorylate fructose 1,6-bisphosphate (FBP). The pyrophosphate (PPi) produced by PfkA could be converted into ATP, recovering some of the energy spent on CO₂ fixation. The role of PfkA in the CBB cycle was studied in the chemosynthetic symbiont of the bivalve, *Solemya velum*, and in a facultatively photolithoautotrophic bacterium, *Allochromatium vinosum*. The expression of *pfkA* and the other genes in the CBB cycle was among the highest in the symbionts within freshly-collected bivalves. PfkA activity, measured as PPi and fructose 6-phosphate formation on addition of FBP, was detected in symbiont-containing gill tissue but absent in symbiont-free tissue. The obtained rates (40 μmol/min/g of protein) agreed closely with published rates of symbiont CO₂ fixation. The symbiont PfkA was cloned in *E. coli*, expressed, purified, and biochemically characterized (K_m FBP - 27 μM, P_{O₄} - 2.3 mM). To study the ability of PfkA to support CO₂ fixation in the absence of FBPase, *fbp* and *pfkA* genes were deleted in *A. vinosum*, which, unlike the symbionts, is genetically tractable. While mutants lacking either *fbp* or *pfkA* grew autotrophically, the double mutant lacking both genes did not. This suggested that *pfkA* is sufficient and essential for autotrophic growth. Work is in progress testing the ability of the symbiont *pfkA* to restore autotrophic growth of the double mutant. To determine the potential of PfkA for recovering some of the ATP spent on CO₂ fixation, the *A. vinosum* mutants lacking either *fbp* or *pfkA* are being characterized in terms of their ATP production and

relative fitness. To examine the evolutionary role of PfkA vs. FBpase in the CBB cycle, an ancestral state reconstruction was performed. It indicated that the PfkA version of the cycle has evolved multiple times in diverse lineages of chemosynthetic symbionts and thus may have facilitated the origin and maintenance of these symbioses.

Author Disclosure Block:

O. Dmytrenko: None. **F.J. Stewart:** None. **D.R. Utter:** None. **C.M. Cavanaugh:** None.

Session Number:

101

Session Title:

Expanding Host Capabilities through the Microbiome

Publishing Title:

Metabolite Exchange within the Microbiome Influences *Drosophila* Behavior

Author Block:

C. N. Fischer¹, E. Trautman¹, J. M. Crawford¹, E. V. Stabb², N. A. Broderick³, J. Handelsman¹; ¹Yale Univ., New Haven, CT, ²Univ. of Georgia, Athens, GA, ³Univ. of Connecticut, Storrs, CT

Abstract Body:

Although communities are shaped by interactions between members, the molecular mechanisms that mediate host-microbial community association rarely account for microbe-microbe interactions. To elucidate mechanisms by which hosts associate with beneficial microbial communities, we used the genetically tractable insect *Drosophila melanogaster* along with its simple, cultivable microbiome of yeasts, lactic acid bacteria, and acetic acid bacteria. *Drosophila* locates yeasts via olfactory behavior yet it is unknown whether *Drosophila* behavior actively facilitates acquisition of its bacterial microbiome. Furthermore, members of the *Drosophila* microbiome interact metabolically within fermenting fruit, but it is unknown whether products of these interactions are detected by *Drosophila*. To address this knowledge gap, we adapted an olfactory behavioral assay to measure *Drosophila* preference for cultures of microbiome members grown individually or in communities. *Drosophila* prefers cultures of several microbiome members grown in communities relative to cultures of the same organisms grown individually and mixed, and we detailed one such *Drosophila*-microbial community interaction. *Drosophila* preferred a yeast-*Acetobacter* co-culture to a mixture of the same microorganisms grown individually, a behavior we found was partially mediated by the conserved olfactory receptor *Or42b*. *Drosophila* attraction correlated with three emergent properties of the co-culture: ethanol catabolism, a unique profile of volatile emissions, and yeast population decline. A 24-metabolite mixture resembling the mixture of volatile compounds from the co-culture similarly attracted *Drosophila*. *Acetobacter* ethanol metabolic derivatives were necessary and acetaldehyde metabolic derivatives were sufficient to generate *Drosophila* preference for the co-culture. In sum, our data support a model in which *Drosophila* selects its microbiome with specific metabolic characteristics by olfactory detection of emergent microbial community properties.

Author Disclosure Block:

C.N. Fischer: None. **E. Trautman:** None. **J.M. Crawford:** None. **E.V. Stabb:** None. **N.A. Broderick:** None. **J. Handelsman:** None.

Session Number:

101

Session Title:

Expanding Host Capabilities through the Microbiome

Publishing Title:

Transcriptomic Response of *Serratia marcescens* to the Amphibian Fungal Pathogen *Batrachochytrium dendrobatidis*

Author Block:

J. D. Madison, J. Kerby; Univ. of South Dakota, Vermillion, SD

Abstract Body:

Understanding the role of the amphibian microbiome in disease resistance is becoming increasingly important in development of disease treatment. Amphibian declines worldwide have been directly linked to the emerging chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). While reduced morbidity is linked to bacterial community composition, the underlying mechanisms of these microbial interactions are unclear. In order to understand these interactions, we characterized the transcriptomic response of a commonly identified member of the amphibian microbiome, *Serratia marcescens* which shows strong anti-*Bd* inhibition in vitro. *S. marcescens* were collected from a relict population of *Agalachnys annae* in Costa Rica. Cultures were grown in liquid TGhL broth using *Bd*, no *Bd*, and heat-killed *Bd* experimental treatments. Biological replicates (2) of each treatment were lysed, rRNA depleted, and library prepped (indexed). These samples (6 total) were used in RNA-seq on an Illumina MiSeq instrument. Analysis of FASTQ output files was done using Rockhopper 2 assuming a negative binomial distribution. Differentially expressed genes were identified with a q-value less than 0.05. Expression verification was carried out on a subset of identified genes using RT-qPCR. Pathway analysis was completed using KEGG. *Bd* exposed bacteria exhibited an upregulation of 56 genes as compared to control treatments. These genes (mapped to a de novo assembly) are involved in metabolism and signal transduction pathways including nitrogen, carbon, fatty-acid, and carbohydrate metabolism; oxidative phosphorylation, transcription regulation, and the *S. marcescens* two-component system. We also found upregulation of anti-sense RNA genes associated with genes that relate to transcription and ones with uncharacterized gene products. The results of this study suggest there is a transcriptomic response to *Bd*. Genes that were significantly upregulated are likely part of a global response. The possibility of *S. marcescens* anti-*Bd* action due to basally produced anti-*Bd* metabolites remains a possibility. Additionally, the upregulation of

asRNAs as a mechanism of an anti-*Bd* response is possible and at this point not fully understood. These results will aid in a deeper understanding of the interactions in this disease system and a potential microbial based treatment.

Author Disclosure Block:

J.D. Madison: None. **J. Kerby:** None.

Session Number:

101

Session Title:

Expanding Host Capabilities through the Microbiome

Publishing Title:

Estradiol Modifies Gut Microbiota Responses to a High-fat Diet in Female Mice

Author Block:

M. K. Nagyal, X. Gao, P. Ram, E. P. Bless, K. D. Acharya, M. J. Tetel, V. KLEPAC-CERAJ; Wellesley Coll., Wellesley, MA

Abstract Body:

Introduction: Estrogens protect against high-fat diet (HFD)-induced obesity in women and female mice. However, the mechanisms by which estrogens prevent diet-induced obesity are not well understood. Diet has been shown to play an important role in the shaping of the gut microbiome, which has been linked to weight gain. We hypothesized that one mechanism by which estradiol-treated mice fed a HFD resist weight gain is through alterations in the gut microbiome. **Methods:** To investigate the impact of estradiol on the female mouse gut microbiome, we analyzed microbial communities from fourteen adult C57BL6 mice that were ovariectomized and subcutaneously implanted with capsules containing either 17 β -estradiol (E2, 50 ug) or oil (Veh). All mice were fed a standard rodent chow for 10 days followed by HFD for 25 days. We analyzed the longitudinal 16S rRNA gene data from fecal pellets to identify and compare microbial community composition of samples across treatment and over time using QIIME, LefSe, and PICRUSt. **Results:** Veh-treated mice gained 35% more weight than E2-treated mice over the 35 days (student's t-test, $p < 0.05$). Interestingly, the gut microbiota in Veh-treated mice responded immediately after the switch to HFD. Over the course of the HFD, there was a 51.6% decrease in relative abundance of S24-7 family in the order Bacteroidales (Bacteroidetes) (Spearman's $\rho = 0.98$, $p < 0.01$) and a 25.2% increase in relative abundance of *Allobaculum* genus in the order Erysipelotrichales (Firmicutes) (Spearman's $\rho = -0.68$, $p < 0.01$) in Veh-treated mice. The diet-induced changes in the gut microbiome were more gradual in the E2-treated mice with a 33.7% decrease in S24-7 relative abundance and 7.6% increase in *Allobaculum* relative abundance over the course of the HFD. **Conclusion:** Estradiol-treated mice resist high-fat diet-induced changes in the gut microbiome, a potential mechanism, by which this hormone prevents diet-induced obesity.

Author Disclosure Block:

M.K. Nagyal: None. **X. Gao:** None. **P. Ram:** None. **E.P. Bless:** None. **K.D. Acharya:** None. **M.J. Tetel:** None. **V. Klepac-ceraj:** None.

Session Number:

102

Session Title:

Friends, Foes, and Frenemies: Phage-Host Dynamics

Publishing Title:

Crispr Escape Mutants Provide Insight Into a Mechanism for Phage Resistance in *Propionibacterium acnes*

Author Block:

L. J. MARINELLI¹, S. Fitz-Gibbon¹, G. F. Hatfull², R. L. Modlin¹; ¹UCLA, Los Angeles, CA, ²Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Propionibacterium acnes is a ubiquitous skin commensal that has also been implicated in the pathogenesis of the inflammatory skin disease, acne vulgaris. Phages infecting *P. acnes* reside with their host bacteria inside pilosebaceous follicles in sebum-rich regions of the skin in both healthy individuals and acne patients. We have shown that these phages display limited genetic diversity and broad host ranges against *P. acnes* isolates¹. Notably, a subset of *P. acnes* strains contains intact CRISPR (clustered regularly interspaced short palindromic repeat) loci^{2,3}, which, in other organisms, have been shown to confer resistance to phages and mobile genetic elements, such as plasmids. CRISPR-containing *P. acnes* isolates display elevated phage resistance, which correlates with matching spacer-protospacer (PS) pairs¹, and comparative genomic analysis supports the hypothesis that the CRISPR spacers are responsible for phage resistance in this organism. In order to test this and better understand the role of CRISPRs in *P. acnes*, we have isolated a collection of phage mutants that escape CRISPR resistance. Mutants were detected at varying frequencies, ranging from 10^{-3} - 10^{-7} , depending on the parent phage and host strain, and all display elevated plating efficiencies on CRISPR-containing isolates, which remain highly resistant to the parent phage; infectivity against other non-CRISPR isolates is unaffected. Critically, escape mutants contain point mutations in their PS and PS-associated motif (PAM) regions, consistent with the hypothesis that CRISPRs are involved in *P. acnes* phage resistance. We also observe that PS mutations are enriched near the 5' end of PSs, possibly indicative of the presence of a 'seed sequence', a PAM-proximal region with strict spacer/PS matching requirements that plays a critical role in the initial scanning of phage DNA by the CRISPR/Cas machinery, such as has been identified in *Escherichia coli*. Collectively, our data suggest that CRISPR resistance

plays an important role in governing the interactions between *P. acnes* and its phages, potentially influencing the structure and composition of the cutaneous microbiome.

Author Disclosure Block:

L.J. Marinelli: None. **S. Fitz-Gibbon:** None. **G.F. Hatfull:** None. **R.L. Modlin:** None.

Session Number:

102

Session Title:

Friends, Foes, and Frenemies: Phage-Host Dynamics

Publishing Title:

The Application of a Phage Cocktail to Prevent Infectious Disease: Characterizing the Impact of Virulent Phages on *Vibrio cholerae* Infection

Author Block:

L. S. Cairns¹, M. Yen¹, A. Camilli²; ¹Tufts Univ., Boston, MA, ²Tufts Univ. and Howard Hughes Med. Inst., Boston, MA

Abstract Body:

Cholera, caused by the facultative pathogen, *Vibrio cholerae*, is a major infectious disease in developing and disaster-stricken countries. There are an estimated 1-4 million cases resulting in 42,000-100,000 deaths per year. Household contacts of an individual suffering from cholera are 10-100 times more likely to contract the infection themselves. Household transmission could therefore present a critical point for clinical intervention by administration of prophylactic therapies. Virulent bacteriophages impact the dynamics of cholera infections. Three virulent phages, ICP1, ICP2 and ICP3, previously isolated from patient stool samples in Bangladesh, specifically target *V. cholerae*. We proposed that the combination of these three phages in a cocktail could be used as a prophylactic measure to prevent cholera. As a highly specific antimicrobial strategy, phage therapy has gained traction in recent years due to the current antibiotic crisis. By using a cocktail approach, we hope to limit the survival of genetically resistant *V. cholerae* cells. Using the infant mouse model of *V. cholerae* colonization, we have shown that the ICP cocktail is more effective in preventing colonization of the intestinal tract than any of the phages in isolation. Furthermore, the ICP cocktail effectively reduces colonization when administered up to 24 hours before infection with *V. cholerae*. Using the infant rabbit model of *V. cholerae* infection, we showed that the ICP phages significantly reduce the load of *V. cholerae* in the intestine when administered up to 24 hours prior to infection. Crucially, the phages are also effective in preventing the onset of cholera symptoms in these animals, namely; weight loss, accumulation of cecal fluid and the excretion of rice-water stool. Our data also provide insights into the impact of these phages on the host intestinal microbiota. Collectively, these experiments provide a proof-of-principle study into the usefulness of a phage cocktail in preventing an infectious bacterial disease.

Author Disclosure Block:

L.S. Cairns: None. **M. Yen:** None. **A. Camilli:** None.

Session Number:

102

Session Title:

Friends, Foes, and Frenemies: Phage-Host Dynamics

Publishing Title:

Bacteriophage Dynamics and Their Role in Bacterial Communities in Hypersaline Environment of Great Salt Lake

Author Block:

A. Mohaghegh Motlagh, A. Bhattacharjee, R. Goel; Univ. of Utah, Salt Lake City, UT

Abstract Body:

Introduction: Bacteriophages as the most abundant biological entities play a significant role in microbial population dynamics. Although bacteriophages in natural ecosystems has been studied in general, but still not much is known about the phages' diversity in hypersaline environments. In addition, regardless of the growing appreciation on the ecological role played by phages, only very little is known about their dynamics in natural ecosystems. Hence, it is imperative to be able to classify these organisms to determine their diversity and dynamics in hypersaline ecosystems such as Great Salt Lake. **Method:** Following phage extraction from the sediment sample collected from deep brine layer, CsCl gradient purification and DNase treatment were performed to ensure removal of any residual bacterial DNA. Phage DNA was extracted using Norgen phage DNA isolation kit followed by next-generation sequencing on Illumina MiSeq sequencer. Raw reads were quality filtered using CLC Workbench followed by scaffold assembly. ORF identification and genes were predicted with MetaGeneMark and taxonomic binning was performed using MEGAN. To understand the phage dynamics, the abundance of phages was compared with publicly available metagenomes of marine, freshwater, and lake sediment samples. Raw reads of aquatic viromes available on Metavir were mapped to assembled phage contigs with length longer than 20 kb using Bowtie v.2. Contig abundances were obtained by counting the number of reads mapped to each contig and the obtained abundance matrix was plotted as a heatmap in which samples and contigs were clustered according to their Euclidean distance. **Results:** For our study, we developed a high resolution insight into the viral biogeography by comparing the abundance of the novel unclassified *Caudovirales* isolated from Great Salt Lake sediments. The abundances of these novel phages were calculated among 140 phageomes of freshwater lakes, lake sediments, and hypersaline marine environments available in Metavir. **Conclusion:** As the Great Salt Lake is a hypersaline lake fed with

fresh water, it was interesting to observe from the high resolution abundance heatmap that these novel unclassified *Caudovirales* Great Salt Lake phages tend to be more abundant in marine environments than in hypersaline lakes, despite GSL being a land-locked hypersaline lake.

Author Disclosure Block:

A. Mohaghegh Motlagh: None. **A. Bhattacharjee:** None. **R. Goel:** None.

Session Number:

102

Session Title:

Friends, Foes, and Frenemies: Phage-Host Dynamics

Publishing Title:

The Race for Survival: Anti-Crispr and Crispr Dynamics in *Pseudomonas aeruginosa*

Author Block:

S. Y. Stanley¹, J. Bondy-Denomy², A. R. Davidson¹; ¹Univ. of Toronto, Toronto, ON, Canada, ²Univ. Of California, San Francisco, San Francisco, CA

Abstract Body:

The battle for survival between bacteria and phages has led to an evolutionary arms race where bacteria defend themselves against phages and phages overcome these defenses. CRISPR-Cas systems represent one of the most widespread mechanisms by which bacteria protect themselves from phage infection. Our lab discovered the first examples of phage-encoded inhibitors of CRISPR-Cas systems in *Pseudomonas aeruginosa*^{1,2}. These “anti-CRISPRs” must act during the process of infection to protect phages targeted by the *P. aeruginosa* CRISPR-Cas system. Here, we investigate how anti-CRISPRs manifest during the process of infection to overcome CRISPR-Cas activity. Through bioinformatic analysis, we identified promoter elements and operator sites immediately upstream of the anti-CRISPR genes in the phage genome. Furthermore, we identified a putative phage-encoded anti-CRISPR transcriptional regulator at the 3’ end of the anti-CRISPR locus in all anti-CRISPR containing phages. Through a series of *in vitro* studies and transcriptional reporter assays, we found that this transcriptional regulator interacts with the promoter region upstream of the anti-CRISPR genes and modulates its activity. By monitoring anti-CRISPR transcription as well as the fate of phage DNA over the course of an infection, we show that robust activation of anti-CRISPR transcription occurs prior to any significant degradation of phage DNA by CRISPR-Cas machinery. Taken together, these data suggest that an anti-CRISPR associated transcriptional regulatory system ensures timely expression of anti-CRISPRs to protect incoming phages against CRISPR-Cas mediated inactivation. Conceptually, this conserved mechanism present across all known anti-CRISPR phages could become a target for inhibition in the next step of the evolutionary arms race between bacteria and phages.

Author Disclosure Block:

S.Y. Stanley: None. **J. Bondy-Denomy:** None. **A.R. Davidson:** None.

Session Number:

104

Session Title:

Hitchhiking across the Eukaryotic Host Cell: Bacterial Toxin Trafficking and Translocation

Publishing Title:

***Staphylococcus aureus* Targets the Duffy Antigen Receptor for Chemokines (DARC) to Lyse Erythrocytes**

Author Block:

A. N. Spaan¹, T. Reyes-Robles², C. Badiou³, S. Cochet⁴, K. M. Boguslawski², P. Yoong², C. J. Day⁵, C. J. de Haas¹, K. P. Van Kessel¹, F. Vandenesch³, M. P. Jennings⁵, C. Le Van Kim⁴, Y. Colin⁴, J. A. Van Strijp¹, T. Henry³, V. J. Torres²; ¹Univ. Med. Ctr. Utrecht, Utrecht, Netherlands, ²New York Univ. Med. Ctr., New York City, NY, ³INSERM, Lyon, France, ⁴INTS, Paris, France, ⁵Griffith Univ., Gold Coast, Australia

Abstract Body:

Background: In order for *Staphylococcus aureus* to thrive inside the mammalian host, the bacterium has to overcome iron scarcity. By producing toxins that lyse erythrocytes, *S. aureus* releases hemoglobin, the preferred iron source for the pathogen in mammals. **Material/methods:** Here we describe the identification of the Duffy antigen receptor for chemokines (DARC) as the receptor for the hemolytic leukocidins LukED and HlgAB. **Results:** By combining human studies of DARC polymorphisms with gain and loss of function experiments and biochemical analyses, we demonstrate that DARC is necessary and sufficient to render host cells susceptible to these toxins. Importantly, DARC is required for *S. aureus*-mediated lysis of human erythrocytes ex vivo. By targeting DARC, HlgAB and LukED support *S. aureus* growth in a hemoglobin-acquisition dependent-manner. **Conclusions:** Thus, these findings provide the missing link of how *S. aureus* targets and lyses erythrocytes to release one of the scarcest nutrient within the mammalian host.

Author Disclosure Block:

A.N. Spaan: None. T. Reyes-Robles: None. C. Badiou: None. S. Cochet: None. K.M. Boguslawski: None. P. Yoong: None. C.J. Day: None. C.J. de Haas: None. K.P. Van Kessel: None. F. Vandenesch: None. M.P. Jennings: None. C. Le Van Kim: None. Y. Colin: None. J.A. Van Strijp: None. T. Henry: None. V.J. Torres: None.

Session Number:

104

Session Title:

Hitchhiking across the Eukaryotic Host Cell: Bacterial Toxin Trafficking and Translocation

Publishing Title:

Clathrin-Independent Endocytosis of *Clostridium difficile* Toxin A

Author Block:

R. Chandrasekaran, D. B. Lacy; Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract Body:

Clostridium difficile infection (CDI) affects a significant number of hospitalized patients in the United States. Two homologous exotoxins, TcdA and TcdB, are the major virulence factors in *C. difficile* pathogenesis. The toxins are glucosyltransferases that inactivate Rho family-GTPases to disrupt host cellular function and cause fluid secretion, inflammation, and cell death. Toxicity depends on receptor binding and subsequent endocytosis. TcdB has been shown to enter cells by clathrin-dependent endocytosis but the mechanism of TcdA uptake is still unclear. Here, we utilize a combination of RNAi-based knockdown, pharmacological inhibition and cell imaging approaches to investigate the endocytic mechanism(s) that contribute to TcdA uptake and subsequent GTPase inactivation and cell death. We show that TcdA uptake and cellular intoxication is dynamin-dependent but does not involve clathrin- or caveolae-mediated endocytosis. Confocal microscopy using fluorescently labeled TcdA shows that the toxin is internalized into pacsin2-positive membrane tubules that can undergo dynamin-dependent scission and release into the cytosol. Disruption of pacsin2 function by RNAi-based knockdown approaches inhibits TcdA uptake and toxin-induced cell death in Caco-2 cells. We conclude that TcdA and TcdB utilize distinct endocytic mechanisms to intoxicate epithelial cells.¹

Author Disclosure Block:

R. Chandrasekaran: None. **D.B. Lacy:** N. Other; Self; The Lacy laboratory has received funding support from Merck and MedImmune.

Session Number:

104

Session Title:

Hitchhiking across the Eukaryotic Host Cell: Bacterial Toxin Trafficking and Translocation

Publishing Title:

Non-Canonical Toxin Trafficking: *Helicobacter pylori* VacA Targeting of Mitochondria

Author Block:

R. L. Holland, K. D. Bosi, G. H. Harpring, S. R. Blanke; Univ. of Illinois at Urbana Champaign, Urbana, IL

Abstract Body:

Bacterial exotoxins exploit host cell uptake and transport pathways to access their intracellular targets. Toxins that covalently modify their intracellular host targets enzymatically translocate a catalytic component to the cytosol as a prerequisite for toxin activity. In contrast, endomembrane-acting, pore-forming toxins are taken up and transported in host cells by poorly understood mechanisms. VacA of the human gastric bacterium *Helicobacter pylori*, is a pore-forming toxin that localizes to mitochondria and causes organelle dysfunction. Mitochondria are central regulatory hubs for metabolic homeostasis, cell death, and innate immunity, and we hypothesize that toxin-mediated mitochondrial dysfunction may suppress normal cellular sensing and response functions within the infection microenvironment. Previous studies have failed to reveal VacA translocation to the cytosol, suggesting that VacA may transport to mitochondria by an alternative mechanism. Here, we evaluated the time-dependent transport of VacA from the host cell surface to mitochondria. By synchronizing the uptake of cell-surface bound VacA into gastric cells, we observed a rapid uptake of VacA into punctate organelles enriched in early endosomal markers, with arrival of VacA to mitochondria within 15 min. During this period, VacA was never detected in the cytosol, but always in vesicular structures we designated as VacA-containing vacuoles (VCVs), suggesting that VacA may be trafficked from the cell surface to mitochondria within transport vesicles. However, in contrast to canonical vesicular trafficking, VacA localization to and disruption of mitochondria were independent of canonical microtubule-based vesicular transport, but rather dependent upon actin. Magnetic based isolation of VCVs from cells intoxicated with ferromagnetic bead-labeled VacA, revealed VCVs to be complex, hybrid compartments, enriched in proteins associated with vesicular trafficking and mitochondria. These data support a model that VCVs are rapidly and dynamically remodeled from canonical early endosomal compartments to a new class of transport

vesicles that are competent for targeting mitochondria. Our data also suggest the existence of vesicular trafficking from the cell surface to mitochondria, which has not previously been described in eukaryotic cell biology.

Author Disclosure Block:

R.L. Holland: None. **K.D. Bosi:** None. **G.H. Harpring:** None. **S.R. Blanke:** None.

Session Number:

104

Session Title:

Hitchhiking across the Eukaryotic Host Cell: Bacterial Toxin Trafficking and Translocation

Publishing Title:

Genome-Wide CRISPR Screen Reveals Novel Host Factors Required for *Staphylococcus aureus* α -Hemolysin-Mediated Toxicity

Author Block:

S. Virreira Winter, A. Zychlinsky, **B. W. Bardoel**; Max Planck Institute for Infection Biology, Berlin, Germany

Abstract Body:

Staphylococcus aureus causes a wide variety of infections and antibiotic resistant strains are a major problem in hospitals. One of the best studied virulence factors of *S. aureus* is the pore-forming toxin alpha hemolysin (α HL) whose mechanism of action is incompletely understood. We performed a genome-wide loss-of-function screen using CRISPR/Cas9 technology using to identify host targets required for α HL susceptibility. The human myeloid cell line U937 was transduced with a library containing over 120,000 different gRNAs¹. Transduced Cells surviving intoxication with α HL were analyzed for CRISPR-targeted genes by parallel sequencing. We selected genes with an enrichment of at least two-fold of four gRNAs compared to unstimulated cells for validation and further analysis. Besides a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), the host receptor for α HL, we identified three proteins, Sys1 golgi trafficking protein (SYS1), ADP-ribosylation factor 1 (ARFRP1), and tetraspanin-14 (TSPAN14) which regulate the presentation of ADAM10 on the plasma membrane post-translationally. Targeting these genes prevents binding of α HL to the cells resulting in resistance to the toxin. Interestingly, we also showed that cells lacking sphingomyelin synthase 1 (SGMS1) resist α HL intoxication, but have only a slightly reduced ADAM10 surface expression. SGMS1 regulates lipid raft formation, suggesting that α HL requires these membrane microdomains for attachment and cytotoxicity. In the current study, we identified using a CRISPR screen different genes involved in regulation of ADAM10 expression. Besides the role of ADAM10 in *S. aureus* infections via α HL, the identified genes that regulate surface expression of ADAM10 are of interest to other diseases in which this receptor plays a role. The requirement of SGMS1 illustrates the importance of membrane composition for α HL-

mediated cytotoxicity. These finding help to further improve our understanding of the molecular pathogenesis of *S. aureus* infections.

Author Disclosure Block:

S. Virreira Winter: None. **A. Zychlinsky:** None. **B.W. Bardoel:** None.

Session Number:

106

Session Title:

Let's Start at the Very Beginning: What's New with Bacterial Transcription Initiation

Publishing Title:

An Antibacterial Agent Inhibited Bacterial Transcription by Targeting Primary Sigma Factor and Preventing Open Complex Formation

Author Block:

A. Paudel, H. Hamamoto, K. Sekimizu; Univ. of Tokyo, Tokyo, Japan

Abstract Body:

Background: We previously reported identification of novel antibacterial agent targeting bacterial SigA, compound 363, using silkworm infection model (54th ICAAC: F-975). SigA is unique to bacterial RNA polymerase thus making it a suitable target of antimicrobial agents. Despite this, only few antimicrobials have been identified that inhibit SigA. We found that the compound inhibited promoter-specific *in vitro* transcription by *Escherichia coli* RNA polymerase and showed therapeutic activity in mouse infection model. In this symposium, we report the mechanism of inhibition of transcription in *Staphylococcus aureus* by compound 363. **Methods:** *In vitro* transcription assay was performed with [α -³²P] UTP and synthesized run-off transcripts were electrophoresed in polyacrylamide gel and analysed by autoradiography. Recombinant his-tagged SigA was purified using Probond nickel chelating resin. SigA was bound to magnetic beads and treated with compound 363. After wash and elution, HPLC analysis was performed to check binding. RNA-seq analysis of *S. aureus* in presence and absence of the compound was performed by next generation sequencer. **Results:** Compound 363 inhibited the promoter-specific transcription in *S. aureus*. This inhibition was profound in the wild type whereas mutant was resistant. Further, we added the compound at various stages during transcription and found that it inhibited the open complex formation thus inhibiting initiation of transcription. Moreover, we found that SigA bound to compound 363. RNA-seq analysis revealed that expression of genes related to pyrimidine biosynthesis were down-regulated in the presence of the compound. **Conclusion:** Thus, an antibacterial agent that inhibited the open complex formation during transcription in *S. aureus*, was identified using silkworm infection model. To our knowledge, this is the first synthetic small molecule with therapeutic activity inhibiting function of the primary sigma factor of bacterial RNA polymerase.

Author Disclosure Block:

A. Paudel: None. **H. Hamamoto:** None. **K. Sekimizu:** C. Consultant; Self; Genome Pharmaceuticals.

Session Number:

106

Session Title:

Let's Start at the Very Beginning: What's New with Bacterial Transcription Initiation

Publishing Title:

Functional and Regulatory Studies of YbiH, an Induced Hypothetical Transcription Factor in Uropathogenic *Escherichia coli* during Infection

Author Block:

A. K. Chaurasia, S. Ravichandran, K. K. Kim; Dept. of Molecular Cell Biology, Sch. of Med., Sunkyunkwan Univ., Suwon, Korea, Republic of

Abstract Body:

Increase of antibiotic resistance in uropathogenic *E. coli* CFT073 (*UPEC*) raised an urgent need to identify novel cellular targets and their interacting drug partner to combat the *UPEC* pathogenesis. With the advent of genome sequencing and x-omics approach, a large number of hypothetical transcriptional regulators have been shown to induce during infection. YbiH, a hypothetical transcription factor has been identified to induce during infection. Here, we show that the YbiH is induced by the host in *UPEC* to inhibit *UPEC* infection potential. The deletion mutant of *ybiH* does not show any growth disadvantage while the multi-copy mutant complementation or overexpression of YbiH showed significant growth disadvantage and extremely reduced invasion potential compared to wild type or mutant. These results suggest that *ybiH* is host inducible host-offensive-defense virulence factor. Furthermore, *ybiH* expression is controlled by putative G-quadruplex (PG4) structure in the promoter (P_{ybiH}). The step-wise point mutations in G-quadruplex forming G-tracks were tested under *in vitro* conditions using CD spectroscopy and identified the key nucleotides which destabilize the G4 structure in P_{ybiH} . The *in vitro* results were further verified under *in vivo* conditions by constructing reporter strain using enhanced green fluorescent protein (EGFP) tagged with P_{ybiH} with or without G4 forming DNA motif. Moreover, cloning of G4-adaptor motif in front of strong T5 promoter stopped the expression of EGFP proving G4 mediated attenuation of *ybiH* under control conditions. The RNA sequencing data wild type versus mutant and wild type versus overexpressing *UPEC* strains would enable to establish the novel virulence-network under the control of YbiH transcription factor.

Author Disclosure Block:

A.K. Chaurasia: None. **S. Ravichandran:** None. **K.K. Kim:** None.

Session Number:

106

Session Title:

Let's Start at the Very Beginning: What's New with Bacterial Transcription Initiation

Publishing Title:

A Novel Heme-Responsive Regulator Controls Synthesis of Staphyloferrin B in *Staphylococcus aureus*

Author Block:

H. A. Laakso, C. L. Marolda, T. B. Pinter, M. J. Stillman, D. E. Heinrichs; Univ. of Western Ontario, London, ON, Canada

Abstract Body:

Iron acquisition is critical to the pathogenesis of *Staphylococcus aureus*, a major human pathogen responsible for a wide range of infections, including endocarditis, meningitis, and bacteremia. *S. aureus* has evolved several means of iron acquisition that enable it to overcome the limited concentration of free iron during infection. Mechanisms of iron acquisition utilized by *S. aureus* include heme-uptake systems, such as the iron surface determinant (Isd) system, and two high affinity iron-scavenging siderophores, staphyloferrin A (SA) and staphyloferrin B (SB). Although all systems are induced under iron-restricted conditions, it is thought that heme, which represents ~70% of iron within the host, is the preferred iron-source of *S. aureus*. The mechanisms that regulate this preference, however, remain unknown. In this study, we identify *sbnI* as encoding a transcription factor-like protein that is required for expression of genes in the *sbn* operon, the biosynthetic operon for synthesis of the endogenous *S. aureus* siderophore SB. Determined by qPCR, expression of *sbn* is drastically decreased in an *sbnI* mutant, and hence the mutant does not synthesize detectable SB during early phases of growth. Thus, SB-mediated iron acquisition is impaired in an *sbnI* mutant strain. We show that the protein forms dimers and tetramers in solution and electrophoretic mobility shift assays showed that SbnI binds to DNA within the downstream genes of the operon. Moreover, we show that SbnI binds heme, and that heme-bound SbnI does not bind DNA. Last, we showed through disc-diffusion bioassays, that providing exogenous heme to *S. aureus* growing in an iron-free medium results in delayed synthesis of SB. This is the first study in *S. aureus* that identifies a DNA-binding regulatory protein that senses heme to control gene expression for siderophore synthesis. Thus this study proposes a novel mechanism in which *S. aureus* controls SB synthesis in response to heme, which is of interest as both

SB- and heme- mediated iron uptake have been implicated in virulence in *S. aureus* models of infection.

Author Disclosure Block:

H.A. Laakso: None. **C.L. Marolda:** None. **T.B. Pinter:** None. **M.J. Stillman:** None. **D.E. Heinrichs:** None.

Session Number:

106

Session Title:

Let's Start at the Very Beginning: What's New with Bacterial Transcription Initiation

Publishing Title:

A Molecular Explanation for the Toxicity of Horizontally Acquired AT-rich Genes in Bacteria

Author Block:

S. S. Singh; Univ. of Birmingham, Birmingham, United Kingdom

Abstract Body:

Genetic elements can be transferred horizontally between bacteria. Often, horizontally transferred genes are AT rich and literature shows that AT-rich sequences are deleterious for fitness. Therefore, bacteria use mechanisms to minimise the harmful effects of foreign genes. Our work shows that the toxicity of AT-rich genes results from uncontrolled transcription within genes that result in titration of RNA polymerase and a global downshift in host cell transcription. H-NS, a DNA binding protein plays a role in silencing this intragenic transcription, thus counteracting the toxicity caused by horizontally acquired DNA. We have also found that mutations in RNA polymerase compensate for loss of H-NS by favouring regulated transcription. Accordingly, this work gives a detailed molecular description for the toxicity of horizontally acquired DNA and explains mechanisms employed by the bacteria to compensate for this toxicity.

Author Disclosure Block:

S.S. Singh: None.

Session Number:

107

Session Title:

Meet Me at the Membrane

Publishing Title:

A Role for Membrane Protein SpoIIQ in the Specific Gene Regulation of an Anti-Sigma^G Factor During *Bacillus subtilis* Sporulation

Author Block:

K. A. Flanagan¹, A. Camp, 01075²; ¹Mt. Holyoke Coll., South Hadley, MA, ²Mount Holyoke Coll., South Hadley, MA

Abstract Body:

SpoIIQ (“Q”) is an essential constituent of a channel connecting the developing forespore to the mother cell in sporulating *Bacillus subtilis*. Here, we present evidence that Q is a multifunctional protein, which we investigated in order to ascertain additional mechanisms by which Q regulates gene expression in the developing forespore. In addition to its established role as a “feeding tube” channel component, we have found that Q Tyr-28 is also required for a previously unknown regulatory pathway that blocks late, σ^G -dependent transcription of the gene encoding the σ^G inhibitor, CsfB. We used reporter assays to demonstrate that *csfB* is ordinarily transcribed only at early times by σ^F . However, in Q^{Y28A} cells, *csfB* is also aberrantly transcribed later by σ^G . Other σ^F -dependent promoters do not display aberrant σ^G -dependent activation in the Q^{Y28A} mutant, indicating that Q Tyr-28 specifically regulates *csfB* expression. Consistent with overproduction of CsfB, a Q^{Y28A} mutant fails to activate σ^G to wild-type levels. Bioinformatic and mutational analysis of the *csfB* promoter indicate a conserved promoter element that is both necessary and sufficient for Q Tyr-28-mediated inhibition. We used fluorescent microscopy and immunoblot analysis to demonstrate that Q Tyr-28 is also specifically required for proper localization and stability of the SpoIIIE phosphatase, raising the intriguing possibility that these two multifunctional proteins cooperate to fine tune developmental gene expression in the forespore at late times. Altogether, our data support a model in which Q Tyr-28 is required for a DNA-binding repressor to limit the levels of *csfB* expression during σ^G activity, a regulatory pathway that may involve a functional role for Q-dependent stabilization of SpoIIIE.

Author Disclosure Block:

K.A. Flanagan: None. **A. Camp:** None.

Session Number:

107

Session Title:

Meet Me at the Membrane

Publishing Title:

Bridging the Gap: Protein Interactions in a Mycobacterial Lipid Transport Pathway

Author Block:

M. H. Touchette, E. R. Van Vlack, L. Bai, **J. C. Seeliger**; Stony Brook Univ., Stony Brook, NY

Abstract Body:

The cell wall-associated lipoprotein LprG has been implicated in the transport and display of outer membrane lipids that are required for *Mycobacterium tuberculosis* virulence. We have shown *in vitro* that LprG transfers lipids between membranes independent of additional factors or energy sources.¹ In cells, however, LprG likely cooperates with other proteins to accomplish its critical functions, but the transport pathway and its components have not been elucidated. Since phenotypic assays specific for LprG function and conducive to genetics screens are not available, we pursued a biochemical approach to identifying proteins that interact with LprG, as candidates for partners that facilitate lipid transport. Specifically, we used site-specific unnatural amino acid incorporation and UV crosslinking in live mycobacteria to capture native interactions in the cell wall. The quantity and identity of crosslinked proteins were specific to particular sites within LprG, indicating multiple and overlapping interaction interfaces. Interacting proteins were identified by quantitative proteomics and included cell wall- and membrane-associated proteins. Several are lipoproteins of previously unknown function; others are associated with cell wall biosynthesis. Selected partners were further confirmed and biochemically characterized for their interactions with LprG. These results not only identify novel potential components of a virulence-associated lipid transport pathway, but also provide new evidence for protein complexes that integrate biosynthesis and transport processes, pointing to mechanisms that coordinate cell envelope expansion and thus facilitate polar growth in mycobacteria.

Author Disclosure Block:

M.H. Touchette: None. **E.R. Van Vlack:** None. **L. Bai:** None. **J.C. Seeliger:** None.

Session Number:

107

Session Title:

Meet Me at the Membrane

Publishing Title:

Actinorhodopsin in Freshwater Aci-Actinobacteria: Evidence for a Biological Role and a Novel Accessory Antenna

Author Block:

J. Dwulit-Smith, F. Moya, B. Oyserman, S. He, J. Hamilton, K. McMahon, **K. Forest**;
Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:

Actinobacteria of the acI lineage are ubiquitous and abundant in freshwater lakes. Several years ago, genomic evidence emerged that acI may pump protons through their plasma membranes by harvesting light with actinorhodopsin (actR). Single cell genome sequences are now available that confirm certain tribes have the capacity to synthesize actR. Moreover, analysis of lake water RNA samples demonstrates actR is a highly transcribed gene. Bioinformatic mining of the single cell genomes has yielded a cassette of putative carotenoid biosynthesis enzymes that likely generate actinorhodopsin's cofactors, retinal and a novel accessory antenna. Enzyme sequences were PCR-amplified from cDNA of a single acI-A cell and cloned into constructs that supply various carotenoid precursors. Expression of these gene products in *E. coli* followed by HPLC-MS analysis of whole cell extracts validated predicted enzymatic activities. Efforts to detect the complete antenna pigment and confirm its structure from both size-fractionated lake water and *E. coli* expressing all the predicted pathway enzymes are underway. Implications for ecology of acI Actinobacteria in light of these findings will be discussed.

Author Disclosure Block:

J. Dwulit-Smith: None. **F. Moya:** None. **B. Oyserman:** None. **S. He:** None. **J. Hamilton:** None. **K. McMahon:** None. **K. Forest:** None.

Session Number:

107

Session Title:

Meet Me at the Membrane

Publishing Title:

Delivering Lipoproteins to the Outer Membrane When Nobody'S Home: Bypassing the Essentiality of LolB

Author Block:

M. Grabowicz, T. J. Silhavy; Dept. of Molecular Biology, Princeton Univ., Princeton, NJ

Abstract Body:

Lipoproteins are a family of secreted proteins that have been acylated in the cytoplasmic membrane. In Gram-negative bacteria, many lipoproteins are destined for the outer membrane (OM) where they fulfil essential functions. Delivery of these highly hydrophobic molecules across the aqueous periplasm poses a challenge. In *E. coli*, the LolABCDE proteins are tasked with lipoprotein trafficking to the OM. Lipoproteins are extracted from the cytoplasmic membrane by LolCDE and then transferred to LolA which chaperones them across the periplasm. LolB is the sole member of the pathway that resides in the OM. Lipoproteins are delivered from LolA to LolB which then catalyzes their insertion into the OM bilayer. Most members of the Lol pathway are highly conserved among Gram-negative bacteria, but LolB is only found in β - and γ -proteobacteria. Hence, other organisms appear to lack the protein responsible for the final step in the Lol pathway. Yet, such organisms clearly produce lipoproteins with functions at the OM that are essential for viability. This paradox raises several questions: how, then, are lipoproteins trafficked in these organisms? Have divergent strategies for lipoprotein trafficking evolved? And, can the Lol pathway function without LolB? We investigated the essentiality of LolB in *E. coli*. We found that cells survive LolB-deplete conditions if the highly abundant OM lipoprotein Lpp is absent and one of the envelope stress responses is also inactivated. Moreover, inducing a second envelope response allows a complete bypass of LolB essentially. We show that essential lipoproteins still reach the OM and perform their functions. For example, we detect no significant defects in β -barrel protein folding, a process relying on four OM lipoproteins. Indeed, we find that the cell envelope is surprisingly robust despite the loss of *lolB*. Our data reveal that LolB in fact does not perform an essential function in the delivery of lipoproteins to the OM. Rather, LolB acts to substantially increase the efficiency of this trafficking. Our

results demonstrate the existence of a novel second pathway for lipoprotein trafficking to the OM that is sufficient to support viability. More broadly, our studies support the idea that in divergent organisms, LolB homologs might be neither required nor present. In these organisms, we suggest the second pathway functions in isolation.

Author Disclosure Block:

M. Grabowicz: None. **T.J. Silhavy:** None.

Session Number:

108

Session Title:**Microbial Electric Grids: Electromicrobiology, Fuel Cells, Nanowires, and Cable Bacteria****Publishing Title:****Discovery of a Versatile *Geobacter sulfurreducens* Electron Conduit Complex Responsible for Electron Transfer to Electrodes****Author Block:****F. Jimenez Otero, D. R. Bond; Univ. of Minnesota, Saint Paul, MN****Abstract Body:**

Electron transfer across the outer membrane during extracellular metal reduction has been proposed to use cytochrome *c*-based electron conduits¹ consisting of a β -barrel embedded in the outer membrane through which heme cofactors of periplasmic and extracellular cytochromes are aligned. The tandem duplication of conduits containing *omcB* or *omcC* ('*omcBC* cluster') is involved in electron transfer to metals by *G. sulfurreducens*², but the conduit(s) involved in electrode reduction have not been identified. In addition to mutants lacking the *omcBC* cluster, mutants lacking each of three uncharacterized gene clusters hypothesized to form electron conduits were constructed, as well as all possible combinations of multiple conduit deletions. Deleting the gene cluster *extABCD* decreased electron transfer to electrodes by 80% compared to wild type, a phenotype which could be rescued by complementation, while deleting the *omcBC* cluster had no effect. Additionally, a mutant lacking four conduits but retaining only *extABCD* ('*extABCD*⁺') grew 30% faster than wild type on electrodes, and reached a final current density 40% higher than wild type. To further examine the substrate specificity of *extABCD*, soluble and insoluble forms of Fe(III) were used as electron acceptors. As expected, the triple mutant containing only *omcBC*⁺ was able to reduce both Fe(III) substrates. Surprisingly, a quadruple mutant containing only *extABCD*⁺ grew at wild-type rates using Fe(III)-citrate as the electron acceptor. While no other conduits were able to facilitate electron transfer to Fe(III), all mutants containing only each one of the gene clusters were able to reduce Mn(IV)-oxide. A quintuple mutant lacking all gene clusters was unable to reduce either Fe(III) or Mn(IV). These results indicate that each of these clusters can be involved in extracellular electron transfer under specific circumstances. Of all of them, however, *extABCD* encodes the most versatile electron conduit in *G. sulfurreducens* which is sufficient for Fe(III)-citrate and Mn(IV)-oxide

reduction and essential for efficient electrode reduction, making it a promising candidate for bioengineering of microbial electrochemical systems.

Author Disclosure Block:

F. Jimenez Otero: None. **D.R. Bond:** None.

Session Number:

108

Session Title:

Microbial Electric Grids: Electromicrobiology, Fuel Cells, Nanowires, and Cable Bacteria

Publishing Title:

pH Shifts in the Anode Potential Response from *Thermincola ferriacetica* Suggest the Presence of a Rate Limiting Proton Coupled Electron Transfer Protein

Author Block:

B. G. Lusk; Arizona State Univ., Tempe, AZ

Abstract Body:

Background: *Thermincola ferriacetica*, a thermophilic, Gram-positive, anode respiring bacterium (ARB) was grown in biofilms in microbial electrochemical cells (MXCs) to investigate its external electron-transport (EET) limitations. Electrochemical studies, including low scan cyclic voltammetry (LSCV), are often used to elucidate the rate limiting step of electron transport in ARB biofilms. Previously reported CV analysis of *T. ferriacetica* biofilms indicated a sigmoidal Nernst-Monod response in electrical current (j) to changes in anode potential (V). This response suggests that a single proton (H^+) coupled electron ($n = 1$) transport reaction is responsible for the rate-limiting step in *T. ferriacetica* metabolism. The specific protein responsible for this response is thought to be a c -type cytochrome. Although *T. ferriacetica* has been shown to contain 35 c -type cytochromes, the one(s) responsible for EET has yet to be identified. **Methods:** To determine the effect of pH on Eka, biofilms were grown at 50 mM bicarbonate buffer and 25 mM acetate as the electron donor. After achieving a steady j , pH was altered by the addition of HCl or NaOH. Then, LSCV was performed to determine the effect of pH on Eka. To assess the effect of bicarbonate buffer on Eka, biofilms were grown with 10, 25, 50, and 100 mM bicarbonate with 25 mM acetate as the electron donor. After achieving a steady j , LSCVs were performed at 1.0 mV s^{-1} and 10 mV s^{-1} . This was repeated at 10, 25, 50, or 100 mM bicarbonate by either starting at 10 mM and increasing to 100 mM or by starting at 100 mM and decreasing to 10 mM. **Results:** *T. ferriacetica*'s response under certain growth conditions is composed of at least two separate $n = 1$ Nernst-Monod relationships; suggesting the presence of more than one pathway for anode respiration. Altering bulk pH reveals that biofilms in neutral to high pH (6.9-8.3) show a very broad redox peak while biofilms in low pH (5.2) reveal multiple redox peaks. Altering bicarbonate buffer concentration shows a similar trend, with lower bicarbonate leading to

the presence of multiple redox peaks; consistent with pH gradients developing inside the *T. ferriacetica* biofilm. **Conclusions:** *T. ferriacetica* contains more than one H⁺ coupled EET pathway and EET pathways within *T. ferriacetica* are sensitive to changes in bulk pH.

Author Disclosure Block:

B.G. Lusk: None.

Session Number:

108

Session Title:

Microbial Electric Grids: Electromicrobiology, Fuel Cells, Nanowires, and Cable Bacteria

Publishing Title:

Extracellular Electron Uptake by *Desulfovibrio ferrophilus* Strain IS5 via Outer-Membrane C-Type Cytochrome

Author Block:

a. okamoto, X. Deng, K. Hashimoto; The Univ. of Tokyo, Tokyo, Japan

Abstract Body:

Background: Microbially induced corrosion (MIC) under anaerobic conditions damages important energy infrastructure such as underground oil and gas pipelines, resulting in enormous economic losses that are estimated to be in the billions of dollars annually. Sulfate-reducing bacteria (SRB) commonly play a major role in promoting MIC in these environments; in particular, SRB are thought to not only produce corrosive H₂S during dissimilatory sulfate reduction but also deplete molecular hydrogen formed on iron or FeS surfaces, thereby enhancing rates of anaerobic corrosion. However, the relatively slow kinetics of hydrogen evolution have raised questions regarding the role of hydrogen as a major electron carrier for MIC processes. Recently, using Fe(0) as a sole electron donor, Dinh *et al.* isolated several novel SRB strains that induce corrosion significantly faster than hydrogen-consuming microbes. Here, we report that an intensely iron-corroding microbe, *Desulfovibrio ferrophilus* strain IS5, is capable of extracting electrons from an indium tin-doped oxide electrode via outer-membrane *c*-type cytochromes without consuming electrochemically generated hydrogen as an electron carrier. **Methods:** Electrochemical measurements using intact microbes were conducted in a single-chamber anaerobic 3-electrode system with *D. ferrophilus* strain IS5 precultivated in butyl-rubber-stoppered glass vials in DSMZ medium 195c at 303 K with an anoxic headspace of CO₂/N₂ (20:80, v/v) for 3 days. **Results:** When sulfate was presented as a metabolic electron acceptor, significant cathodic current production was observed at an onset potential of -200 mV vs. SHE, which was approximately 750 mV more positive than the onset for hydrogen evolution in our experimental condition. This finding indicates that hydrogen is not required for the cathodic reaction of IS5, suggesting that IS5 accelerates anaerobic iron corrosion through direct electron uptake. We will present our whole genome sequence of IS5 and analysis of the outer-membrane fractions

by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and biofilm voltammetric data, suggesting microbial electron uptake was conducted through the *c*-type cytochromes located at cell surface in IS5.

Author Disclosure Block:

A. okamoto: None. **X. Deng:** None. **K. Hashimoto:** None.

Session Number:

108

Session Title:

Microbial Electric Grids: Electromicrobiology, Fuel Cells, Nanowires, and Cable Bacteria

Publishing Title:

Effect of Oxygen on *Shewanella oneidensis* Mr-1 Per-Cell Extracellular Electron Transfer Rate

Author Block:

M. Lu, G. Wanger, S. Chan, S. Babanova, O. Bretschger; J Craig Venter Inst., San Diego, CA

Abstract Body:

Shewanella oneidensis is a facultative anaerobe that can generate current through extracellular electron transfer (EET) to solid conductive surfaces, under both anaerobic and aerobic conditions. Current production by *Shewanella* can be affected by environmental factors, such as pH, temperature, and oxygen concentration. Studies on the effect of oxygen on current production by *Shewanella* have shown inconsistent results. Generally, oxygen intrusion will decrease or even eliminate current production since oxygen is a preferred electron acceptor for *Shewanella* relative to a solid electrode. However, several researchers observed enhanced current production with oxygen exposure [1-3], which is hypothesized to be a result of promoted growth of *Shewanella* under aerobic conditions. However, the oxygen effect on per-cell EET behavior is still unclear since it has been difficult to study the real-time single cell behavior in operating bioelectrochemical systems (BESs). Here, we use optically accessible BESs to independently investigate the effect of oxygen on the growth and the per-cell EET rates of *S. oneidensis* MR-1 in real time. Our experiments confirmed that oxygen exposure impairs per-cell EET rate, though it promotes biomass growth, and the overall effect on current production is a competition between the increased cell population and decreased per-cell EET rate. Our results also indicated that the effect of riboflavin as an effective electron shuttle varies with different oxygen concentration. We also discovered that the per-cell EET rate can be recovered as oxygen concentration was switched from a high to low level. Thus it is possible to enhance current production by introducing oxygen in the BES during the initial stage to promote biomass growth, and reduce the dissolved oxygen (DO) value later to achieve high per-cell EET rate. Our preliminary results showed that by maintaining 1.26 mg/L DO for 8.5 hours and then reducing to 0.40 mg/L DO, a 57%

increase in current production was achieved, compared to the BES operated with DO maintained at 0.42 mg/L.

Author Disclosure Block:

M. Lu: None. **G. Wanger:** None. **S. Chan:** None. **S. Babanova:** None. **O. Bretschger:** None.

Session Number:**109****Session Title:****Ocean's Sixteen: New Discoveries in Marine Microbiology****Publishing Title:****Novel Archaeal-Bacterial Interactions and Functions Revealed via Activity-Based Sorting and Comparative Genomics of Individual Methane-Oxidizing Consortia****Author Block:**

R. Hatzenpichler¹, G. Chadwick¹, C. Skennerton¹, D. Goudeau², R. R. Malmstrom², T. Woyke², V. Orphan¹; ¹California Inst. of Technology, Pasadena, CA, ²Joint Genome Inst., Walnut Creek, CA

Abstract Body:

The anaerobic oxidation of methane accounts for the removal of ~90% of methane produced below the seafloor. In marine seeps AOM is predominantly catalyzed by a symbiosis of anaerobic methane-oxidizing euryarchaeotes (ANME) with sulfate-reducing bacteria. The potential for niche partitioning within these diverse, yet seemingly functionally redundant associations is not well understood and other archaeal-bacterial associations may await their discovery. We used bioorthogonal non-canonical amino acid tagging (BONCAT) for the in situ tracking of translational activity of AOM-consortia. By combining BONCAT-staining of active consortia with fluorescence-activated cell-sorting (FACS), whole genome amplification, and 16S rRNA gene sequencing, we resolved the identities of hundreds of individual, biosynthetically active partnerships. This approach revealed that representatives of all major ANME-clades occurring in a single seep sample were active under controlled incubation conditions. It also led to the discovery of a previously unrecognized interaction of ANME with members of the environmentally highly abundant, yet poorly understood phylum Verrucomicrobia. In order to shed light onto the functional potentials of this novel inter-domain association as well as the different ANME-SRB consortia observed in our sample, we reconstructed the genomes of 45 individual microbial consortia that were active at the time of sampling. Comparative genomics allowed us to compile a draft set of genes shared between all ANME-subgroups and identify potentially niche-differentiating features of specific ANME-SRB consortia.

Author Disclosure Block:

R. Hatzenpichler: None. **G. Chadwick:** None. **C. Skennerton:** None. **D. Goudeau:** None. **R.R. Malmstrom:** None. **T. Woyke:** None. **V. Orphan:** None.

Session Number:

109

Session Title:

Ocean's Sixteen: New Discoveries in Marine Microbiology

Publishing Title:

Under the Sea - *Roseobacter* Gardening of Microalgae

Author Block:

E. C. Sonnenschein, C. Phippen, M. Bentzon-Tilia, S. Rasmussen, K. Nielsen, L. Gram; Technical Univ. of Denmark, Kgs. Lyngby, Denmark

Abstract Body:

Interactions between microalgae and bacteria play a central role in global nutrient cycling. The versatility of these interactions has been studied for a haptophyte and its 'gardener', the *Roseobacter* clade species *Phaeobacter inhibens*: The algae likely provide the bacteria with nutrients, while the bacteria produce the antibacterial compound tropodithietic acid (TDA). Subsequently, the algal breakdown products induce the production of algicidal compounds, roseobactinoids, in the bacteria enhancing the algal decay. While structurally similar to TDA, their biosynthesis has not been fully elucidated. The purpose of this study was to investigate the distribution and genomic background of roseobactinoids in TDA-producing alphaproteobacteria and to compare the effect of producers and non-producers on microalgae. Chemical extracts were analyzed by UHPLC-DAD-QTOFMS and the closed genome sequences were investigated using CGView and OrthoVenn. Also, we are studying the survivability in co-cultures of microalgae and bacteria. All but one of the 19 *Phaeobacter* strains produced roseobactinoids, whilst four strains not belonging to the genus did not. This one non-producing *Phaeobacter* strain shared an average nucleotide identity of >98% with three producer strains and was isolated from the same location. While the closest producer strain has six plasmids, the non-producer carried seven plasmids: three being homologous, three carrying similar regions and one being absent in the producer. Comparison of the predicted proteins revealed 41 orthologous protein clusters unique to the producers. Most protein sequences could be traced back to a genomic region encoding a prophage. Furthermore, we found ethanol to be inducing roseobactinoid biosynthesis, while other stressors such as antibiotics or nutrient depletion did not. Concluding, TDA-producing alphaproteobacteria do not necessarily biosynthesize roseobactinoids or production is induced by unknown factors. This ability is potentially linked to the high genomic diversity within even closely related *Roseobacter* strains. The results of the co-

culture experiments will give indications on the environmental importance of roseobacticides and thus, the role of *Roseobacter* strains as ‘gardener’ of microalgae in the oceans.

Author Disclosure Block:

E.C. Sonnenschein: None. **C. Phippen:** None. **M. Bentzon-Tilia:** None. **S. Rasmussen:** None. **K. Nielsen:** None. **L. Gram:** None.

Session Number:

109

Session Title:

Ocean's Sixteen: New Discoveries in Marine Microbiology

Publishing Title:

Stable And Transient Members In The Marine Copepod Microbiome

Author Block:

P. H. Moisander¹, M. C. Daley¹, C. Dziallas², R. E. Scavotto³, A. D. Sexton⁴, K. M. Shoemaker¹, L. Riemann²; ¹Univ. of Massachusetts Dartmouth, North Dartmouth, MA, ²Univ. of Copenhagen, Helsingor, Denmark, ³Univ. of Massachusetts Dartmouth, North Dartmouth, MA, ⁴Univ. of New Hampshire, Durham, NH

Abstract Body:

Marine copepods (Arthropoda) are an important part of the marine food webs, serving as a major food source for fish larvae and other larger zooplankton. The composition, function, and controls of microbial communities found in association of copepods are currently not well understood. Both stable (potentially symbiotic) and transient (such as food-associated and randomly attached) components of the microbiome are expected. Many marine copepods feed on detrital bacteria-rich material, and thus food-associated bacteria could play a major role in the microbiome composition and function. We investigated stable and transient components of the copepod microbiome from the North Atlantic Ocean waters by studying copepods with full or voided guts by amplicon sequencing, and assessing presence of genes in microbial protein coding genes mediating steps in the nitrogen cycle. The stable gut-associated communities were investigated from fecal pellets produced when copepods were feeding on axenic phytoplankton. There were differences among starved and full gut copepod microbiomes assessed with 16S rRNA amplicon sequencing, and several microbial *nifH* gene phylotypes were detected in full gut copepods and seawater particles but not in starved copepods. These results suggest that food-associated bacteria form a major component in the copepod microbiome. In addition, while these small marine crustaceans require oxygen to survive, they appear to be hosting anaerobic bacteria in their guts. If the microbial component in the copepod food is digested, it could shunt carbon and nitrogen away from the protist-driven, traditional marine microbial loop.

Author Disclosure Block:

P.H. Moisander: None. **M.C. Daley:** None. **C. Dziallas:** None. **R.E. Scavotto:** None. **A.D. Sexton:** None. **K.M. Shoemaker:** None. **L. Riemann:** None.

Session Number:

109

Session Title:

Ocean's Sixteen: New Discoveries in Marine Microbiology

Publishing Title:

Indole-3-Acetic Acid is Produced by *Emiliana huxleyi* Coccolith-Bearing Cells and Triggers a Physiological Response in Bald Cells

Author Block:

L. Labeeuw, J. Khey, A. R. Bramucci, H. Atwal, P. de la Mata, J. Harynuk, **R. J. Case**;
Univ. of Alberta, Edmonton, AB, Canada

Abstract Body:

Indole-3-acetic acid (IAA) is an auxin produced by terrestrial plants which influences development through a variety of cellular mechanisms, such as altering cell orientation, organ development, fertility, and cell elongation. IAA is also produced by bacterial pathogens and symbionts of plants and algae, allowing them to manipulate growth and development of their host. They do so by either producing excess exogenous IAA or hijacking the IAA biosynthesis pathway of their host. The endogenous production of IAA by algae, however, remains contentious. Using *Emiliana huxleyi*, a globally abundant marine haptophyte, we investigated the presence and potential role of IAA in algae. Homologs of genes involved in several tryptophan-dependent IAA biosynthesis pathways were identified in *E. huxleyi*. This suggests that this haptophyte can synthesize IAA using various precursors derived from tryptophan. Addition of L-tryptophan to *E. huxleyi* stimulated IAA production, which could be detected using Salkowski's reagent and GC×GC-TOFMS in the C cell type (coccolith-bearing), but not in the N cell type (bald). Various concentrations of IAA were exogenously added to these two cell types to identify a physiological response in *E. huxleyi*. The N cell type, which did not produce IAA, was more sensitive to it, showing an increased variation in cell size and membrane permeability, and a corresponding increase in the photosynthetic potential quantum yield of Photosystem II (PSII). A bacterial symbiont of *E. huxleyi*, *Ruegeria sp.* R11, previously shown to produce IAA, was co-cultured with *E. huxleyi* C and N cells. IAA could not be detected from these co-cultures, and even when stimulated by addition of L-tryptophan, they produced less IAA than axenic C type culture similarly induced. This suggests that IAA plays a novel role, signalling between different *E. huxleyi* cell types, rather than between a bacterial symbiont and its algal host.

Author Disclosure Block:

L. Labeuw: None. **J. Khey:** None. **A.R. Bramucci:** None. **H. Atwal:** None. **P. de la Mata:** None. **J. Harynuk:** None. **R.J. Case:** None.

Session Number:

110

Session Title:

pH Up, pH Down: The Bacterial Response

Publishing Title:

The Effect of Cyclic Di-AMP on Pneumococcal Ion Transport

Author Block:

T. M. Zarrella, J. Yang, D. W. Metzger, G. Bai; Albany Med. Coll., Albany, NY

Abstract Body:

Streptococcus pneumoniae is able to cause otitis media, bacteremia, and pneumonia. Previously, two phosphodiesterases, Pde1 and Pde2, were found to be essential for pneumococcal virulence in mouse models of disease. These proteins cleave cyclic diadenosine monophosphate (c-di-AMP), which is a newly discovered second messenger in bacteria. However, its role as a messenger is not well understood. We hypothesize that c-di-AMP modulates protein activity within the pneumococcus, and that c-di-AMP-mediated regulation is essential for pneumococcal pathogenesis. We identified two c-di-AMP binding proteins, CabP and TrkA. They are both Trk-family proteins which are implicated in potassium transport. Genetically, CabP and TrkA are clustered with Trk-family potassium transporters TrkH and TrkG, respectively. We show that CabP and TrkH are essential for bacterial growth in low potassium media, but TrkA and TrkG are not. When pneumococcal Trk-family mutants were grown in media dropped out of all sodium salts, but sodium was then supplemented as NaCl, $\Delta cabP\Delta trkH$ and $\Delta trkA\Delta trkG$ displayed reverse dose-dependent growth. Surprisingly, we found that these growth phenotypes were not due to the altered concentration of sodium, but rather of chloride. Elevated levels of intracellular c-di-AMP hindered growth in high chloride concentrations, and displayed a phenotype similar to $\Delta cabP\Delta trkH$. However, the interaction of TrkA and TrkG is also disrupted in the presence of c-di-AMP. Taken together, these data suggest that CabP and TrkA have distinct functions. Overall, we report that ion homeostasis is regulated by c-di-AMP in *S. pneumoniae*. Future work will focus on the signaling cascades by which c-di-AMP controls pneumococcal physiology and pathogenesis.

Author Disclosure Block:

T.M. Zarrella: None. **J. Yang:** None. **D.W. Metzger:** None. **G. Bai:** None.

Session Number:

110

Session Title:

pH Up, pH Down: The Bacterial Response

Publishing Title:

Functional Analysis of *Staphylococcus aureus* Urease

Author Block:

C. Zhou, P. D. Fey; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract Body:

Staphylococcus aureus is one of the most important pathogens that cause opportunistic infections in both the community and health care facilities. Urease, which catalyzes the conversion of urea into ammonia and CO₂, is crucial to niche adaptation of some organisms such as *Helicobacter pylori* and *Staphylococcus saprophyticus*. However the function of urease in the pathogenesis or host colonization of *S. aureus* is relatively unknown. We hypothesize that urease functions to facilitate pH homeostasis in niches of the host where urea is abundant. To investigate this hypothesis, growth experiments were performed with *S. aureus* USA 300 LAC JE2 wildtype and urease deficient strains (JE2 Δ ureB) measuring pH, optical density at 600nm (OD₆₀₀) and colony-forming unit (cfu) following growth in Tryptic Soy Broth (TSB) containing either 14 or 45mM glucose with or without the addition of 10mM urea. Furthermore, the Nebraska Transposon Mutant Library (NTML) was screened to isolate mutants with differential urease activity using Christensen's urea agar. To confirm the screening results, real-time quantitative reverse transcription PCR or β -galactosidase assays were performed. JE2 Δ ureB showed a significant defect compared to wild type in growth yield, viability, and ability to restore a neutral pH in acidic conditions created by 45mM glucose. In contrast, the addition of 5mM arginine did not rescue viability or restore pH neutrality of JE2 grown in TSB containing 45mM glucose suggesting that arginine deiminase may not have as significant a function in pH homeostasis as urease in *S. aureus* USA300. Screening of the NTML revealed 33 mutants with decreased urease activity and 12 with increased urease activity. Potential urease regulators include CodY, MalR, ClpP, and PurA. Both qRT-PCR and a β -galactosidase assay confirmed these results. In conclusion, our data demonstrate that urease generated ammonia is important for pH homeostasis in niches where urea is abundant. Furthermore, the transcriptional regulation of urease by CodY suggests that urease may also be important in nitrogen metabolism.

Author Disclosure Block:

C. Zhou: None. **P.D. Fey:** None.

Session Number:

110

Session Title:

pH Up, pH Down: The Bacterial Response

Publishing Title:

Ph And Antibiotic Pressure Govern The Structure And Function Of The Cystic Fibrosis Lung Microbiome

Author Block:

R. A. Quinn¹, S. Widder², D. Conrad¹, J. LiPuma³, F. Rohwer⁴, R. Knight¹, P. Dorrestein¹; ¹Univ. of California at San Diego, La Jolla, CA, ²Univ. of Vienna, Vienna, Austria, ³Univ. of Michigan, Ann Arbor, MI, ⁴San Diego State Univ., San Diego, CA

Abstract Body:

Cystic fibrosis (CF) is a fatal genetic disease that results in buildup of thick mucus in the lungs. This mucus becomes infected with a complex community of microorganisms resulting in life-long chronic infections. Our group has been studying the physiology and dynamics of the microbial community focusing on exacerbations, which are events of CF disease characterized by an increase in symptom severity. Co-occurrence networking of 16S rDNA microbiome data (n=126 sputum samples) and metagenomic data (19 sputum samples) identified the existence of two contrasting communities in the CF lung. These were a community of classic CF pathogens (ie. *Pseudomonas aeruginosa*) utilizing respiration and consuming amino acids, and a fermentative community, comprised of mostly oral anaerobes. There was a higher relative abundance of the fermentative community during exacerbation events than during or after treatment with antibiotic therapy (Tukey's test ANOVA, p<0.05). We then tested the hypothesis that dysbiosis at exacerbation was due to the physiology of the two communities having an opposite effect on lung mucus pH. Using a novel culture model that mimics the lung environment, we show that at lower pH the anaerobic/fermentative community dominates, while at higher pH, *P. aeruginosa* and classic CF pathogens take over. Mass spectrometry-based metabolomics further supported this hypothesis by showing that *P. aeruginosa* virulence factors and quorum-sensing metabolites were elevated at higher media pH, but not present at low pH. Our findings demonstrate that the CF microbial community exists in stable and disturbed states that are determined by antibiotic pressure and pH. This study also shows how the core metabolism of an infectious microbiome can drive its structure and dynamics.

Author Disclosure Block:

R.A. Quinn: None. **S. Widder:** None. **D. Conrad:** None. **J. LiPuma:** None. **F. Rohwer:** None. **R. Knight:** None. **P. Dorrestein:** None.

Session Number:

110

Session Title:

pH Up, pH Down: The Bacterial Response

Publishing Title:

NhaC Cation/Proton Antiporters of *Staphylococcus aureus*

Author Block:

M. Vaish, A. Jereen, T. A. Krulwich; Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract Body:

Structurally distinct families of cation/proton antiporters (CPAs) in bacteria play major roles in cytoplasmic pH homeostasis in alkaline ranges of pH. They take up external protons utilizing inward proton gradients generated by respiration or other specific proton pumps. Concomitantly, the antiporter catalyzes efflux of cytoplasmic cations that are potentially toxic, e.g. Na^+ , Li^+ or excess K^+ (1). Such antiporters were first observed in alkaliphilic *Bacillus firmus* OF4 (later renamed *B. pseudofirmus* OF4) (2), were named NhaC and now have 4 numbered sub-sets, e.g. containing antiporter variants such as the H^+ -malate/ Na^+ -lactate antiporters or new species (3). Here, we report on a study of the physiological role and catalytic properties of 3 NhaC antiporters in *Staphylococcus aureus*, designated NhaC-1, NhaC-2 and NhaC-3. Each of the three single cistron-encoded antiporters was cloned into a pBad vector and transformed into a CPA-deficient *E. coli* KNabc host. Assays of Na^+/H^+ and K^+/H^+ antiport were carried out on everted membrane vesicles. All three antiporters NhaC-1, NhaC-2 and NhaC-3 exhibited Na^+/H^+ and K^+/H^+ antiporter activity in the pH range from pH 7 to pH 9.5. Activity of all three antiporters was most robust at elevated pH (8.5-9.5) and high concentration of sodium and potassium salt (750 mM). Studies have been initiated on the physiological roles of NhaC-1-3 by making individual knock-out strains in *S. aureus* Newman as well as double mutations with the other five antiporters, 3 CPA and 2 Mnh, which may reveal vulnerable areas of the antiport network.

Author Disclosure Block:

M. Vaish: None. **A. Jereen:** None. **T.A. Krulwich:** None.

Session Number:

112

Session Title:

Protein Destruction by Protein Machines

Publishing Title:

A Rufomycin Analogue Is an Anti-Tuberculosis Drug Lead Targeting ClpC1 - An Update

Author Block:

M. P. Choules¹, Y. Yu¹, S-H. Cho², J. R. Anderson², W. Gao², D. C. Lankin¹, J-Y. Kim³, J. Cheng³, S. H. Yang³, H. Lee³, J-W. Suh³, B. U. Jaki¹, S. G. Franzblau², G. F. Pauli¹;
¹Coll. of Pharmacy, Univ. of Illinois at Chicago, Chicago, IL, ²Inst. for Tuberculosis Res., Chicago, IL, ³Coll. of Natural Sci., Myongji Univ., Gyeonggi-Do, Korea, Republic of

Abstract Body:

Background: The continued search for new anti-tuberculosis drugs has led to the recent discovery of compounds targeting chaperone protein ClpC1 ATPase complex such as ecumicin (ECU, MW 1599.22, MIC 0.16 μ M), lassomycin, and cyclomarin have identified ClpC1 as a viable target for the treatment of *M. tuberculosis* (*M.tb*). Analogues of the heptapeptide rufomycin (RUF, MW 1041.55, MIC 0.019 μ M) were recently discovered and also shown to target ClpC1. **Methods:** Compound isolation was achieved through liquid-liquid countercurrent separation. Structure elucidation utilized ¹H, ¹³C, 2D NMR, high resolution mass spectrometry, and ¹H iterative full spin analysis (HiFSA). Full biological characterization was carried out with *M.tb* strain H37Rv, other mycobacteria strains, as well as non-mycobacteria. Activity against spontaneously generated mutants was used to confirm the absence of cross resistance between ECU and RUF. Checkerboard assays were used to determine potential for synergy **Results:** Seven (7) unique mutations in clpC1 were identified with >10x increase in MIC when treated with RUF-I. These point mutations were found to be distinct from those observed for ECU. The checkerboard assay indicated a lack of antagonism between ECU and RUF-I with potential for synergistic effects. **Conclusions:** Preliminary data suggests that the RUF analogues, at about half the size of ECU analogues, could be a potential drug lead utilizing ClpC1 as a multifaceted drug target for multidrug resistant *M. tuberculosis*.

Author Disclosure Block:

M.P. Choules: None. **Y. Yu:** None. **S. Cho:** None. **J.R. Anderson:** None. **W. Gao:** None. **D.C. Lankin:** None. **J. Kim:** None. **J. Cheng:** None. **S.H. Yang:** None. **H. Lee:** None. **J. Suh:** None. **B.U. Jaki:** None. **S.G. Franzblau:** None. **G.F. Pauli:** None.

Session Number:

113

Session Title:**Single Cell Behavior****Publishing Title:****A Multi-omics Approach to Disentangle Strain-specific Phenotypes in Bacterial Pathogens with a Pan-Genome****Author Block:**

Z. Zhu¹, P. A. Jensen², T. van Opijnen¹; ¹Boston Coll., Chestnut Hill, MA, ²Univ. of Illinois at Urbana Champagne, Urbana, IL

Abstract Body:

The increasing availability of fully sequenced bacterial genomes has demonstrated a distinction between a species' core genome (genes shared by all strains of the species) and pan-genome (species' global gene repertoire). Such genomic fluidity is remarkable, since genes are embedded in complex gene networks; the gain or loss of a gene can thus rewire an existing network and affect associated phenotypes. However, functional characterization of pan-genomes is lacking, partially due to the technical difficulties with conducting genome-wide experiments on a species-wide scale. Here we present an integrated systems biology approach to contextualize differences in growth profiles and nutrient requirements among 3 strains of the bacterial pathogen *Streptococcus pneumoniae*. By employing two genome-wide tools: Tn-Seq and RNA-Seq, we collected over 2 million data points of gene fitness and over 71,000 transcription profiles representing 8 distinct environments. These profiles are integrated into strain-specific FBA models consisting of 1002 metabolic reactions and 462 enzyme-coding genes to computationally characterize inter-strain differences. Over 10% of the core genome (~170 genes), mostly genes encoding metabolic enzymes, transporters and regulators, display strain-dependent fitness, meaning that an important gene for survival in one strain is dispensable in others under a specific condition. With simultaneous analyses of the expression data, we categorize these strain-dependent genes into: 1) strain-dependent fitness and expression (e.g. *purE*, *purK*, *purN* in the purine operon in D39), 2) strain-dependent fitness and conserved expression (e.g. genes for L-Asp to L-Thr conversion in TIGR4), and 3) conserved fitness and strain-dependent expression (e.g. the *trp* operon and chorismate mutase in aromatic amino acid synthesis in 19F). Overall, we show that central metabolism is rewired at both gene function and expression levels with compensatory changes in surrounding networks in a genetic context-dependent manner.

Our study generates fundamental knowledge on strain-specific gene networks, and is a first step towards dissecting complex phenotypes including antibiotic resistance and virulence. Moreover, this integrated approach is easily transferrable to other species with a large pan-genome.

Author Disclosure Block:

Z. Zhu: None. **P.A. Jensen:** None. **T. van Opijnen:** None.

Session Number:

113

Session Title:

Single Cell Behavior

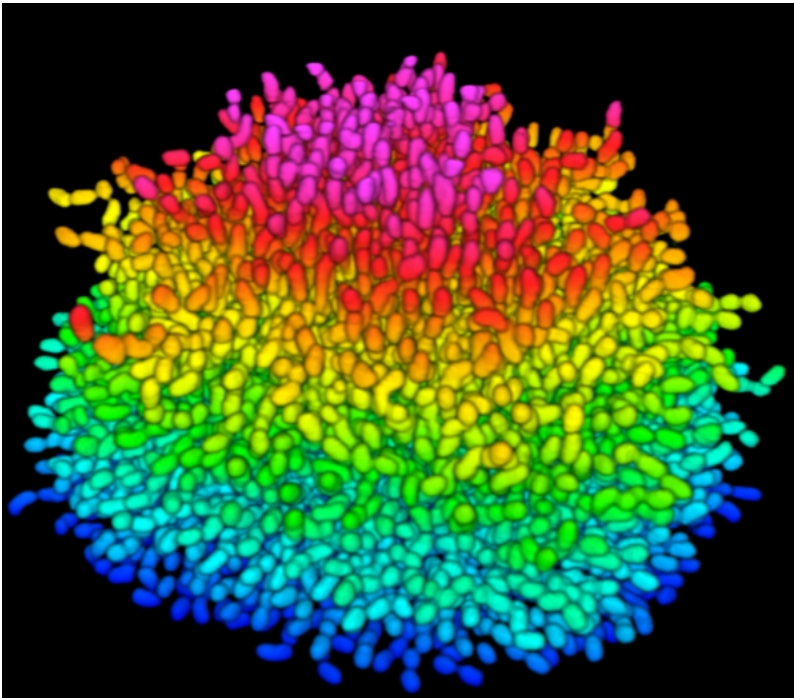
Publishing Title:

Single-cell Imaging of Living *Vibrio cholerae* Biofilms Reveals Collective Cellular Ordering

Author Block:

J. Yan, A. G. Sharo, H. A. Stone, N. S. Wingreen, B. L. Bassler; Princeton Univ., Princeton, NJ

Abstract Body:



Biofilms are surface-associated communities of bacteria that are a predominant form of bacterial life on Earth. Key facts are known about regulation of biofilm formation and production of the extracellular matrix that binds cells together and to the surface, however, little is known about internal biofilm structure at the single-cell level. Here, we image the growth of *living* biofilms of *Vibrio cholerae* from single founder cells to ten thousand cells at

single-cell resolution and with a temporal resolution of one cell division cycle. We discover a transition from two-dimensional (2D) branched growth to a dense three-dimensional (3D) cluster in which cells at the biofilm center exhibit collective vertical alignment and local nematic packing while cells at the periphery are arranged horizontally in radial fashion. Mutagenesis and *in situ* immunostaining enable us to trace the origin of biofilm cell ordering to a competition between biovolume expansion and cell-to-surface adhesion, the outcome of which is modulated by cell-to-cell adhesion. The extracellular matrix proteins Bap1 and RbmC drive cell-to-surface adhesion and RbmA controls cell-to-cell adhesion. We underpin our experimental results with an agent-based simulation incorporating the specific matrix protein functions. We suggest that biofilm cells exploit this biomechanical mechanism to balance the need for strong surface adhesion with the need for access to three-dimensional space. The ability to resolve and track the activity of individual cells within living bacterial biofilms should enable a transformation in our understanding of fundamental principles of biofilms.

Author Disclosure Block:

J. Yan: None. **A.G. Sharo:** None. **H.A. Stone:** None. **N.S. Wingreen:** None. **B.L. Bassler:** None.

Session Number:

113

Session Title:**Single Cell Behavior****Publishing Title:****Cell-to-cell Variability in Expression of the Multiple Antibiotic Resistance Activator Confers Transient Antibiotic Resistance****Author Block:**

I. El Meouche, Y. Siu, M. J. Dunlop; Univ. of Vermont, Burlington, VT

Abstract Body:

Within an isogenic bacterial population, individual organisms often exhibit noise in gene expression. This noise may lead to phenotypic heterogeneity, which generates divergence of bacterial cells responses in the presence of a stressor such as an antibiotic. Notably, transient antibiotic resistance allows microorganisms to temporarily survive lethal concentrations of antibiotics even without genetic changes. To date, research on transient, stochastic bet-hedging strategies has focused primarily on bacterial persistence, where a subpopulation of cells enters a dormant, drug-tolerant state for a finite time. Less is known about how stochastic effects lead to populations with broad, diversified levels of antibiotic resistance. In this work we focused on the multiple antibiotic resistance activator MarA. Already known for its major role in antibiotic resistance induction at the population level, we asked if stochastic MarA expression, when uninduced, can also generate variable resistance levels among growing cells in a population. By using single cell time-lapse microscopy we showed that isogenic cells express heterogeneous, dynamic levels of MarA. Critically, we found that MarA variability was correlated with survival to antibiotic exposure. Cells expressing higher initial MarA levels were likely to grow in filaments. These filamented cells were able to grow normally when returned to conditions without the stress and were equally susceptible when antibiotics were reintroduced. Our findings suggest that stochastic expression of the transcription factor MarA allows cells to transiently induce downstream resistance genes. Antibiotic resistance strategies that stochastically generate a continuum of resistance levels have important implications for public health, as mechanisms that generate elevated levels of resistance can facilitate evolutionary adaptation and serve as a stepping stone towards higher, permanent levels of drug resistance.

Author Disclosure Block:

I. El Meouche: None. **Y. Siu:** None. **M.J. Dunlop:** None.

Session Number:

113

Session Title:

Single Cell Behavior

Publishing Title:

Spatial and Temporal Expression Dynamics of a Global Bacterial Transcriptional Regulator during Pneumonic Plague

Author Block:

J. T. Ritzert¹, L. Sobieraj¹, J. T. Koo², W. W. Lathem¹; ¹Northwestern Univ., Chicago, IL, ²Wheaton Coll., Wheaton, IL

Abstract Body:

Yersinia pestis is the causative agent of pneumonic plague, an acute, rapidly progressing respiratory syndrome with a fatality rate approaching 100% if untreated. While the pulmonary compartment during pneumonic plague shifts from an anti-inflammatory to a pro-inflammatory environment during the course of the disease, little is known as to how *Y. pestis* responds to this changing lung environment to survive and proliferate. Therefore, we sought to determine the spatial and temporal responses of *Y. pestis* gene expression at the single-cell level during the progression of pneumonia. To do so, we examined the expression dynamics of the gene encoding the transcriptional regulator Crp. Crp regulates the expression of hundreds of genes in *Y. pestis*, and its expression and activity are known to change in response to environmental conditions. Fluorescent reporter constructs containing the *crp* promoter fused to the Gfp coding sequence were integrated onto the chromosome of virulent *Y. pestis* and mice were infected via the intranasal route. Cross-sections of lungs excised from mice at multiple time points post-infection were imaged to capture both spatial and temporal changes in reporter expression. *crp* promoter activity was quantified by measuring fluorescence from the reporter (GFP) at the single cell level from over 50,000 bacilli and normalized to constitutive RFP fluorescence. Mean reporter fluorescence increased over time, indicating that *crp* is upregulated as the infection progresses. Furthermore, we found that *Y. pestis* contained within large bacterial aggregates localized to the interior of inflammatory lesions showed higher reporter expression than isolated cells at the periphery of the same lesions. These results indicate that *crp* expression by *Y. pestis* is not stochastic within the lung; rather, its expression is dynamic and responsive to environmental cues present as the pulmonary environment shifts from the anti-inflammatory to the pro-inflammatory phase, and also within inflammatory foci

themselves. These results highlight the usefulness of high-resolution microscopy for uncovering temporal and spatial changes in gene expression at the single cell level during animal infections.

Author Disclosure Block:

J.T. Ritzert: None. **L. Sobieraj:** None. **J.T. Koo:** None. **W.W. Lathem:** None.

Session Number:

214

Session Title:

Environmental Control of Bacterial Cell Cycle

Publishing Title:

A Dynamic Redox Switch for the Topoisomerase IV Activity during Cell Cycle

Author Block:

S. Narayanan, **S. Radhakrishnan**; Indian Inst. of Sci. Ed. and Res., Thiruvananthapuram, India

Abstract Body:

Topoisomerase IV, an essential factor during chromosome segregation in bacteria, decatenates the catenated chromosomes at the end of each replication cycle. It has remained unclear as to how the decatenating activity of the topoisomerase IV is regulated during the early stages of chromosome cycle, despite the topoisomerase IV being in association with the chromosome. Herein we report the identification of a novel cell cycle regulated protein, NstA, as a negative regulator of the decatenation activity of the topoisomerase IV, during the early stages of cell cycle in the dimorphic bacterium, *Caulobacter crescentus*. The presence of NstA, during cell cycle, is tightly controlled at the level of transcription by GcrA and CcrM, and at the level of protein abundance by the protease ClpXP. We demonstrate that intermolecular cysteine disulfide controls the activity of NstA, and that NstA exerts its function by binding to the ParC subunit of the topoisomerase IV. Most importantly, we uncover a dynamic oscillation of the intracellular redox during cell cycle, which correlates with and controls the activity of NstA on topoisomerase IV, and is vital for the cell cycle progression. Thus, we propose that predetermined dynamic intracellular redox fluctuations regulate bacterial cell cycle progression.

Author Disclosure Block:

S. Narayanan: None. **S. Radhakrishnan:** None.

Session Number:

214

Session Title:

Environmental Control of Bacterial Cell Cycle

Publishing Title:

Novel Roles for FtsEX in *Caulobacter Crescentus*: Z-Ring Architecture and Chromosome Segregation

Author Block:

E. L. Meier, E. D. Goley, 21205; Johns Hopkins Sch. of Med., Baltimore, MD

Abstract Body:

In bacteria, a multiprotein complex called the divisome orchestrates invagination of the membranes and synthesis and remodeling of the cell wall. The tubulin-like GTPase FtsZ plays an essential role in bacterial cytokinesis as a scaffold for the assembly of the division machinery and, possibly, as a constrictive force generator. FtsZ forms an annulus (Z-ring) under the membrane at midcell and recruits all downstream components of the divisome, a subset of which regulate FtsZ activity and structure. FtsEX is a broadly conserved member of the divisome but its exact role during division remains poorly understood. FtsX spans the inner membrane and FtsE resides in the cytoplasm, together forming a membrane-embedded heterodimer. Research in numerous bacteria implicates FtsEX in the regulation of cell wall hydrolysis. In addition, *Escherichia coli* FtsE has been reported to bind to FtsZ, leading to the proposal that FtsEX coordinates Z-ring dynamics with cell wall remodeling. Most research on FtsEX has focused on the interplay between FtsEX and cell wall remodelers, however, leaving the significance of the putative FtsE and FtsZ interaction unclear. We study FtsEX function during division in the Gram-negative, α -proteobacterium *Caulobacter crescentus*, noted for its obligate asymmetric division and ease of synchronization. In *C. crescentus*, *ftsX* is essential while Δ *ftsE* cells are severely chained and display clusters of FtsZ foci. Overexpressing either *ftsE* or *ftsEX* causes rapid filamentation and lysis. Intriguingly, high levels of FtsE cause puncta of FtsZ to appear along the length of filaments, whereas high levels of FtsEX produce wide bands of FtsZ. These strikingly different FtsZ structures suggest that FtsE:FtsX stoichiometry affects FtsZ localization and architecture. Due to phenotypic similarities between Δ *ftsE* and cells lacking FtsK's DNA translocase domain, we hypothesized a role for FtsEX in chromosome segregation. Bacterial two hybrid results indicate an interaction between FtsE and MipZ, a DNA and FtsZ binding protein that coordinates chromosome segregation with division. Overproduction of FtsE or FtsEX

causes MipZ mislocalization with decreased and increased levels of MipZ and FtsZ, respectively. We hypothesize that *C. crescentus* FtsEX plays a novel role in ensuring proper cell separation and chromosome segregation, likely in an FtsZ and MipZ dependent manner.

Author Disclosure Block:

E.L. Meier: None. **E.D. Goley:** None.

Session Number:

214

Session Title:

Environmental Control of Bacterial Cell Cycle

Publishing Title:

Determining the Function of the Histidine Kinase Mora in *Sinorhizobium Meliloti*

Author Block:

J. L. Dombach, K. E. Gibson, 02125; Univ. of Massachusetts Boston, Boston, MA

Abstract Body:

The Gram-negative alphaproteobacterium *Sinorhizobium meliloti* can be found free-living in soil or within root nodules of various legumes where it performs nitrogen fixation. During free-living growth, it undergoes a tightly controlled cell cycle in which the chromosome is replicated once, and only once, and cell division produces asymmetric daughter cells. While *S. meliloti* is a model organism for chronic host invasion, there remains a great deal unknown about its cell cycle regulation. In the related species *Caulobacter crescentus*, a model for cell cycle control and asymmetric cell division, it is known that the histidine kinases (HKs) DivJ and PleC are responsible for regulation of DivK, which is part of a two-component signaling pathway that regulates a master regulator CtrA. Bioinformatics identified *S. meliloti* orthologs of DivJ and PleC as well as two novel HKs, CbrA and MorA. Our previous work has shown that CbrA, as predicted, is required for cell cycle progression and functions in the CtrA regulatory pathway as a DivK kinase. This work aims to determine how MorA functions in the cell cycle. We have taken the genetic approaches of epistasis analysis and cross-complementation as well as the biochemical approaches of *in vitro* kinase assays and *in vivo* transcription analyses. Preliminary results show that a *morA* null strain is defective for motility, but has wildtype production of flagella, and is normal for cell cycle progression. However, the $\Delta cbrA \Delta morA$ double mutant has additive cell cycle defects compared to the $\Delta cbrA$ mutant. In addition, ectopic expression of *morA* complements the cell cycle defects of a *cbrA* null strain, suggesting that MorA does function to regulate cell cycle progression in a manner that is redundant with CbrA. We therefore hypothesize that MorA function is restricted to aberrant growth or environmental conditions, and is therefore a novel regulator of the cell cycle. Further work will use a bacterial-2-hybrid system and protein pull down followed by mass spectrometry to determine the interaction partners of MorA in order better elucidate its function in *S. meliloti*.

Author Disclosure Block:

J.L. Dombach: None. **K.E. Gibson:** None.

Session Number:

214

Session Title:

Environmental Control of Bacterial Cell Cycle

Publishing Title:

Iron Starvation Inhibits Late Stages of Bacterial Cell Division and Arrests Cytokinesis

Author Block:

T. Santos, Y-J. Eun, M. Zhou, M. Lammers, K. Hurley, Q. Cui, D. Weibel; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:

Background: Bacterial cell division requires accurate placement of the division machinery at the division site and coordinated constriction of the cell envelope. Micronutrients, such as iron, are essential for cell division. In contrast to detailed knowledge of the mechanisms controlling division in bacteria, the effects of micronutrient starvation on this process are not well understood. Here we characterize the connection between iron starvation and bacterial cell division using divin, a small molecule that disrupts iron homeostasis and inhibits late stages of division. **Methods:** We used functional, fluorescently labeled versions of cell-division proteins to monitor spatiotemporal localization of the divisome. Fluorescence recovery after photobleaching and cell wall labeling enabled us to study cytoplasm compartmentalization and peptidoglycan remodeling at the division site. We performed structure-activity relationship (SAR) studies and molecular modeling to analyze the chemical properties of divin. In addition, we used in vivo protein labeling, quantitative reverse transcription PCR, and target-multicopy suppression screen to identify potential molecular targets and understand the downstream consequences of target inhibition. **Results:** Divin arrests division after initial constriction at the division site and prior to compartmentalization of the cytoplasm. It prevents the assembly of cell-division proteins and reduces peptidoglycan remodeling at the division site. Spectroscopic studies and in vivo protein labeling indicated that divin has metal-chelating properties and binds intracellular iron with high affinity. SAR studies and molecular modeling confirmed the chelating properties of divin. Consistent with these observations, addition of excess iron to divin-treated cells restored cytokinesis. Finally, we showed that expression of genes encoding cell-division proteins is significantly reduced in divin-treated cells and overexpression of these genes suppresses the biological activity of divin. **Conclusions:** Divin is a potent iron

chelator that perturbs bacterial iron homeostasis and arrests cytokinesis. Our results provide insight into the potential mechanisms by which iron homeostasis and cell division are coordinated in bacteria, and suggest potential strategies for antibiotic development.

Author Disclosure Block:

T. Santos: None. **Y. Eun:** None. **M. Zhou:** None. **M. Lammers:** None. **K. Hurley:** None. **Q. Cui:** None. **D. Weibel:** None.

Session Number:

215

Session Title:

Evolution of Microbial Consortia

Publishing Title:

Invasion and Interaction Networks in Microbial Communities Composed of Antibiotic Producers

Author Block:

E. S. Wright, K. H. Vetsigian; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:

Knowledge of the patterns of ecological interactions and their frequency dependence within and between species is central to understanding ecological and evolutionary dynamics in communities. At the same time, the type and pattern of microbial interactions that exist in natural communities are poorly understood, which is limiting our comprehension of ecological systems and our ability to engineer microbial consortia. Here we determined the network of invasions and inhibitions among a panel of 18 naturally antibiotic producing bacteria. We performed a tournament between all species pairs beginning from two extremes of relative abundance, and determined whether the least abundant strain could invade using high-throughput sequencing. In addition to survival of the fittest, we observed many cases of ‘survival of the common’ where the most abundant strain is the winner. Surprisingly, we observed few cases of cycles of dominance, and no instances of the well-studied rock-paper-scissors loop. The invasion network was shaped by inhibitory interactions between strains, which also promoted survival of the common. These findings have several immediate implications for how we view the assembly, structuring, and diversity of microbial communities. They indicate that pairwise interactions exhibit inherent nonlinearities that predispose communities toward multiple stable states. This makes microbial communities intrinsically sensitive to initial conditions during community assembly but, at the same time, makes them more resistant to change once they are established. The frequent ‘survival of the common’ that we identified may also promote mosaic distributions with different microbial populations dominating different spatial patches or hosts despite similar abiotic conditions.

Author Disclosure Block:

E.S. Wright: None. **K.H. Vetsigian:** None.

Session Number:

215

Session Title:

Evolution of Microbial Consortia

Publishing Title:

Siderophore Loss and Exploitation among Natural *Pseudomonas* Isolates

Author Block:

E. Butaite, R. Kümmerli; Univ. of Zurich, Zurich, Switzerland

Abstract Body:

Fluorescent pseudomonads typically secrete the siderophore pyoverdine (PVD) to scavenge iron from the environment. PVD non-producers often evolve in the laboratory because PVD is either not needed under the given culturing conditions, or the mutants act as cheats exploiting the PVD as a public good. PVD non-producers are found also among natural isolates. However, factors driving their evolution and their role in natural communities remain poorly studied. We investigated the pattern of PVD loss and exploitation among 920 natural *Pseudomonas* strains, isolated from soil and pond habitats. We found that PVD non-producers or low producers were significantly more common in habitats with higher iron content and lower pH, where iron is more bioavailable. This suggests that loss of PVD production is driven by disuse. However, we also found that PVD producers and non-producers often co-existed in the same community. A mixture of cross-feeding and competition assays revealed that many non-producers could exploit the PVD produced by other community members. We also found evidence for local antagonistic interactions because non-producers were better at using the PVD from non-community than community members. Altogether, our data suggest that evolution of PVD non-producers in natural communities is driven by both social and ecological factors and that PVD exploitation can select for novel, more exclusive variants of PVD. PVD secretion has become a model trait to study bacterial cooperation involving public goods in laboratory settings. Our findings now highlight that complex social interactions also play a role in natural bacterial communities, where they likely represent an important driver of strain diversification. Moreover, siderophore production is also relevant for virulence and heavy-metal detoxification, and it has been shown that the evolution of siderophore non-producers can significantly disturb these processes. Thus, our findings are interesting for applied research that seeks to develop therapeutic approaches promoting the spread of the less virulent non-producers in infections, or that seek to suppress the spread of non-producers in siderophore-based bioremediation processes.

Author Disclosure Block:

E. Butaite: None. **R. Kümmerli:** None.

Session Number:

215

Session Title:**Evolution of Microbial Consortia****Publishing Title:****Enhanced Cooperative Nitrogen Exchange between Syntrophic Bacterial Partners Leads to Less Carbon Assimilation****Author Block:**

B. LaSarre, 47405, A. L. McCully, 47405, **J. B. McKinlay**; Indiana Univ., Bloomington, IN

Abstract Body:

Synthetic microbial cocultures are useful for addressing ecological questions that are difficult to address in natural environments. However, maintaining stable relationships has been a challenge in establishing experimental cocultures. We developed a stable coculture between fermentative *Escherichia coli* and an engineered strain of phototrophic *Rhodospseudomonas palustris* (Nx). The two bacteria form a syntrophic relationship wherein *E. coli* ferments glucose and excretes essential carbon (organic acids) for *R. palustris* Nx while *R. palustris* Nx fixes N₂ and excretes essential nitrogen (NH₄⁺) for *E. coli*. Growth and metabolic trends are highly reproducible, allowing us to development of an ecological model that accurately describes our coculture observations. While exchange of metabolites is clearly the basis for many mutualistic microbial systems, the impact metabolite exchange rates on those relationships is difficult to address. We therefore used experimental and computational approaches with our coculture to address how the NH₄⁺ excretion rate affects the population of each species and coculture metabolism. To our surprise, the model predicted that a higher NH₄⁺ excretion rate would not affect the final *E. coli* cell density but would result in fewer *R. palustris* cells with unassimilated carbon left over as organic acids. Essentially, higher levels of NH₄⁺ would stimulate rapid *E. coli* growth with organic acids being produced faster than *R. palustris* could consume them, resulting in a growth-inhibiting acidic pH. To test this prediction, we generated a ‘hyper-cooperator’ *R. palustris* mutant that excreted 3-fold more NH₄⁺ than the Nx parent. Cocultures with the hyper-cooperator confirmed the predictions, as there were fewer *R. palustris* cells, a higher residual organic acid concentration, and a more acidic pH. Nonetheless, the hyper-cooperator and *E. coli* stably coexisted over serial transfers. These results illustrate how nutrient excretion rates can alter a syntrophic relationship. In this case, we found that organic acids play a dual role, providing either a benefit or detriment

to *R. palustris* depending on their level of accumulation. Enhancing the cooperative trait of NH_4^+ excretion shifted the role of organic acids towards being detrimental and resulted in less efficient utilization of carbon by our synthetic ecosystem.

Author Disclosure Block:

B. LaSarre: None. **A.L. McCully:** None. **J.B. McKinlay:** None.

Session Number:

215

Session Title:

Evolution of Microbial Consortia

Publishing Title:

Adaptative Landscapes of Tem-1 and Ctx-M-15 Beta-Lactamases

Author Block:

A. Birgy, k. panigoni, c. pintard, a. launay, o. tenaillon, **H. Jacquier**; INSERM, IAME, UMR 1137, Univ Paris Diderot, Paris, France

Abstract Body:

Background: The TEM-1 beta-lactamase has been well described in clinical isolates for 50 years. The emergence of mutations, conferring a spectrum extended to third generation cephalosporins or resistance to beta-lactamase inhibitors highlight its strong adaptability. Moreover, a lot of knowledges concerning thermodynamics, 3D-structure and enymology have been collected. For all these reasons, TEM-1 has quickly been considered as a model for evolutionary biologist. Conversely, the extended spectrum beta-lactamase CTX-M-15, has been well described in epidemiology studies, but a lot of evolutionary data are still lacking. In this study, we performed random mutagenesis of these two enzymes to compare common and different properties. **Methods:** TEM-1 and CTX-M-15 mutants were constructed using GeneMorph II Random Mutagenesis Kit (Stratagene) to obtain an average of one mutation per gene. The mutagenized amplicons were cloned into a modified pUC19 plasmid containing the pMB1 origin of replication from pBR322. The mutation effects were determined by measuring the Minimum Inhibitory Concentration of amoxicillin by agar dilution. **Results:** Out of the 10,000 TEM mutants and 2300 CTX-M-15 mutants performed, we obtained respectively 990 and 503 single missense mutants. Among these mutations, 240 were located in the same residues, with 83 common mutations. If we look at all common mutations, most mutations (85%) confer similar effects in the two backgrounds ($R^2=0.74$), suggesting that most mutations are not context-dependant. This rate was higher for mutations affecting conserved sites (89%), more buried in the proteins, and lower for variable sites (78%), located in more exposed sites. **Conclusions:** Most mutations in TEM-1 and CTX-M-15 beta-lactamases share similar effects. The context-dependence of mutations seems to be due the conservation of the mutated site, and their exposition in the protein.

Author Disclosure Block:

A. Birgy: None. **K. panigoni:** None. **C. pintard:** None. **A. launay:** None. **O. tenaillon:** None. **H. Jacquier:** None.

Session Number:

216

Session Title:

Getting into a Rhythm: Microbial Influence on Biological Clocks

Publishing Title:

Fine-Scale Phylogenetic Resolution of Community-Wide Diel Transcriptional Rhythms of Microbial Communities in the North Pacific Subtropical Gyre

Author Block:

F. O. Aylward, D. R. Mende, J. M. Eppley, T. Nielsen, E. F. DeLong; Ctr. for Microbial Oceanography Res. and Ed., Univ. of Hawaii at Manoa, Honolulu, HI

Abstract Body:

Introduction: Planktonic microbial communities inhabiting oligotrophic gyres of the world's oceans are critical drivers of global biogeochemical cycles. Recent evidence has suggested that whole-community metabolic activities in these planktonic assemblages vary in a consistent manner across diel cycles [1,2], but the precise phylogenetic groups involved as well as their specific metabolic activities are not well characterized. **Methods:** In this study we analyzed whole-community metatranscriptomic datasets collected every four hours over eight days during the Hawaii Ocean Experiment Legacy II cruise in the North Pacific Subtropical Gyre (NPSG). To increase our ability to assign transcripts to specific phylogenetic groups we compiled a reference gene library consisting of > 8 million genes predicted from over one hundred metagenomes sequenced from NPSG waters across different depths and seasons. **Results:** Our combination of large-scale metagenomic and metatranscriptomic sequencing allowed for unprecedented phylogenetic resolution of whole-community transcriptional dynamics that would not have been possible using traditional reference genome-base approaches. In particular, the transcriptional activities of sub-clades within the prevalent bacterial groups SAR11, SAR116, and *Roseobacter* were analyzed in detail. Moreover, a network-based approach to study interactions between microbial groups revealed numerous potential microbe-microbe and host-phage interactions of likely ecological importance. **Conclusions:** Our use of a comprehensive metagenome-based reference for transcript mapping substantially increased the phylogenetic resolution of this study and provided unprecedented insight into whole-community metabolic and ecological dynamics over diel cycles. In addition to confirming the importance of diel cycles in structuring the broader whole-community ecological dynamics, this approach allowed us to shed light on the impact of microdiversity in shaping community functioning.

Author Disclosure Block:

F.O. Aylward: None. **D.R. Mende:** None. **J.M. Eppley:** None. **T. Nielsen:** None. **E.F. DeLong:** None.

Session Number:

216

Session Title:

Getting into a Rhythm: Microbial Influence on Biological Clocks

Publishing Title:

The Dynamic Nature of the *Arabidopsis* Rhizosphere Microbiota

Author Block:

L. Pasa-Tolic¹, A. Ferrieri¹, C. Ansong¹, H. Brewer¹, A. Norbeck¹, M. Markillie¹, R. Ferrieri², S. G. Tringe³, M. Sadowsky⁴, C. Staley⁴, Y. Cui⁵, G. Stacey⁶; ¹Pacific Northwest Natl. Lab., Richland, WA, ²University of Missouri, Columbia, MO, ³DOE Joint Genome Inst., Walnut Creek, CA, ⁴Univ. of Minnesota, St. Paul, MN, ⁵Univ. of Missouri, Columbia, MO, ⁶Univ. of Missouri, Columbia, MO

Abstract Body:

The plant rhizosphere contains a complex microbial community that directly impacts plant growth, health, and development. Climate change factors, such as elevated CO₂ and soil temperature, are likely to impact carbon sequestration into recalcitrant pools, and the movement of essential nutrients, which control plant and microbial growth and productivity. We examined the rhizosphere community dynamics of *Arabidopsis thaliana* in natural soil using a range of techniques including radioisotopic analyses, high throughput metagenomic and 16S rRNA amplicon sequence analyses, metaproteomics, metametabolomics, and a variety of imaging techniques. We performed a time course experiment (in triplicate) using wild-type (WT) plants and mutants affected in carbon storage harvested after 5 weeks at 12 time points (every 6 to 72 hours). Results of this study showed that rhizosphere microbiota composition varied according to the light/dark cycle and differed between mutants and WT plants. The 16S rRNA amplicon sequencing results showed that, of the 21 major groups (phyla and classes of Proteobacteria) identified, 19 phyla exhibited distinct microbial diurnal cycles in the rhizosphere of WT plants. However, the diurnal cycling was not seen in an acyclic *Arabidopsis* line, indicating that the cycling of specific microbiota taxa is dependent on the plant circadian cycle. The proteomics and metabolomics analyses of community structure provided direct protein-level evidence for active biological processes within the community. This research provides an unprecedented view of the impact of diurnal cycles and changes in root C partitioning on the root microbiota. Our results suggest that the rhizosphere microbial community is very dynamic responding to both biotic and abiotic factors.

Author Disclosure Block:

L. Pasa-Tolic: None. **A. Ferrieri:** None. **C. Ansong:** None. **H. Brewer:** None. **A. Norbeck:** None. **M. Markillie:** None. **R. Ferrieri:** None. **S.G. Tringe:** None. **M. Sadowsky:** None. **C. Staley:** None. **Y. Cui:** None. **G. Stacey:** None.

Session Number:

217

Session Title:

Growing the Tree of Life

Publishing Title:

Assembling Whole Genomes from Mixed Microbial Communities Using Hi-C

Author Block:

I. Liachko¹, J. N. Burton¹, L. K. Sycuro², A. H. Wiser², D. Fredricks², M. J. Dunham¹, J. Shendure¹; ¹Univ. of Washington, Seattle, WA, ²Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract Body:

Background: Assembly of whole genomes from next-generation sequencing is inhibited by the lack of contiguity information in short-read sequencing. This limitation also impedes metagenome assembly, since one cannot tell which sequences originate from the same species within a population. We have overcome these bottlenecks by adapting a chromosome conformation capture technique (Hi-C) for the deconvolution of metagenomes and the scaffolding of *de novo* assemblies of individual genomes. **Methods:** In modeling the 3D structure of a genome, chromosome conformation capture techniques such as Hi-C are used to measure long-range interactions of DNA molecules in physical space. These tools employ crosslinking of chromatin in intact cells followed by intramolecular ligation, joining DNA fragments that were physically nearby at the time of crosslink. Subsequent deep sequencing of these DNA junctions generates a genome-wide contact probability map that allows the 3D modeling of genomic conformation within a cell. The strong enrichment in Hi-C signal between genetically neighboring loci allows the scaffolding of entire chromosomes from fragmented draft assemblies. Hi-C signal also preserves the cellular origin of each DNA fragment and its interacting partner, allowing for deconvolution and assembly of multi-chromosome genomes from a mixed population of organisms. **Results:** We have used Hi-C to scaffold whole genomes of animals, plants, fungi, as well as prokaryotes and archaea. We have also been able to use this data to annotate functional features of microbial genomes, such as centromeres in many fungal species. Additionally, we have applied our technology to diverse metagenomic populations such as craft beer, bacterial vaginosis infections, soil, and tree endophyte samples to discover and assemble the genomes of novel strains of known species as well as novel prokaryotes and eukaryotes. **Conclusion:** The high quality of Hi-C-based assemblies allows the simultaneous assembly of numerous unculturable

genomes, placement of plasmids within host genomes, and microbial strain deconvolution in a way not possible with other methods.

Author Disclosure Block:

I. Liachko: A. Board Member; Self; Phase Genomics, Inc.. D. Employee; Self; Phase Genomics, Inc.. K. Shareholder (excluding diversified mutual funds); Self; Phase Genomics, Inc. **J.N. Burton:** K. Shareholder (excluding diversified mutual funds); Self; Phase Genomics, Inc.. **L.K. Sycuro:** None. **A.H. Wiser:** None. **D. Fredricks:** None. **M.J. Dunham:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Phase Genomics, Inc.. K. Shareholder (excluding diversified mutual funds); Self; Phase Genomics, Inc. **J. Shendure:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Phase Genomics, Inc.. K. Shareholder (excluding diversified mutual funds); Self; Phase Genomics, Inc..

Session Number:

217

Session Title:**Growing the Tree of Life****Publishing Title:****Microfluidics Based Mini-Metagenomics Improves the Discovery of Novel Microbial Organisms****Author Block:**

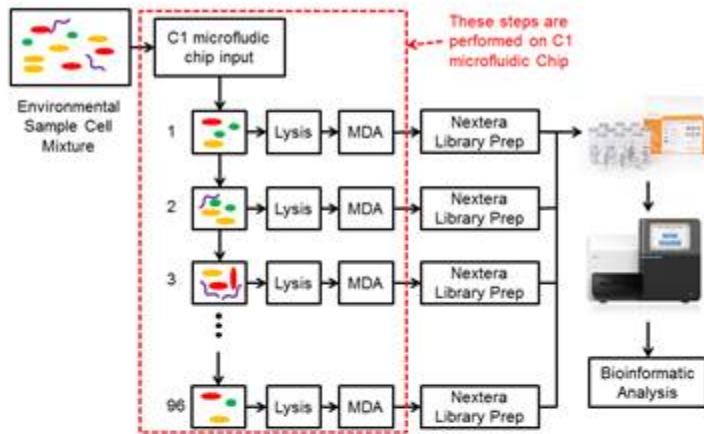
F. Yu¹, R. Malmstrom², T. Woyke², M. Horowitz¹, S. Quake¹; ¹Stanford Univ., Stanford, CA, ²Joint Genome Inst., Walnut Creek, CA

Abstract Body:

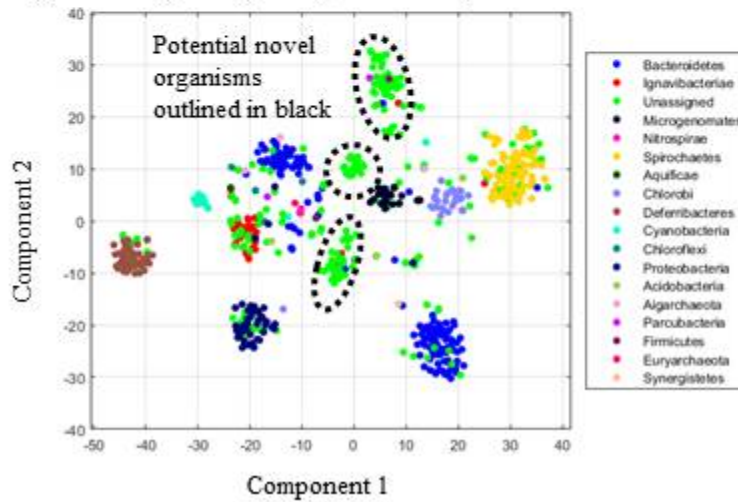
Even though many techniques exist for the analysis of metagenomic materials, the majority of environmental microbial diversity remains uncharacterized. Conventional shotgun and single cell metagenomic techniques are often limited by sample complexity and throughput respectively. In this work, we present mini-metagenomics, a technique that combines advantages of both shotgun and single cell methods for discovering novel organisms. This technique isolates populations of around 10 cells from an environmental sample, significantly reducing the complexity of each isolate. Lysis, whole genome amplification, and sequencing are performed on each isolate. Then, contigs are assembled using standard bioinformatics tools. Because we process 96 isolates in parallel, each containing 10 cells, throughput is improved. Leveraging automation capabilities, the sample preparation portion of our mini-metagenomic technique is implemented on a modified Fluidigm C1 microfluidic platform (Figure 1a). Using this method, we analyzed a hot spring sample from Yellowstone National Park. 100 million paired end 2x150bp reads were generated using Illumina's Nextseq platform. 643 contigs greater than 10kbp were assembled and 30% could not be assigned to a phylum. Since cells are randomly partitioned, each contig typically appears in multiple mini-metagenomic isolates. Thus, presence of a contig across isolates provides sequence independent information for binning unknown sequences into phylogenetic groups. Using this information, we efficiently separated contigs belonging to 17 phylums and recovered 3 potential unknown phylums (Figure 1b). Our analysis demonstrates the power of the mini-metagenomic

approach in discovering novel organisms.

(a) Mini-Metagenomic Pipeline



(b) Clustering Contigs Longer than 10kbp



Author Disclosure Block:

F. Yu: None. **R. Malmstrom:** None. **T. Woyke:** None. **M. Horowitz:** None. **S. Quake:** None.

Session Number:

217

Session Title:

Growing the Tree of Life

Publishing Title:

Gene Acquisitions From Bacteria At The Origin Of Major Archaeal Clades Are Vastly Overestimated

Author Block:

M. Groussin¹, B. Boussau², G. Szollosi³, L. Eme⁴, M. Gouy², C. Brochier-Armanet², V. Daubin²; ¹Massachusetts Inst. of Technology, CAMBRIDGE, MA, ²Univ. Lyon 1, CNRS, UMR 5558, Laboratoire de Biometrie et Biologie Evolutive, Villeurbanne, France, ³ELTE-MTA “Lendulet” Biophysics Res. Group, Budapest, Hungary, ⁴Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Background: Recent evolutionary studies of prokaryotic genomes highlighted the high rates of Horizontal Gene Transfer (HGT). HGT is now considered as a major process entailing genome evolution, providing metabolic innovations and promoting new adaptations. However, the contribution of HGT during the formation of major prokaryotic lineages is poorly understood. In a recent article, Nelson-Sathi et al. (NS, Nature, 2015, 517:77-80) report that the origins of Major Archaeal Lineages (MAL) correspond to massive group-specific gene acquisitions (2,264) via HGT from bacteria. If correct, this would have fundamental implications for the process of diversification in microbes. **Methods:** A re-examination of these data shows that the methodology used by NS systematically inflates the number of genes acquired at the root of each MAL, and incorrectly assumes bacterial origins for these genes. We re-analyzed their data with appropriate phylogenetic models in parsimony and Maximum Likelihood, accounting for the dynamics of gene gain and loss between lineages along the MAL phylogenies. **Results:** We show that most of the gene acquisitions identified by NS are not located at the origin of MALs, but are spread over long periods in their evolution. Thus, 75% of the 2,264 genes were acquired during the diversification of MALs, and not at their origin. For instance, the number of gene acquisition at the root of Methanosarcinales drops from 338 to 15. Additionally, we show that NS’s assumption that the 2,264 genes were transferred from Bacteria to Archaea rather than the reverse is unfounded. **Conclusion:** Gene transfer seems to have occurred continuously across the tree of Archaea. Both the quantification and the argument for a systematic bacterial origin

defended by NS are erroneous. Our results strongly claim for the use of accurate phylogenetic methods and models to understand the evolutionary history of adaptations in relation to gene transfer.

Author Disclosure Block:

M. Groussin: None. **B. Boussau:** None. **G. Szollosi:** None. **L. Eme:** None. **M. Gouy:** None. **C. Brochier-Armanet:** None. **V. Daubin:** None.

Session Number:

217

Session Title:

Growing the Tree of Life

Publishing Title:

Microbial Genome Atlas: Standardizing Genome-Based Taxonomic Analyses for *Archaea* and *Bacteria*

Author Block:

L. M. Rodriguez-R¹, S. Gunturu², C. Luo¹, J. Tiedje², J. Cole², K. T. Konstantinidis¹;
¹Georgia Inst. of Technology, Atlanta, GA, ²Michigan State Univ., East Lansing, MI

Abstract Body:

Background: The small subunit ribosomal RNA gene (16S rRNA) has been successfully used to catalogue and study the diversity of microbial species and their communities to date. Nonetheless, several aspects of the rRNA-based studies remain problematic. Most importantly, how to better resolve microbial communities at the levels where the 16S rRNA gene provides inadequate resolution, namely species and finer levels, and how to best catalogue whole-genome diversity and fluidity. Further, the deluge of genomic data in the last three decades has promoted the development of a rapidly growing set of bioinformatic techniques and implementations. However, the lack of standards in genomic analyses complicates data sharing and comparisons across research projects. **Methods:** Here, we introduce the Microbial Genome Atlas (MiGA), a genomic data management and processing tool integrating best practices in genomic analyses with recent and novel developments in whole-genome-based taxonomy and classification. Data is processed according to presets tuned for isolate genomes, single-cell sequences, metagenome-derived bins, or metagenomes. MiGA features an indexing system based on medoid clustering over sparse matrices of Average Nucleotide and Amino acid Identity (ANI/AAI) guided by heuristic approximations, enabling the fast classification of query genomes, as well as calculation of pangenome and assessment of horizontal gene transfer among the ~40,000 genomes included in its database. **Results:** End users can interact with MiGA through the Web Interface, the built-in Command Line Interface, or the Ruby Application Interface. Analysis of available *Bacillus anthracis* genomes (n = 300) with MiGA showed that it is possible to distinguish between these very closely related genomes (ANI>99.8%), identified previous as well as new clades within the species, detected obvious misclassification based on previous MLVA or SNP approaches, and provided new insights into the degree of biogeography of North American

genomes. **Conclusions:** MiGA represents the genome equivalent of the Ribosomal Database Project and aims to facilitate classification and diversity studies at the genome level. MiGA is currently available through the webserver of our laboratory (<http://enve-omics.gatech.edu/>).

Author Disclosure Block:

L.M. Rodriguez-R: None. **S. Gunturu:** None. **C. Luo:** None. **J. Tiedje:** None. **J. Cole:** None. **K.T. Konstantinidis:** None.

Session Number:

218

Session Title:

Keeping the Batteries Charged

Publishing Title:

Investigation of the Formate and Hydrogen Metabolism of Different *Thermococcus* Species

Author Block:

K. Trchounian¹, S. Spaans², A. Trchounian¹, A. Stams², S. Kengen²; ¹Yerevan State Univ., Yerevan, Armenia, ²Wageningen Univ., Wageningen, Netherlands

Abstract Body:

Formate is an important intermediate in the anaerobic mineralization of organic matter in hydrogenogenic and methanogenic environments. Formate is a substrate for growth of methanogens, acetogens and sulfate-reducing bacteria. Under standard conditions this reaction is thermodynamically unfavorable ($\Delta G^{\circ} = +4.5$ kJ/mol). Recently, it has been shown that the hyperthermophilic archaeon *Thermococcus onnurineus* strain NA1 is able to grow on formate and convert it to H₂ and CO₂ [1]. In this work, three *Thermococcus* strains (*T. kodakaraensis*, *T. gammatolerans*, *T. barophilus*) were compared with respect to growth on formate as sole carbon and energy source. Different growth conditions were tested (absence/presence of sulfur and/or formate, yeast extract (YE) in different concentrations). Based on optical density and protein measurements, none of the strains showed significant growth on formate. Even in the presence of S⁰, which may act as terminal electron acceptor, growth on formate was not obvious. Nevertheless, formate was converted with concomitant H₂ production by *T. gammatolerans* and *T. barophilus* in the absence S⁰, but not by *T. kodakaraensis*, provided that YE was present (5g/l). Remarkably, in the presence of S⁰, all strains showed formate to H₂ conversion, with *T. gammatolerans* performing best (45 mM formate converted). Formate conversion and H₂ production in *T. barophilus* and *T. kodakaraensis* leveled off after 48 h, whereas *T. gammatolerans* continued for up to 72 h. In the presence of S⁰ and YE acetate was produced in all strains. No acetate was produced in the absence of YE, suggesting that part of the observed production of H₂ was also caused by YE. It is noteworthy that the lowest amount of acetate (4 mM) is found for *T. gammatolerans*, which produced the most H₂ (50 mM). These data suggest that, in the presence of S⁰, all strains ferment YE to acetate, but also formate is converted to H₂, with *T. gammatolerans* performing best. Taken together it can be concluded that all strains tested do not show significant growth

on formate. Nevertheless, all strains are able to convert formate to H₂ in the presence of S⁰. *T. gammatolerans* shows the highest level of formate to H₂ conversion, even in the absence of S⁰. Further study is required to explain the observed differences and to link these to the available genome information.

Author Disclosure Block:

K. Trchounian: None. **S. Spaans:** None. **A. Trchounian:** None. **A. Stams:** None. **S. Kengen:** None.

Session Number:

218

Session Title:

Keeping the Batteries Charged

Publishing Title:

Metagenomic and Metatranscriptomic Analysis of an Electromethanogenic Biofilm

Author Block:

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Abstract Body:

Electromethanogenesis is the process whereby microbes utilize electricity as an energy source and reduce carbon dioxide to methane in a bioelectrochemical system (BES). This process may have several benefits toward the sustainable production of biofuels; however, a fundamental understanding about the mechanisms underlying how microbial communities utilize electricity to fix CO₂ may have broader implications towards the synthesis of several useful products including high value chemical precursors, as well as revealing new insights about how solid-phase electron donors (like metal oxides) may play a role in carbon cycling in soils and sediments. Researchers have described the mechanisms of extracellular electron uptake from solid surfaces in model electrogens like *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* as well as providing evidence for direct electron uptake for some methanogenic strains in BESs. While studying model organisms has led to a deeper understanding of extracellular electron uptake mechanisms and CO₂ conversion, microbes rarely thrive as monocultures and prefer to live in diverse microbial communities. Here we utilized a novel stimulus-induced metatranscriptomic approach in combination with metagenomics to identify the dominant microbial taxa in an electromethanogenic biofilm and quantify the dynamic responses of key genes associated with electron uptake, hydrogen production/utilization and carbon dioxide fixation in duplicate BES reactors. Results from the metagenomic analyses show that the same strains of methanogens, sulfate reducers and fermenters were present in both reactors; however, they were present in different relative abundances, which may explain the performance differences. Further, stimuli applied to both reactors induced different functional responses from the respective communities, which also indicated that the relative abundance of species impacts how they functionally respond in BESs. Future work will address the specific interactions that correlate with these

performance differences. These data yield new perspectives on the functional and taxonomic dynamics of electromethanogenic microbial communities.

Author Disclosure Block:

O. Bretschger: None. **S. Babanova:** None. **K. Carpenter:** None. **S. Ishii:** None. **S. Suzuki:** None. **M. Flynn:** None. **J. Hogan:** None.

Session Number:

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Session Title:**May the Force Be With You: Microbial Mechanosensing****Publishing Title:****Cyclic-Di-Gmp as a Mechanically-activated Toggle Switch****Author Block:**

C. A. Rodesney, N. Dhamani, R. E. Du, V. D. Gordon; Univ. of Texas at Austin, Austin, TX

Abstract Body:

When planktonic cells of *Pseudomonas aeruginosa* attach to a surface, intracellular levels of the second messenger cyclic-di-GMP increase and initiate biofilm development. What specific cue(s) initiate cyclic-di-GMP signaling are not well understood. We vary mechanical shear stress by varying the flow rate of liquid medium over surface-attached bacteria and use a GFP reporter for the *cdrA* gene, which other researchers have validated as a reporter for intracellular cyclic-di-GMP levels. We continually image bacteria on a 30-minute cycle using a confocal microscope on which the stage is enclosed in an incubator. We find that the cyclic-di-GMP signaling response of populations of bacteria to adhesion to a surface is non-monotonic - the population's average signal level first increases by 40% and then decreases to a level within 20% of the initial, planktonic state. This suggests that cyclic-di-GMP may act as a toggle switch, wherein transient high signal levels set gene expression patterns to the biofilm state, which is then maintained at lower signal levels. Increasing the shear stress on bacterial cells increases the average peak intracellular levels of cyclic-di-GMP. A shear stress of $0.012 \text{ pN}/\mu\text{m}^2$ is associated with peak cyclic-di-GMP levels that are 40% higher than peak levels under no flow. We also find that expression of the polysaccharide Pel, which we have earlier shown increases the mechanical and geometric coupling of *P. aeruginosa* to the surface (Cooley *et al.*, 2013 Soft Matter), increases peak cyclic-di-GMP levels by 20%. These data show that cyclic-di-GMP production by *P. aeruginosa* is activated when a mechanical input is sensed. Unlike previous work showing that mechanosensing by PilY can activate cyclic-AMP signaling in *P. aeruginosa* (Persat *et al.*, 2015 PNAS), surface sensing leading to cyclic-di-GMP production does not require pili.

Author Disclosure Block:

C.A. Rodesney: None. **N. Dhamani:** None. **R.E. Du:** None. **V.D. Gordon:** None.

Session Number:

220

Session Title:

May the Force Be With You: Microbial Mechanosensing

Publishing Title:

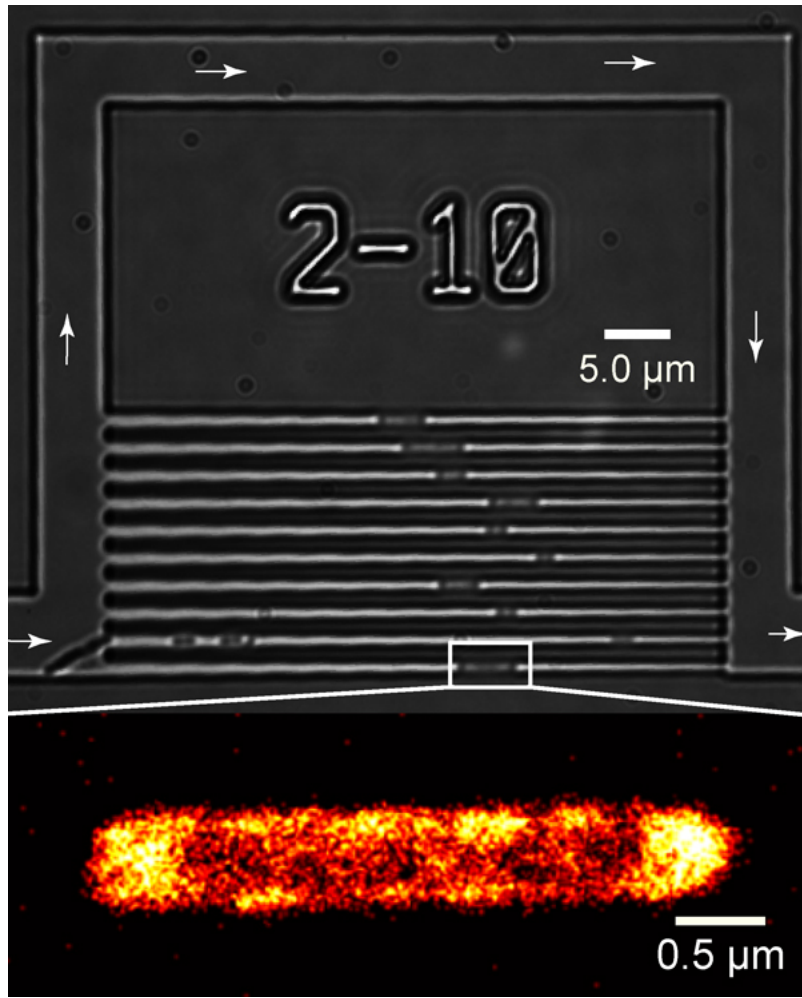
A Microfluidic Platform for Generating Non-Uniform Mechanical Stress in Cell Envelopes of Live Bacteria

Author Block:

M. F. Roberts, A. Srivastava, X. Sun, L. Kreminski, L. Ling, L. Wang, P. Chen, C-Y. Hui, C. J. Hernandez; Cornell Univ., Ithaca, NY

Abstract Body:

Mechanical stress is a powerful stimulus for cell growth in eukaryotic cells. The effects of mechanical stress on prokaryotic growth remain poorly understood because existing methods of generating mechanical stress in the bacteria also alter cell physiology (osmotic shock) or immobilize cells. Here we describe and validate a method of generating mechanical stress in live bacteria. The technique involves flowing bacteria in liquid suspension into a microfluidic device where they become trapped in tapered channels (smallest dimension 250 nm). We refer to this loading mode as “extrusion loading.” The cell walls of trapped bacteria experience mechanical stresses related to external fluid pressure and can be observed under mechanical loading using traditional and super-resolution microscopy. As a demonstration of the technique we examined *E. coli* transformed to express a photoswitchable fluorescent probe on the Tsr membrane protein and observed cell morphology with PALM (Fig. 1). To characterize the mechanical stress state within trapped bacteria we present an analytical mechanical model derived from fundamental elasticity using a linear elastic constitutive equation. The analysis indicates that bacteria submitted to extrusion loading experience predominately tensile stresses and that peak cell wall mechanical stress occurs at the upstream points of contact with the channel walls. The extrusion loading approach allows for subcellular characterization of mechanical stress in any rod-like bacterial



species.

Fig. 1. (Top) The microfluidic device is shown with arrows indicating direction of flow. Bacteria either flow into tapered channels or upward through the bypass channel. (Bottom) A trapped bacteria is observed using PALM.

Author Disclosure Block:

M.F. Roberts: None. **A. Srivastava:** None. **X. Sun:** None. **L. Kreminski:** None. **L. Ling:** None. **L. Wang:** None. **P. Chen:** None. **C. Hui:** None. **C.J. Hernandez:** None.

Session Number:

220

Session Title:

May the Force Be With You: Microbial Mechanosensing

Publishing Title:

The Psp Envelope-Stress-Response System of Gram-Positive Bacteria

Author Block:

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Abstract Body:

The phage shock protein (Psp) stress-response system, which has been extensively studied in Gram-negative bacteria, protects bacteria from envelope stress through a cascade of protein interactions that stabilize the cell membrane. In enterobacteria, the multi-gene Psp system participates in membrane polarization maintenance, divalent metal ion transport, and bacterial virulence. The key component of this system is PspA, a protein that stabilizes the inner leaflet of the plasma membrane when envelope structure/composition is altered. Homologs of PspA are found in diverse bacterial phyla, archaea, cyanobacteria, and chloroplasts. In contrast, PspF (the transcriptional regulator of the Psp family) and other accessory Psp proteins are conserved in Proteobacteria but sparsely found in other phyla. Recent work with Gram-positive bacteria and Actinobacteria suggests that even though the PspA function appears to be maintained across phyla, the structure of the accessory Psp proteins, the regulatory mechanisms, and the system's stress response dynamics vary among bacterial species. These variations likely reflect different envelope structures and bacterial lifestyles. We will present a critical analysis of the phylogenetic distribution and sequence/structural aspects of the Psp system in Gram-positive bacteria, and discuss the significance of the evolution of the Psp system in Gram-positive and Gram-negative bacteria.

Author Disclosure Block:

J. Ravi: None. **P. Datta:** None. **A. Barton:** None. **R. Manganelli:** None. **M.L. Gennaro:** None.

Session Number:

220

Session Title:

May the Force Be With You: Microbial Mechanosensing

Publishing Title:

The Surface Movements of Bacteria Follow Aligned Polymers

Author Block:

D. J. Lemon¹, X. Yang², P. Srivastava³, Y-Y. Luk¹, M. C. Marchetti¹, A. Garza¹;
¹Syracuse Univ., Syracuse, NY, ²Northwestern Univ., Evanston, IL, ³The Francis Crick Inst., London, United Kingdom

Abstract Body:

Behavioral changes to chemical signals have been well characterized in many systems, but correspondingly little is understood about how cell behavior is affected by physical changes or biophysical signals. In recent years, there has been increasing interest in how biofilm formation is affected by changes in the physical properties of synthetic substrates such as those found in medical implants, which can become contaminated with pathogenic biofilms. Because biofilm-forming bacteria typically produce a polysaccharide matrix that coats the surface on which they live, our work has focused on the physical signals derived from changes in natural polysaccharide substrates. In particular, we have been examining how bacteria respond to deformations of agar, which is primarily composed of linear polymer agarose, using *Myxococcus xanthus* as our model. *M. xanthus* forms circular colonies on unstressed agar, but forms elliptical colonies on compressed agar such that their major axes are perpendicular to the axis of compression. From the aspect ratio of these colonies we infer the strength of the compression-induced response and find that it correlates with the strain within the substrate, which is predicted by numerical simulation. We found that agar is not birefringent before compression, but becomes birefringent after compression and in roughly the same regions where strain is predicted, indicating an increase in the molecular order of these regions. This compression-induced change in birefringence is not unique to agar, as other polysaccharide hydrogels show a similar change. Furthermore, multiple rod-shaped, biofilm-forming, surface motile species of bacteria show this asymmetric spreading on compressed agar. Based on these findings, we propose that the randomly oriented polysaccharide fibers (or bundles) in unstressed agar become aligned by compression, and that bacterial movements on these substrates follow these aligned polysaccharide fibers.

Author Disclosure Block:

D.J. Lemon: None. **X. Yang:** None. **P. Srivastava:** None. **Y. Luk:** None. **M.C. Marchetti:** None. **A. Garza:** None.

Session Number:

221

Session Title:

Microbes that Link C, N, and P Cycles

Publishing Title:

Using Occam's Razor: Biochemical Control of Relative Entropy in Methane-producing Archaea

Author Block:

N. R. BUAN; Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract Body:

Methane-producing archaea (methanogens) are key organisms in the global carbon cycle responsible for producing 2 gigatons of methane gas annually. Methanogens are remarkable for surviving near the “thermodynamic limit of life”. The Gibbs’ free energy yield of a methanogenic lifestyle is low, typically between -20 to -80 kJ/mol. Hence methanogens only produce 0.5-2 mol ATP per mol substrate compared to 36 mol ATP yield for *E. coli*. We hypothesize methanogens optimize coupling between methane production and growth by minimizing the relative entropy at each step in the electron transport system and central metabolism. This hypothesis is supported by several lines of biochemical and physiological evidence including detection of protein:protein interactions between enzymes involved in methanogenesis and acetyl-CoA synthesis, membrane electron carrier synthesis, and metabolic engineering experiments. Here we report the quantification of methanophenazine in cells during exponential and stationary phase growth and the effect of reducing or increasing methanophenazine levels on growth and methanogenesis rates. Methanophenazine is a membrane electron carrier in Methanosarcinales family methanogens analogous to quinones in bacteria and eukarya. We observed an increase in methanophenazine membrane concentrations as cells enter stationary phase in *Methanosarcina acetivorans* and *Methanosarcina barkeri*, similarly to how quinone levels increase in stationary-phase *E. coli* cells. In contrast, *Methanosarcina mazei* cells exhibit a decrease in methanophenazine concentration at stationary phase, indicating isopotential electron hopping is improbable in *M. mazei* membranes. These results suggest methanophenazine synthesis and energetic coupling are linked, regulated, and may be manipulated. To test this idea, we genetically varied membrane methanophenazine concentrations by up to +/-50% versus parent *M. acetivorans* cells and observed the expected corresponding changes in methanogenesis and growth rates. These results support our hypothesis that manipulating relative entropy at key steps in

methanogen metabolism can be used to control flux through biomass synthesis and/or methane production pathways.

Author Disclosure Block:

N.R. Buan: None.

Session Number:

221

Session Title:

Microbes that Link C, N, and P Cycles

Publishing Title:

Short and Fat, Skinny and Tall: Rates and Mechanisms of Carbon Fixation among Various *Ridgeia piscesae* Vent Tubeworm Morphotypes

Author Block:

P. R. Girguis; Harvard Univ., Cambridge, MA

Abstract Body:

The siboglonid tubeworms *Riftia pachyptila* and *Ridgeia piscesae* are among the best-studied hydrothermal vent symbioses. These worms have no mouth, gut or anus and are among the fastest growing organisms on Earth. Many studies have looked at aspects of primary productivity, including assessments of carbon fixation pathways, stable carbon isotope ratios, and net carbon fixation rates. In recent years, metagenomic, metaproteomic and enzymatic studies have suggested that *Riftia* and *Ridgeia* possess two different carbon fixation pathways: the Calvin-Benson-Bassham (CBB) and the reductive Tricarboxylic Acid (rTCA) cycles. Very few organisms are known to possess and use two different carbon fixation pathways. To date, it remains unclear if and when *Riftia* or *Ridgeia* are using both pathways to fix carbon, and accordingly we have conducted a series of experiments on *Ridgeia piscesae* to better understand A) if both modes of carbon fixation are active in these worms; B) if they are more active at different environmental conditions; and C) if differences in activity could serve to explain the range of stable carbon isotope ratios seen among *Ridgeia* morphotypes. We collected and incubated ~40 worms in our high-pressure aquaria, and conducted a series of mass spectrometric, metagenomic and metaproteomic analyses. The results strongly suggest that there is differential expression of the rTCA and CBB pathways among the various morphotypes and, surprisingly, that the differences in protein expression are consistent with changes in the geochemical regime at which the different morphotypes are found. Moreover, the different modes of carbon fixation influenced the rates of nitrate and ammonia assimilation, with the onset of rTCA leading to a massive increase in nitrate uptake. All together, these data suggest that *Ridgeia* tubeworm symbionts may have co-opted one of these carbon fixation pathways in response to the differences in physiological demand (and thus selective pressures) across the range of geochemical conditions in which the worms thrive.

Author Disclosure Block:

P.R. Girguis: None.

Session Number:

221

Session Title:

Microbes that Link C, N, and P Cycles

Publishing Title:

Phylogeny and Structure of the Archaeal Assemblage in Arctic Lakes

Author Block:

P. B. Matheus Carnevali¹, C. Herbold², K. P. Hand³, A. E. Murray⁴; ¹Univ. of California, Berkeley, Berkeley, CA, ²Univ. of Vienna, Vienna, Austria, ³Jet Propulsion Lab., California Inst. of Technology, Pasadena, CA, ⁴Desert Res. Inst., Reno, NV

Abstract Body:

Two contrasting shallow lakes in the Coastal Plain of Alaska were studied: Siqlukaq (Siq) and Sukok (Suk), including a site ~ 10 ft. from a thermogenic methane gas seep (SukS) and ~ 1 km away from the seep (SukB). A higher content of total organic carbon (TOC), pore water dissolved iron concentration, and potential for biological methane production was found in Siq. Overall, nitrate and sulfate concentrations were higher in Suk, while methanogenesis was observed only in small proportion in SukB. In this study we were interested in the effects that these differences in geochemistry may have on the structure and composition of the archaeal assemblage, and how the Archaea contribute to them. Partial length (< 800 bp) SSU rRNA gene sequences in addition to Illumina Tag sequences of the SSU rRNA gene, total RNA, and amplified SSU rRNA (aRNA) were obtained from sediment cores collected late October-early November 2011. A comprehensive phylogenetic tree including the < 800 bp sequences and neighbors of the iTag sequences in the Silva database was constructed using the RAxML algorithm. The high diversity of Archaea in these lakes was reflected in their phylogenetic affiliations to almost all known phyla of Archaea known to date, including Bathyarchaeota, Thaumarchaeota, Crenarchaeota, Euryarchaeota, Lokiarchaeota, Woesearchaeota and Pacearchaeota. Moreover, a two-dimensional hierarchical clustering (average linkage) analysis based on non-parametric Spearman's rank correlations showed a distinct distribution of these phyla among sites, reflecting the differences in geochemistry. Thermokarst lakes on the North Slope of Alaska have thaw bulbs that may be especially susceptible to a warming climate, and the microbial community will adapt accordingly. An increased availability of organic matter from increased thawing leads to increased microbial activity. Understanding the composition, function and structure of this dynamic

microbial community is thus critical to improving our ability to predict changes in the greenhouse gas inventory.

Author Disclosure Block:

P.B. Matheus Carnevali: None. **C. Herbold:** None. **K.P. Hand:** None. **A.E. Murray:** None.

Session Number:

221

Session Title:**Microbes that Link C, N, and P Cycles****Publishing Title:****Growth of the Marine Bacterium *Phaeobacter inhibens* Dsm 17395 with More Than 200 Different N:P Ratios****Author Block:****K. Trautwein**, C. Feenders, R. Hulsch, A. Strijkstra, B. Blasius, R. Rabus; Carl von Ossietzky Univ. Oldenburg, Oldenburg, Germany**Abstract Body:**

Marine bacteria are major contributors to the global carbon, nitrogen and phosphorus cycle. The concentration and bioavailability of these elements varies largely in the ocean (spatially, seasonally) and controls the activity and growth of marine microorganisms. *Phaeobacter inhibens* DSM 17395 is an aerobic, heterotrophic alphaproteobacterium, belonging to the globally distributed and abundant marine *Roseobacter* clade. Within the last years, this organism was studied intensively with respect to biochemical pathways involved in the mineralization of prominent marine carbon sources (e.g. amino acids, sugars), and its physiological and molecular response to high-nutrient conditions (e.g. simulating the nutritional complexity of collapsing algal blooms). The aim of this study was to characterize the physiological response of *P. inhibens* DSM 17395 to a wide variety of different ratios of nitrogen (supplied as NH_4Cl) and phosphorus (supplied as KH_2PO_4). The concentration of both nutrients ranged from 50 μM to 250 mM for ammonium and 1 μM to 3 mM for phosphate, while the concentration of the carbon source glucose was constant (11 mM). In total, 416 different ammonium and phosphate concentrations were tested (at least in triplicates), corresponding to 233 different N:P ratios. Growth was monitored by measuring the optical density (OD) over the time course of growth and by determining the biomass concentration at the transition of cultures into the stationary growth phase. Optimal growth was observed at N:P ratios of 30-200 within a clearly defined concentration range of 1-20 mM ammonium and 30-100 μM phosphate. Within this concentration range, *P. inhibens* DSM 17395 achieved the highest maximal ODs (OD_{max}) and biomass concentrations, as well as highest linear growth rates (exponential growth was short and confined to an early stage during growth). With phosphate concentrations between 100-1000 μM (N:P ratios <30 at 1-20 mM ammonium), growth performance decreased considerably. Nitrogen-controlled growth

limitation occurred at concentrations <1 mM ammonium, while phosphorus-controlled growth limitation was observed at phosphate concentrations <10 μ M. At concentrations of >120 mM ammonium or 3 mM phosphate, growth of *P. inhibens* DSM 17395 was completely inhibited.

Author Disclosure Block:

K. Trautwein: None. **C. Feenders:** None. **R. Hulsch:** None. **A. Strijkstra:** None. **B. Blasius:** None. **R. Rabus:** None.

Session Number:

222

Session Title:

Microbial Communication via Surface Structures

Publishing Title:

Extracellular Rna Serves as a Building Material in Bacterial Habitats

Author Block:

A. Chiba, K. Yonemoto, S. Sugimoto, Y. Mizunoe; The Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract Body:

Background: Microbial cells within a biofilm are embedded in an extracellular matrix (ECM) composed of proteins, polysaccharides, and/or DNA, and develop high resistance against the host immune system and antibiotics. Thus, biofilm-forming bacteria often cause various human chronic infectious diseases. Knowledge of how these habitats develop is important for combating these problems; however, biofilm formation mechanisms are poorly understood. In this study, we explored the presence of extracellular RNA (eRNA) in bacterial biofilms and analyzed its roles in biofilm development. **Methods:** Several clinically isolated strains of *Staphylococcus aureus* and *S. epidermidis* and *Pseudomonas aeruginosa* PAO-1. ECM components were extracted from the biofilms by a short incubation with 1.5 M NaCl and were subsequently analyzed by conventional electrophoresis such as agarose gel electrophoresis and polyacrylamide gel electrophoresis as recently reported^{1,2)}. We examined localization of eRNA in the biofilms by fluorescence microscopy combined with the RNA detection method using Thioflavin T³⁾. To clarify the physiological role of eRNA in the biofilm ECM, we investigated the effects of RNase A and other ECM-degrading enzymes on biofilm formation and dispersal. **Results:** Nucleic acids were detected in the ECM of *S. aureus* and *S. epidermidis*, major bacteria causing biofilm-associated infections. The nucleic acids were degraded by RNase A but neither by dispersin B, proteinase K, nor DNase I, indicating the presence of eRNA in the ECM. The molecular size of the eRNA was estimated 20 to 200 nucleotides by denaturing polyacrylamide gel electrophoresis. RNase A inhibited biofilm formation and dispersed pre-formed biofilms, representing the importance of eRNA in the structural integrity of the biofilms. The results of time-course experiments suggested the requirement for eRNA at various stages of biofilm formation. We further showed that extracellular polysaccharides co-localize with and stabilize eRNA in the biofilm. eRNA was also identified in a biofilm formed by *Pseudomonas*

aeruginosa, indicating that this phenomenon is not limited to staphylococcal biofilms.

Conclusions: Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology and the treatment of biofilm-associated problems.

Author Disclosure Block:

A. Chiba: None. **K. Yonemoto:** None. **S. Sugimoto:** None. **Y. Mizunoe:** None.

Session Number:

222

Session Title:**Microbial Communication via Surface Structures****Publishing Title:****Small Molecule Induced Outer Membrane Vesicle Biogenesis in Gram-Negative Bacteria****Author Block:****J. W. Schertzer**; Binghamton Univ., Binghamton, NY**Abstract Body:**

Outer Membrane Vesicle (OMV) trafficking has been shown to be important across species in such diverse functions as threat avoidance, toxin delivery, biofilm development and cell-cell signaling. With such wide-ranging potential impacts, it is imperative that we understand the molecular mechanisms by which OMVs are formed and the factors that facilitate/induce this process. A fortuitous overlap between cell-cell communication and OMV biogenesis in *Pseudomonas aeruginosa* has allowed us to use this organism as a model for small molecule induced OMV formation, which we believe may represent a much more general paradigm. The Pseudomonas Quinolone Signal was shown to induce OMV formation in a manner related to its biophysical characteristics rather than its quorum sensing potential. We followed this up using surrogate membranes to show that PQS was capable of inducing membrane curvature in a heterologous system. Based on this, we proposed the Bilayer-Couple model of OMV biogenesis whereby hydrophobic small molecules would insert into the outer membrane (OM) and, by virtue of their preference for one leaflet over the other, cause asymmetric leaflet expansion leading to curvature and OMV biogenesis. Here we describe several experiments designed to test this model. We examined *P. aeruginosa* grown under conditions of high and low OMV production. Though overall PQS production was comparable, we showed that the level of OMV biogenesis was directly correlated to the amount of PQS that was able to reach the OM. This further supports the model prediction that PQS interaction with the OM, rather than association with its QS receptor, drives OMV formation. Next, we took steps to address whether small molecule induced OMV biogenesis may plausibly exist in other Gram-negative species. Using a panel of recipient organisms, we showed that PQS induces OMV biogenesis in all species tested. The organisms used are not known to produce PQS or to have a PQS receptor. Combined with our earlier data, these results confirm that PQS is capable of inducing curvature in diverse membranes whose

compositions are either radically different or largely similar to the *P. aeruginosa* OM. What's more, characteristics of OMVs produced in response to the same small molecule but in various species showed interesting differences. This suggests that other aspects of OMV biogenesis (besides curvature induction) may be controlled in a species-specific manner.

Author Disclosure Block:

J.W. Schertzer: None.

Session Number:

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Session Title:

Microbial Communication via Surface Structures

Publishing Title:

High Resolution Imaging of Aqueous Biofilms by Atmospheric Scanning Electron Microscopy

Author Block:

S. Sugimoto¹, K-i. Okuda¹, R. Miyakawa¹, M. Sato², A. Chiba¹, C. Sato², Y. Mizunoe¹;
¹The Jikei Univ. Sch. of Med., Tokyo, Japan, ²Natl. Inst. of Advanced Industrial Sci. and Technology (AIST), Tsukuba, Japan

Abstract Body:

Background: Biofilms are complex communities of microbes that attach to biotic or abiotic surfaces causing chronic infectious diseases. Within a biofilm, microbes are embedded in a self-produced soft extracellular matrix (ECM), which protects them from the host immune system and antibiotics. The nanoscale visualization of native biofilms in liquid is challenging. In this study, we developed atmospheric scanning electron microscopy (ASEM)¹⁾ to visualize Gram-positive and -negative bacterial biofilms immersed in aqueous solution. **Methods:** *Staphylococcus aureus* biofilms were cultured in BHI medium supplemented with 1% glucose in the specialized ASEM dishes. *E. coli* colony biofilms were also placed on the ASEM dish after cultivation on YESCA plate. The biofilms were fixed with glutaraldehyde and paraformaldehyde and were subsequently labelled with heavy metals and charged Nanogold. The labelled biofilms on electron-transparent film at the center of the ASEM dish were directly imaged from below using the inverted SEM. A universal immuno-labelling system was also developed to investigate localization of proteins and extracellular DNA in these biofilms. **Results:** We visualized intercellular nanostructures and the exocytosis of membrane vesicles, and linked the latter to the trafficking of cargos, including cytoplasmic proteins and the toxins hemolysin and coagulase in the *S. aureus* biofilms. A thick dendritic nanotube network was observed between *S. aureus* cells, suggesting multicellular communication in the biofilms. In the ECM, fine DNA and protein networks were visualized and the precise distribution of protein complexes was determined (*e.g.*, extracellular adherence protein, termed Eap, and excreted cytoplasmic molecular chaperones in *S. aureus*²⁾ and spiral flagella in *E. coli*). **Conclusions:** Our observations provide structural insights into

bacteria-substratum interactions, biofilm development and the internal microbe community.

Author Disclosure Block:

S. Sugimoto: None. **K. Okuda:** None. **R. Miyakawa:** None. **M. Sato:** None. **A. Chiba:** None. **C. Sato:** None. **Y. Mizunoe:** None.

Session Number:

222

Session Title:**Microbial Communication via Surface Structures****Publishing Title:****Characterization of Iron-Doped Apatite Nanoparticle Enhancement of Phage Infections Using Phd7 Phage Display and Bioinformatic Analysis****Author Block:**

J. M. Andriolo¹, M. L. Pedulla², M. K. Hailer², J. P. Murphy², J. L. Skinner²; ¹Univ. of Montana, Missoula, MT, ²Montana Tech, Butte, MT

Abstract Body:

Previous work established that adding iron-doped apatite nanoparticles (IDANPs) to gram positive bacteria prior to phage introduction, increases resultant death zone counts (plaques) up to 2.28 times that of controls.¹ Critical to the use of IDANP-mediated enhancement of antibacterial agents or to exploit the mechanisms involved, underlying interactions responsible must be characterized. *In vitro* experimentation demonstrates IDANP composition, size, and shape, as well as exposure time to bacteria are all involved in the observed effect. Additionally, SEM shows extracellular structures may interact with IDANPs dependent on IDANP composition. It is assumed the interaction observed between bacteria and IDANPs that cause the greatest increases in plaques is most ideal for consequent cell death. To reveal extracellular structures involved, phage display was utilized. IDANP synthesis, plating methods, and SEM sample preparation have been published.¹ Standard New England Biolabs (NEB) Phd7 phage display surface panning methods² were followed with the exception of an added fourth panning step. During the fourth pan, isolation step was modified to include only those filamentous phage with fused peptides that interact with IDANPs effective at increasing plaques, but not those which interact with IDANPs that do not increase plaques. Filamentous M13 phage collected in final pan were amplified in *Escherichia coli*, prepared according to NEB protocols, sequenced at Functional Biosciences, and annotated using various online bioinformatic tools. Phage display results indicate IDANPs bind to a peptide found in choline binding proteins (CBPs) which are found in a number of respiratory tract pathogens. CBPs are bound to phosphorylcholine of the cell wall noncovalently through a choline binding domain.³⁻⁶ CBPs, along with many other surface anchored proteins, have been implicated in adhesion, virulence, and pathogenesis-related functions of gram positive bacteria.^{3,7} If CBP anchoring is found to be involved in IDANP-mediated

increases in phage infections, it can be hypothesized that obstruction of the cell surface via IDANPs may hinder bacterial viral-defense mechanisms. Future work will involve *in vitro* experimentation with Streptococcal CBP-null strains and further extend to Staphylococcal-SrtA-null strains to determine if CBPs or other surface anchoring structures found in gram positive bacteria are indeed involved

Author Disclosure Block:

J.M. Andriolo: None. **M.L. Pedulla:** None. **M.K. Hailer:** None. **J.P. Murphy:** None. **J.L. Skinner:** None.

Session Number:

223

Session Title:

Microbiology: Let's Spend Some Time Together

Publishing Title:

New Insights Into Rhs Proteins: Highly Potent Toxins Secreted by the Type VI Secretion System

Author Block:

J. Alcoforado Diniz, F. R. Cianfanelli, S. J. Coulthurst; Univ. of Dundee, Dundee, United Kingdom

Abstract Body:

Bacteria are often found in polymicrobial environments and, to guarantee a better fitness advantage, have adopted diverse strategies to fight for their niche. An important mechanism utilised by Gram-negative bacteria is protein secretion, with protein secretion systems efficiently translocating particular proteins to the external medium or directly into a competitor target cell. Recently, much progress has been made towards understanding the role and mechanism of the final major secretion system reported in Gram-negative bacteria, the Type VI secretion system (T6SS). The T6SS is widespread and can deliver toxins into both eukaryotic host cells and rival bacteria cells. In the opportunistic human pathogen, *Serratia marcescens*, we have described a highly effective and offensive anti-bacterial T6SS. Several secreted toxins, or effectors, delivered by this T6SS have already been reported to represent efficient weapons against susceptible target cells, acting on diverse cellular targets and with different modes of action. In previous work, two of these, from a class of specialised effectors, namely polymorphic toxins containing Rhs repeat domains, have been shown to play a central role in intraspecies competition. These Rhs-family proteins possess highly variable C-terminal toxin domains associated with specific cognate immunity proteins. We have shown that one of these Rhs proteins possesses a nuclease toxin at its C-terminus and further reported a new class of conserved accessory protein, EagR (Effector associated gene, with Rhs). EagR proteins are essential for deployment of specific Rhs effectors by the T6SS. Further work has aimed to investigate the mode of action of the second Rhs protein encoded by *Serratia marcescens*, which has a C-terminal domain with no homology to toxins of known function, and to further elucidate the potent anti-bacterial activity of this family of proteins, using biochemical, genetic and microscopic approaches. In addition, we describe a direct interaction between Rhs proteins and their cognate EagR protein,

together with the importance of this accessory protein in the secretion of Rhs proteins by the T6SS. Taken together, our findings help to understand how these important toxins are delivered and act in the target cell.

Author Disclosure Block:

J. Alcoforado Diniz: None. **F.R. Cianfanelli:** None. **S.J. Coulthurst:** None.

Session Number:

223

Session Title:

Microbiology: Let's Spend Some Time Together

Publishing Title:

Individuality, Stability, and Variability of the Plaque Microbiome Analyzed with Single-Nucleotide Resolution

Author Block:

D. R. Utter¹, J. L. Mark Welch², G. G. Borisy³; ¹Harvard Univ., Cambridge, MA, ²Marine Biol. Lab., Woods Hole, MA, ³The Forsyth Inst., Cambridge, MA

Abstract Body:

Although the dental plaque microbiome is intimately associated with human health and oral disease, relatively little information from cultivation-independent, high-throughput analyses has been published on its temporal dynamics. Further, previous studies have characterized oral microbial dynamics paradoxically by the seemingly contradictory qualities of stability and variability. We used Minimum Entropy Decomposition, an information theory-based approach that produces *de novo* partitions called oligotypes with single-nucleotide resolution, to analyze a previously published time series (8 individuals, 8 time points over 3 months) with 16S rRNA sequence data (V4-V5 region) and investigate the dynamics of the plaque microbiome at various analytic and taxonomic levels. At the genus and Operational Taxonomic Unit (OTU) levels of resolution, the range of variation within each individual overlapped that of other individuals in the dataset. When analyzed at the oligotype level, however, the overlap largely disappeared, showing that single-nucleotide resolution enables differentiation of individuals from one another without ambiguity. The overwhelming majority of the plaque community in all samples was made up of bacteria from a moderate number of plaque-typical genera, indicating that the overall community framework is shared among individuals. Each of these genera fluctuated in abundance around a stable mean that varied between individuals, with some genera having higher inter-individual variability than others. Thus, at the genus level, differences between individuals lay not in the identity of the major genera but in differing proportions of these genera. However, at the oligotype level, we detected oligotype “fingerprints,” a highly individual-specific set of persistently abundant oligotypes fluctuating around a stable mean over time. For example, within the genus *Corynebacterium*, more than a dozen oligotypes were detectable in each individual, of which a different subset reached high abundance in any given person. We

conclude that the plaque microbiome is highly individualized at the oligotype level and characterized by stability of community membership, with variability in the relative abundance of community members between individuals and over time.

Author Disclosure Block:

D.R. Utter: None. **J.L. Mark Welch:** None. **G.G. Borisy:** None.

Session Number:

223

Session Title:

Microbiology: Let's Spend Some Time Together

Publishing Title:

Modeling Microbial Communities: Is Pairwise Modeling Adequate?

Author Block:

B. Momeni¹, W. Shou²; ¹Boston Coll., Chestnut Hill, MA, ²Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract Body:

Background: Microbial communities offer efficient solutions for degrading harmful waste and producing clean biofuels. Predicting the properties of a microbial community requires a model that captures the interactions among community species. The majority of previous modeling efforts have focused on *pairwise models*. These models assume that community can be represented as additive superposition of net fitness influences between pairs of species. The popularity of such models has several reasons: they compact interactions into a simple form with only a few parameters; the experimental procedure to estimate model parameters is straightforward, requiring only the measurement of population sizes; and there is a long history of such models, supported notably by the success of Lotka-Volterra models of prey-predation and competition. However, the validity of such models for microbial communities has not been investigated. Additionally, previous studies on plant communities suggest that pairwise models may not be suitable for multispecies communities.**Methods:** Here, starting from mechanistic reference models of microbial communities, we analyze the validity of pairwise modeling for describing chemical-mediated interactions. This analysis is based on comparing the predictions of the mechanistic model with those of its corresponding pairwise model.**Results:** We find that even when only two species interact via a single chemical mediator, pairwise models that best represent consumable or reusable mediators take different forms and a single canonical model operates only under restricted conditions. If one species affects another via multiple mediators, the canonical model becomes even less tenable. In multispecies communities, we identify under-appreciated, commonly-encountered mechanisms that violate the additivity assumption of pairwise models.**Conclusions:** Pairwise models faithfully represent microbial communities only under very restrictive conditions. Thus, one should verify the validity of pairwise modeling before exploiting its simplicity and avoid blanket generalization of conclusions

from such models. We advocate technologies enabling a mechanistic understanding of interactions to represent and explain community behaviors.

Author Disclosure Block:

B. Momeni: None. **W. Shou:** None.

Session Number:

223

Session Title:

Microbiology: Let's Spend Some Time Together

Publishing Title:

Nitric Oxide Targets the *Staphylococcus aureus* AgrA Protein to Inhibit Quorum Sensing and Suppress Virulence

Author Block:

R. Urbano¹, **J. Karlinsey**¹, **P-T. Doulias**², **H. Ischiropoulos**², **F. C. Fang**¹; ¹Univ. of Washington, Seattle, WA, ²Children's Hosp. of Philadelphia Res. Inst., Philadelphia, PA

Abstract Body:

Staphylococcus aureus colonizes the anterior nares of humans, where it encounters the antimicrobial mediator nitric oxide (NO·) produced by the nasal epithelium. Although nasal carriage is asymptomatic, *S. aureus* can transform into a virulent pathogen capable of causing invasive infections. The Agr quorum sensing system that coordinately regulates staphylococcal virulence genes is suppressed during nasal carriage but is activated during invasive infection. Using RT-qPCR, we found that NO· inhibits Agr-dependent virulence gene expression *in vitro* in a dose-dependent manner. NO·-treated bacterial cultures exhibit decreased transcript levels of Agr operon genes, the RNAIII regulator, and the Psm α and Psm β toxins. Biotin-switch assays and mercury resin-assisted capture followed by liquid chromatography-tandem mass spectrometry analysis revealed that the AgrA response regulator is S-nitrosylated at multiple cysteine residues (Cys-6, Cys-123, and Cys-199) located in the receiver and DNA-binding domains. Western blots demonstrated that S-nitrosylation lowers AgrA protein levels by inhibiting auto-activation. Increasing AgrA phosphorylation by treatment of bacterial cultures with spent medium was able to partially rescue inhibition. Reduced AgrA occupancy of the *agrP*II and *agrP*III promoters was demonstrated by chromatin immunoprecipitation in the presence of NO·, suggesting that NO· inhibits Agr function by interfering with promoter binding. NO· inhibition of quorum sensing may play a role in suppressing staphylococcal virulence and maintaining commensalism in the human nose.

Author Disclosure Block:

R. Urbano: None. **J. Karlinsey:** None. **P. Doulias:** None. **H. Ischiropoulos:** None. **F.C. Fang:** None.

Session Number:

225

Session Title:

Nutrient Acquisition by Intravacuolar Pathogens

Publishing Title:

Life in a Bubble: How Intra-Vacuolar Apicomplexan Parasites Gain Access to Host Nutrients

Author Block:

D. A. Gold¹, A. D. Kaplan², R. L. Rasmusson², J. P. J. Saeij¹; ¹UC Davis, DAVIS, CA, ²SUNY-Buffalo, Buffalo, NY

Abstract Body:

Many of the infectious diseases responsible for global mortality are caused by pathogens that spend a critical portion of their lifecycles residing within intra-vacuolar compartments. This vacuolar compartment presents a barrier to the flow of small molecules, such as nutrients, between these pathogens and their host cells. Nearly 20 years ago, it was demonstrated for several Apicomplexa, a phylum of protozoan parasites that includes *Toxoplasma gondii* and *Plasmodium* that their parasitophorous vacuoles (PVs) are selectively permeable to small molecules, akin to a molecular sieve, but the molecular basis for this property had remained elusive. *Toxoplasma* GRA17 and GRA23 are secreted dense granule proteins, related to *Plasmodium* EXP2, which localize to the PV membrane (PVM) and are conserved across PV-residing Apicomplexa. The PVs of GRA17-deficient parasites have aberrant morphology, reduced permeability to small molecules, and structural instability. GRA17-deficient parasites proliferate slowly and are avirulent in mice. These GRA17-deficient phenotypes are rescued by complementation with *Plasmodium* EXP2. GRA17 functions synergistically with GRA23 and exogenous expression of GRA17, GRA23, or *Plasmodium* EXP2 alters the surface membrane conductance properties of *Xenopus* oocytes in a manner consistent with a large non-selective pore. We have uncovered the first molecules underlying this selective permeability by identifying secreted proteins, unique to these parasites, which have pore-forming properties and mediate the flow of small molecules between the parasite compartment and the host cell.

Author Disclosure Block:

D.A. Gold: None. **A.D. Kaplan:** None. **R.L. Rasmusson:** None. **J.P.J. Saeij:** None.

Session Number:

225

Session Title:

Nutrient Acquisition by Intravacuolar Pathogens

Publishing Title:

A Plant-like Ingestion Pathway Delivers Host Proteins to the Intracellular Parasite *Toxoplasma gondii*

Author Block:

O. L. McGovern¹, Z. Dou², M. Meissner³, V. B. Carruthers¹; ¹Univ. of Michigan, Ann Arbor, MI, ²Clemson Univ., Clemson, SC, ³Univ. of Glasgow, Glasgow, United Kingdom

Abstract Body:

Background: The obligate intracellular pathogen *Toxoplasma gondii* resides in a non-fusogenic parasitophorous vacuole (PV) that sequesters this parasite from the host cell cytosol during replication. Within the parasite, canonical endocytic players cathepsin L, dynamin, clathrin, and Rab5 promote maturation and trafficking of regulated secretory proteins, supporting the idea that the *T. gondii* endosomal system was adapted for secretion. *T. gondii* also ingests host cytosolic proteins for digestion in its plant-like lysosome (vacuolar compartment, VAC), indicating it also uses its endosomal system for endocytosis. Disruption of ingestion leads to enhanced susceptibility to IFN γ -mediated killing, attenuated virulence, defective chronic infection; however, how ingested material is trafficked across the PV and to the VAC is unclear. It is hypothesized that host protein containing vesicles generated from the PV are trafficked to the VAC via clathrin-mediated endocytosis. **Methods:** Synchronized cultures of *T. gondii* were utilized to define the trafficking of ingested proteins from the host cell cytosol to the VAC by immunofluorescence microscopy with endosomal markers and dominant negative mutants of putative trafficking facilitators. **Results:** Our earlier studies suggested that vesicular trafficking of ingested proteins across the PV occurred at a tubulovesicular structure of the PV called the intravacuolar network (IVN). However, we find that host cytosolic proteins are ingested within 7 minutes post infection, when a mature IVN is absent. Ingested protein initially occupies an unidentified non-digestive compartment and subsequently localizes to the trans-Golgi network, late endosome and finally the VAC by 30 minutes post infection. **Conclusions:** The endosomal system of *T. gondii* is plant-like in which the trans-Golgi network acts as an early endosome. Similar to other systems, trafficking to the VAC takes about 30 minutes, and ingested proteins also encounter a late endosomal compartment *en route* to the VAC. Current studies aim to determine localization with additional endocytic markers and further interrogate the roles of Rab

GTPases, dynamin and clathrin in trafficking to the VAC. These studies are expected to advance our understanding of endocytosis by *T. gondii* and may reveal new targets for managing toxoplasmosis.

Author Disclosure Block:

O.L. McGovern: None. **Z. Dou:** B. Collaborator; Self; Zhicheng Dou. **M. Meissner:** B. Collaborator; Self; Markus Meissner. **V.B. Carruthers:** None.

Session Number:

225

Session Title:

Nutrient Acquisition by Intravacuolar Pathogens

Publishing Title:

A Functional Respiratory Chain Protects *Listeria monocytogenes* from Bacteriolysis in the Cytosol of Macrophages

Author Block:

G. Y. Chen, M. A. D'Antonio, J-D. Sauer; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:

Listeria monocytogenes (*Lm*), the causative agent of listeriosis, is a Gram-positive bacterium that replicates in the cytosol of a variety of host cells. Non-cytosol adapted bacteria that enter the host cytosol cannot replicate, suggesting that *Lm*, and other cytosol adapted pathogens, possess unique adaptations that facilitate colonization of this replication niche. Although the mechanisms by which *Lm* invades host cells are well known, how *Lm* survives in the cytosol is not well characterized. Using a novel luciferase-based assay, we screened a *Lm* transposon library to identify mutants with defects in cytosolic survival in murine macrophages. Of approximately 6,500 transposon mutants examined, we identified nine genes involved in central metabolism, cell wall homeostasis, or of unknown function that were required for cytosolic survival. Three mutations leading to menaquinone (MQ) auxotrophy were identified in the genetic screen, leading us to examine the role of MQ in *Lm* cytosolic survival. MQ auxotrophs lysed frequently in the cytosol of macrophages and were significantly attenuated for virulence both *ex vivo* and *in vivo*. Interestingly MQ auxotrophs did not lyse during *in vitro* growth or during infection of non-immune cells, signifying that MQ is specifically required for intracellular survival in macrophages and perhaps illustrating a unique cell autonomous defense pathway specific to immune cells. We subsequently determined that mutations that disrupt other components of the electron transport chain led to intracellular bacteriolysis of *Lm*, suggesting that a functional respiratory chain was critical for survival of *Lm* in the cytosol of macrophages. These novel findings demonstrate that *Lm* requires its electron transport chain not just for efficient replication but also for protection against cytosolic stresses/defenses in macrophages. To deepen our understanding of *Lm* cytosolic survival mechanisms, we recently isolated several suppressor mutants that partially restore *ex vivo* virulence and intracellular survival of a *Lm* MQ auxotroph. Importantly these suppressor mutants do not rescue cellular respiration. In summary this research

expands our knowledge of intracellular pathogenesis by unveiling a new link between bacterial metabolism and cytosolic survival of *Lm*.

Author Disclosure Block:

G.Y. Chen: None. **M.A. D'Antonio:** None. **J. Sauer:** None.

Session Number:

225

Session Title:**Nutrient Acquisition by Intravacuolar Pathogens****Publishing Title:****Antimycobacterial Compounds Targeting Cellular Menaquinones via Isoprenoid Biosynthesis****Author Block:**

A. Sajid, H. Boshoff, S. Azeeza, C. E. Barry, III; NIH, Bethesda, MD

Abstract Body:

Tuberculosis, caused by the *Mycobacterium tuberculosis* is a highly contagious disease and is the leading cause of death worldwide in recent years. The major impediments in TB treatment are long duration of therapy and increasing prevalence of multidrug resistant forms. Thus, new drugs are required to combat this disease, which ideally target an essential component of mycobacterial cell machinery to achieve high potency and which are compatible with other drug combinations. In parallel, the compounds must be able to reach sufficient concentrations in host tissues harboring the pathogen without causing toxicity. In this study, we screened a library of compounds against *M. tuberculosis* with the threshold criteria of low to sub-micromolar MIC and a selectivity index of at least 10 as determined by cytotoxicity determination. Based on these criteria, we selected a phenyl-pyrazolo-hydroxypyrimidine core from our list of validated hits. Analogs of this scaffold had whole-cell MIC in low-micromolar range against *M. tuberculosis* H37Rv, were chemically stable in media and had excellent kinetic solubility. The compounds were highly bactericidal to *M. tuberculosis* with 5-log fold killing observed within 7 days. For *in vivo* efficacy, PK studies were done in mice, which showed that compounds had high C_{max} and were able to achieve 10-times MIC levels. Subsequently, mice efficacy was determined with selected compounds. To explore the mechanism of action, macromolecular incorporation assays were performed that indicated a defect in acetate uptake, suggesting inhibition of fatty acid or isoprenoid biosynthesis. Our results indicate that the selected phenyl-pyrazolo-hydroxypyrimidines affect isoprenoid synthesis which is essential for cell survival leading to alteration of cellular menaquinones. Subsequent LC-MS analysis of menaquinones indicated that the compounds induce a defect in isoprenoid chain length of menaquinones, leading to formation truncated species. Thus, this study introduces the novel antimycobacterial

compounds with high efficacy against *M. tuberculosis* and target an essential component of cell.

Author Disclosure Block:

A. Sajid: None. **H. Boshoff:** None. **S. Azeeza:** None. **C.E. Barry:** None.

Session Number:

226

Session Title:

Regulating with RNA

Publishing Title:

Engineering Preq1 Riboswitches as Tools for Inducible Gene Regulation

Author Block:

E. R. Van Vlack¹, S. Topp², D. Iwata-Reuyl³, **J. C. Seeliger¹**; ¹Stony Brook Univ., Stony Brook, NY, ²Univ. of California Berkeley, Berkeley, CA, ³Portland State Univ., Portland, OR

Abstract Body:

We have explored the optimization of preQ1 riboswitches as tools for modulating gene expression. Because mycobacteria do not have genes for queuosine biosynthesis, they are not expected to make the precursor metabolite preQ1 and are thus a suitable host in which to investigate riboswitch application and mechanism. Naturally occurring preQ1 riboswitches, representing a cross-section of classes and types, repressed GFP reporter gene expression in *Mycobacterium smegmatis* in the presence of micromolar preQ1, which was non-toxic up to millimolar concentrations. Repression was time- and dose-dependent and fully reversed following removal of exogenous preQ1. To improve performance for gene knockdown applications, a series of variants derived from a translationally regulated riboswitch were rationally designed to favor the OFF vs. ON conformational state and thereby improve repression. The response ratios of the resulting riboswitches were up to 8-fold and in a range that will be useful for certain knockdown applications. In terms of mechanism, the mutant series responded to preQ1 generally as predicted, confirming that thermodynamics dominates the behavior of these riboswitches in the cell. However, the full dose-response data indicated that additional states make significant contributions, and we developed a multi-state model to describe the observed behavior. These data provide possible *in vivo* corroboration for conformational intermediates previously identified in *in vitro* studies. Overall, this work demonstrates that modified preQ1 riboswitches can be applied to inducible gene regulation in mycobacteria. The resulting model provides an initial framework to aid the predictive design of preQ1 riboswitches with desired characteristics for targeted applications.

Author Disclosure Block:

E.R. Van Vlack: None. **S. Topp:** None. **D. Iwata-Reuyl:** None. **J.C. Seeliger:** None.

Session Number:

226

Session Title:

Regulating with RNA

Publishing Title:

Dual Mechanism for the Control of *mutS* Repair Gene Expression by the Bacterial RNA-Binding Protein Hfq

Author Block:

J. Chen, S. Gottesman; Natl. Cancer Inst., Bethesda, MD

Abstract Body:

Hfq, a bacterial Lsm-family RNA-binding protein, plays pleiotropic roles in bacterial physiology, including cell growth, motility and stress tolerance. Hfq exerts these functions through chaperoning RNA-RNA and RNA-protein interactions, and more specifically, by catalyzing base-pairing interactions between small regulatory non-coding RNAs (sRNA) and mRNAs. Base-pairing between sRNA and its cognate mRNA usually leads to mRNA decay by recruiting the endonuclease RNase E and/or translation inhibition by blocking ribosome loading to the ribosome-binding site on mRNA. Tsui (Tsui et al. 1997) reported Hfq is a strong post-transcriptional repressor of the *mutS* repair gene. Here we report that Hfq deploys a dual mechanism to control *mutS* expression: through ArcZ sRNA-mediated repression and via a direct repression by Hfq. ArcZ sRNA inhibits *mutS* expression through a direct base-pairing interaction, and more interestingly, Hfq binds directly to an (ARN)₃ motif within the *mutS* 5' untranslated region (5'UTR) for repression in the absence of sRNA partners. Furthermore, we demonstrate these are separate repression pathways as each uses distinct Hfq faces and RNA sequence motifs for *mutS* repression. In stationary phase cell growth, absence of Hfq increases MutS protein level by ~6-fold whereas depletion of ArcZ increases MutS by ~2-fold and mutation in the *mutS* (ARN)₃ motif increases MutS by ~4-fold. By mutagenesis assays, we show tight control of the MutS level serves as a switch from high-fidelity DNA replication to stress-induced high-rate mutagenesis. Collectively, our results reveal a novel dual mechanism to control the DNA repair gene, and have implications for the control of stress-induced cellular mutagenesis.

Author Disclosure Block:

J. Chen: None. **S. Gottesman:** None.

Session Number:

226

Session Title:

Regulating with RNA

Publishing Title:

Target Prioritization by the *Escherichia coli* Small RNA Master Regulator of Glucose-Phosphate Stress Response

Author Block:

M. Bobrovskyy, C. K. Vanderpool; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract Body:

The *Escherichia coli* small RNA SgrS controls a response to metabolic stress that occurs upon accumulation of phosphosugar intermediates of glycolysis. SgrS base pairs with and represses translation of at least six mRNAs and activates translation of one mRNA during stress recovery. Regulation of multiple mRNA targets by a single sRNA is commonplace in bacteria. Such regulatory hierarchies may play a role in the physiological outcome of the sRNA-mediated responses, but little is known about how they form and what factors are responsible. We reason that hierarchy can be established at multiple levels of sRNA regulation: 1) sRNA-mRNA annealing facilitated by Hfq, 2) mRNA destabilization and 3) translation inhibition. Although the mechanistic details of each have been well characterized, it is unknown which of these steps contributes most to the final regulatory outcome of the multi-target regulon. In this study we will use a simplified model of several mRNA targets of SgrS arranged in a regulatory hierarchy and will elucidate the steps in the regulatory process that dictate target precedence.

Author Disclosure Block:

M. Bobrovskyy: None. **C.K. Vanderpool:** None.

Session Number:

226

Session Title:**Regulating with RNA****Publishing Title:*****Yersinia enterocolitica* CsrA Regulates Expression of the Ysa and Ysc Type Three Secretion Systems in Unique Ways****Author Block:**

G. Ozturk¹, K. LeGrand¹, S. Petersen¹, Y. Zheng², K. K. Liu¹, J-Y. Chen³, G. M. Young¹; ¹Univ. of California, Davis, CA, ²Shenyang Agricultural Univ., Shenyang, China, ³China Agricultural Univ., Beijing, China

Abstract Body:

Activation of the type three secretion systems (T3SS) genes in the host environment is controlled by a multitude factors. The RNA binding regulator, CsrA is involved in post-transcriptional regulation of many specific genes acting as a mediator of mRNA stability and less often as mediator of translation initiation. This study investigated how CsrA affects expression of the Ysa and Ysc T3SS regulatory cascade that controls Ysps (*Yersinia* secreted proteins) and Yops (*Yersinia* outer proteins) export, respectively. This was motivated by the earlier observation that mutation of *csrA* was unable to export the flagellar T3SS dependent phospholipase, YplA, and affected expression of flagellar genes involved in flagellar T3SS. We first tested the possibility that CsrA affects the expression of Ysps and Yops. To this end bacteria were cultivated to induce either the Ysa or the Ysc T3SS, then the amounts of secreted proteins by wild-type and *csrA* mutant strains were compared by proteomic analysis. The amount of Ysps were higher and Yops were lower, respectively, for the *csrA* mutant. Given that most often CsrA acts as a mediator of mRNA stability that can be monitored using *lacZ* transcriptional fusion, we employed a collection of reporter strains to assess Ysa and Ysc gene expression. Comparison of *csrA* mutants to wild type strain revealed that, in response to respective inducing conditions, genes spanning the Ysa and Ysc gene cascades displayed altered expression. Ysa gene expression increased 2.6-fold and Ysc gene expression increased 3.6-fold. These results support the hypothesis that CsrA affects three different T3SSs in *Yersinia enterocolitica*. They further support the idea that CsrA plays an important role in controlling adaptation of this pathogenic bacterium during its lifecycle shifting between terrestrial and parasitic existence.

Author Disclosure Block:

G. Ozturk: None. **K. LeGrand:** None. **S. Petersen:** None. **Y. Zheng:** None. **K.K. Liu:** None. **J. Chen:** None. **G.M. Young:** None.

Session Number:

228

Session Title:**Sorting Out the Signals: Sensing and Signaling through Second Messengers****Publishing Title:****Mechanisms of C-Di-Gmp-Mediated Swarming Motility Repression in *Pseudomonas Aeruginosa*****Author Block:**

A. E. Baker¹, S. L. Kuchma¹, A. Diepold², J. P. Armitage², G. A. O'Toole¹; ¹Geisel Sch. of Med. at Dartmouth, Hanover, NH, ²Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

c-di-GMP is a broadly conserved intracellular signaling molecule that promotes biofilm formation and represses swarming motility in the opportunistic pathogen *Pseudomonas aeruginosa*. Although the production and breakdown of c-di-GMP are well studied, the mechanism by which this signaling molecule influences motility is poorly understood. Swarming is a form of surface-associated motility that is controlled by flagellar rotation. The single polar flagellum of *P. aeruginosa* can be powered by two distinct stator complexes - one of which, MotCD is required for swarming motility, and the other, MotAB represses swarming. We have previously demonstrated that c-di-GMP levels impact stator localization, suggesting a model by which c-di-GMP influences the occupancies of MotAB and MotCD complexes at the flagellar motor as a means for controlling flagellar function (Kuchma *et al.*, 2015). Here we show that when c-di-GMP levels are elevated, swarming is repressed by Pel polysaccharide production and by the protein FlgZ, which binds c-di-GMP via its PilZ domain. The ability of FlgZ to repress swarming depends on its conserved PilZ domain suggesting that its involvement in swarming repression is a c-di-GMP-dependent response. Using bacterial two-hybrid and co-immunoprecipitation assays, we demonstrate that FlgZ interacts with MotC in a c-di-GMP-dependent manner and does not interact with MotA. The involvement of FlgZ in swarming repression and its ability to interact with MotC suggest that FlgZ influences stator localization and/or function in response to c-di-GMP as a means of motility control. We propose that once bound to c-di-GMP, FlgZ interacts with and sequesters MotCD complexes away from the flagellar motor, and that this reduction in MotCD occupancy at the motor is responsible for decreased flagellar motility.

Author Disclosure Block:

A.E. Baker: None. **S.L. Kuchma:** None. **A. Diepold:** None. **J.P. Armitage:** None. **G.A. O'Toole:** None.

Session Number:

228

Session Title:

Sorting Out the Signals: Sensing and Signaling through Second Messengers

Publishing Title:

Feeling the Heat: Characterizing a Small Rna Involved in the Heat Shock Response in the Water-borne Pathogen *Legionella pneumophila*

Author Block:

N. Mendis, M. Saad, S. P. Faucher; McGill Univ., Sainte-Anne-de-Bellevue, QC, Canada

Abstract Body:

Background: Legionnaires' disease is a life-threatening pneumonia that mainly affect the elderly. The causative agent is the Gram-negative, *Legionella pneumophila* (*Lp*). As a contaminant of man-made water distribution systems, *Lp* encounters a range of environmental stresses, including thermal shock. Small regulatory RNAs (sRNA) can act as post-transcriptional regulators of gene expression. Despite the 70 putative sRNA that are encoded by *Lp*, few have been studied and characterized. To this effect, individual screening of several sRNA mutants revealed a distinct defect in the absence of one sRNA, Lpr0001. **Methods & Results:** CFU counts subsequent to a 1 hour heat shock at 55°C show that the survival rate of the Δ *lpr0001* mutant is 100 fold lower compared to the wild-type (WT). Interestingly, this phenotype seems unique to a water environment, as the survival defect is lost in rich medium. Northern Blot analysis confirms the WT expression of Lpr0001 under thermal stress, which is absent in the mutant. Furthermore, the expression of Lpr0001 monitored by Northern Blot is almost completely inhibited in the absence of the putative response regulator Lpg0879, a yet uncharacterized protein in the *Lp* genome, containing a GGDEF domain. A Δ *lpg0879* mutant exhibits a survival defect similar to Δ *lpr0001* when challenged with a 55°C thermal shock. Northern Blot analysis of Lpr0001 expression also suggests interaction of this sRNA with the RpoS, LetA/S and CpxR regulatory networks in *Lp*. **Conclusion:** We show that Lpr0001 is essential for the survival of *L. pneumophila* at 55°C in the context of a water environment. An important regulator of Lpr0001 expression has been identified in Lpg0879. Finally, we show evidence of Lpr0001 involvement in other major stress response networks. The full sequence of the sRNA is now being investigated using **R**apid **A**mplification of **c**DNA **E**nds (RACE), which will be used to experimentally identify direct mRNA targets of Lpr0001.

Author Disclosure Block:

N. Mendis: None. **M. Saad:** None. **S.P. Faucher:** None.

Session Number:

228

Session Title:**Sorting Out the Signals: Sensing and Signaling through Second Messengers****Publishing Title:****Eukaryotic-Type Serine/Threonine Kinase Mediated Regulation of Phosphodiesterase from *Mycobacterium tuberculosis*****Author Block:****N. Malhotra**, P. Chakraborti; CSIR-Inst. of Microbial Technology, Chandigarh, India**Abstract Body:**

Phosphodiesterases are present from bacteria to human and involved in catalysing hydrolysis of cAMP in a cell. Despite our understanding of phosphorylation mediated regulation of this enzyme in eukaryotes, such a phenomenon remains elusive in prokaryotes. To address this issue, we focused on this enzyme from *Mycobacterium tuberculosis* (mPDE). Utilizing *Escherichia coli* based expression system, we observed that mPDE is transphosphorylated by mycobacterial eukaryotic-type Ser/Thr kinases. Phosphopeptide mapping through LC-MS/MS revealed that Thr-309 is the only phosphosite in mPDE. Immuno-blotting using anti-phosphothreonine antibody further confirmed this to be the predominating phosphorylated residue contributing no role in biochemical activity. Interestingly, our sub-cellular localization studies identified phosphorylated mPDE protein to preferentially associate with the *Mycobacterium smegmatis* cell wall. In contrast, phosphoablative mutant, mPDE-T309A, did not show such behaviour. In addition, biochemical analyses revealed that eukaryotic-type Ser/Thr kinase mediated phosphorylation also has deleterious effects on mPDE enzyme activity. In consonance, expression of kinase(s) in mPDE complemented *E. coli* cells depicted increased cAMP levels. Bioinformatic predictions demonstrated S20, T22, T182 and T240 to be the possible residues contributing to such an effect. Mutagenesis followed by western blot and biochemical analyses confirmed Thr-240 to be the leading residue involved in phosphorylation mediated control of mPDE enzyme activity. Thus, we provide evidence for the first time to show the regulation of phosphodiesterase in prokaryotes by post-translational modification.

Author Disclosure Block:**N. Malhotra:** None. **P. Chakraborti:** None.

Session Number:

228

Session Title:

Sorting Out the Signals: Sensing and Signaling through Second Messengers

Publishing Title:

(p)ppgpp Controls Intracellular Gtp Levels to Promote Antibiotic Tolerance in *Bacillus subtilis*

Author Block:

J. L. Tse¹, K. Liu², D. K. Fung¹, A. N. Bittner¹, J. D. Wang¹; ¹Univ. of Wisconsin-Madison, Madison, WI, ²Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract Body:

The stress-inducible alarmone, guanosine penta- and tetraphosphate or (p)ppGpp, is found in all bacteria and has many effects on bacterial physiology. Recently, (p)ppGpp has been implicated in promoting antibiotic tolerance, the ability to survive antibiotic stress, in Proteobacteria such as *Escherichia coli*, but less is known about the effects of this molecule on antibiotic tolerance in Firmicutes, such as *Bacillus subtilis*. *B. subtilis* contains three (p)ppGpp synthetases and deletion of all three enzymes, designated as (p)ppGpp⁰, cannot synthesize (p)ppGpp. Our previous work showed that (p)ppGpp⁰ cells cannot regulate GTP production, which conditionally leads to high GTP levels and cell death. Here, we show that (p)ppGpp⁰ cells are more susceptible to killing by multiple antibiotics when compared to the wild type, indicating that (p)ppGpp promotes antibiotic tolerance. Induction of (p)ppGpp prior to antibiotic treatment increases tolerance, further supporting that (p)ppGpp is a key player in mediating survival. Decreasing GTP levels in (p)ppGpp⁰ cells restores tolerance to wild type levels. Further decrease of GTP levels resulted in antibiotic hypertolerance. Thus, not only does (p)ppGpp play a critical role in antibiotic tolerance, but decreasing GTP levels, one of the downstream effects of (p)ppGpp, has similar effects. (p)ppGpp control of GTP levels is important for survival of antibiotic treatment. Abolishing (p)ppGpp control of GTP biosynthesis enzymes introduces high GTP levels in cells with intact synthetases and potentiated antibiotic killing is observed. These observations lend themselves to a model wherein (p)ppGpp protects cells from antibiotics by decreasing GTP levels. Absence of (p)ppGpp or abolishing (p)ppGpp regulation of GTP production results in higher GTP levels and thus decreased antibiotic tolerance. Our findings will further the understanding of the differences in mechanisms of antibiotic tolerance between Gram-positive and Gram-negative bacteria.

Author Disclosure Block:

J.L. Tse: None. **K. Liu:** None. **D.K. Fung:** None. **A.N. Bittner:** None. **J.D. Wang:** None.

Session Number:

334

Session Title:

Answering the Threat of Emerging Viruses

Publishing Title:

Alterations in Host Transcriptome Profile Induced by Hepatitis E Virus Like Particles

Author Block:

S. K. Panda, S. P. K. Varma; All India Inst. of Med. Sci., New Delhi, India

Abstract Body:

Hepatitis E virus (HEV) is an enterically transmitted positive stranded RNA virus, encoding three proteins: pORF1 (multi-enzyme polyprotein), pORF2 (capsid protein) and pORF3 (multi-regulatory protein). It mostly causes a self-limiting, sub-clinical to acute hepatitis, widespread throughout the world. Using in vitro assembled HEV virus-like particles (VLP) we have demonstrated HEV entry to be through receptor-dependent clathrin mediated endocytosis. Such VLP's have also been used to deliver foreign RNA to liver cells. Further in the wake of similar HEV VLPs being approved as vaccine, we investigated the transcriptome alterations in VLP infected Huh7 cells. Twenty four hours post infection, cells were harvested using Trizol and mRNA was enriched for analysis. RNA-Seq was performed on Ion-PGM and sequences were aligned to human genome build HG19 using TOPHAT 2 and BOWTIE2 in Partek Flow. Mapped sequences were analysed in Partek Genomic Suite for differential expression. A total of 1806 genes were differentially expressed as compared to control by ≥ 1.5 times ($p < 0.05$). Gene ontology showed maximum alterations in host transcriptional and translational machinery (Ribosomal small and large subunit proteins). Other important altered genes included those involved in endosomal trafficking and biology and few genes affecting Hepatitis B virus (HBV) replication. The data was validated by qPCR for biologically relevant genes (RPL37A, RPL24, RPS11, RPS13, RPL12, LAMTOR5, POL2R1, TGFB3, FMO1). The effect of HEV VLP on HBV replication was experimentally validated. Briefly, HBV transfected Huh7 cells were incubated with HEV VLPs. After 24hrs of HEV VLP incubation, qPCR for HBV copy numbers and HbsAg protein quantification by ELISA was performed. We observed decrease in HBV copy number ($0.908E+06$) and HbsAg levels (0.2758) in HBV transfected cells post HEV-VLP incubation as compared to parallel uninfected HBV transfected cells ($1.24E+06$, 0.4892). This is the first attempt to understand the transcriptome alterations induced by HEV VLP's. Similar recombinant

HEV VLP's have also been licensed as vaccine. Moreover, we demonstrate decreased HBV replication in Huh7 cells upon HEV VLP infection.

Author Disclosure Block:

S.K. Panda: None. **S.P.K. Varma:** None.

Session Number:

334

Session Title:

Answering the Threat of Emerging Viruses

Publishing Title:

Prevalence of Human Metapneumovirus Infection among Egyptian Infants with Acute Viral Bronchiolitis

Author Block:

G. E. A. EL-SAWAF, W. N. F. Raffla, A. A. R. Ghazal, D. R. Metwally, M. M. Eissa;
Med. Res. Inst. - Alexandria Univ., Alexandria, Egypt

Abstract Body:

Background: Despite improved methods for identifying viral pathogens causing acute bronchiolitis, the etiology remains undetermined in a significant number of patients. Human metapneumovirus (hMPV) is one of the emerging respiratory viral pathogen that causes a spectrum of illnesses ranges from asymptomatic infection to severe bronchiolitis. We aimed to identify the prevalence of hMPV that contribute to bronchiolitis in infants and young children in Egyptian populations and to determine the comprehensive clinical characterizations of disease. **Methods:** Nasal swabs for viral detection were obtained from 117 Egyptian infants, clinically diagnosed as acute bronchiolitis at our institutional's Hospital during the period from January to April 2015.. Clinical and demographic data were obtained from parents and medical records, hMPV was detected by means of a reverse-transcriptase polymerase-chain-reaction assay. Indirect immunofluorescent assay (IFA) assay methods were used to detect the presence of any of the most common respiratory viruses (respiratory syncytial virus (RSV), Influenza virus A & B, Parainfluenza virus types 1-3 and adenovirus) that might be involved in infection. **Results:** In our study, 76% of the cases were positive at least to one or more of the seven mentioned viruses. hMPV was detected in 19 (16 %) of the 117 children. The age-related incidence of hMPV infection was higher than that of RSV-infected children. Only 5 patients (4%) had hMPV as the sole respiratory viruses, whilst 14 cases (12%) had a co-infection of hMPV with other respiratory viruses. Clinical symptoms of hMPV were found to be similar to those seen with other respiratory viral infections. The most significant risk factors for acute bronchiolitis in our study groups were young age, exposure to tobacco and living in overcrowded environments. **Conclusions:** hMPV infection is a leading cause of respiratory tract infection in the first 2 years of life, with a spectrum of disease similar to that of RSV. The

identified risk factors in our study require interventional studies to control infections among young children in developing countries. Further investigations to better characterize hMPV infection and its clinical effect are needed.

Author Disclosure Block:

G.E.A. El-sawaf: None. **W.N.F. Raffla:** None. **A.A.R. Ghazal:** None. **D.R. Metwally:** None. **M.M. Eissa:** None.

Session Number:

334

Session Title:

Answering the Threat of Emerging Viruses

Publishing Title:

Molecular Mechanism of Zika Virus-Associated Microcephaly

Author Block:

O. Bagasra, R. Bhattarai, K. Mahalingam; Claflin Univ., Orangeburg, SC

Abstract Body:

Zika virus (ZIKV) is a flavivirus in the same family as yellow fever, dengue, West Nile, and Japanese encephalitis viruses. It is transmitted by various species of mosquitoes. It is named after its discovery almost 70 years ago in Zika forest in Uganda. Since, it is found in many parts of the globe with infected humans suffering from mild fever, rash, arthralgia, and conjunctivitis. Zika fever was first discovered in Uganda in the 1947 and has since become endemic in parts of Africa. It also spread to the South Pacific and areas of Asia, and most recently to Latin America. In the majority of the cases a significant population is infected without reports of any significant illness except most recently. Recently, the Brazilian Government has issues warning to would-be-parents, especially in the country's northeast to use birth control, after health officials have linked Zika viral infection to a surge in newborn microcephaly, a neurological disorder that can result in incomplete brain development. This was prompted by the reports of 2,400 cases of microcephaly in 2015 while there were only 147 cases of microcephaly in 2014. In order to determine a connection between Zika virus and microcephaly at molecular levels, we analyzed the most the genome sequences of Zika virus and the genes linked to human autosomal recessive primary microcephaly (*MCPH*). There are 12 known *MCPH* loci that are linked to microcephaly (i.e., *Microcephalin*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP135*, *CEP152*, *ZNF335*, *PHC1* and *CDK6*). Over 3,000 mature human miRNA sequences were retrieved through a miR-database, and the identification of mature miRNAs were aligned with full length sequences of Zika virus as well genes linked to *MCPH* via computational tools, by which 4 putative miRNAs were found to have near perfect identity at seed sequences with Zika virus as well as *MCPH*. We hypothesize that infection with Zika virus upregulates *MCPH* in Future approaches will focus on experimental validation of these miRNAs in quelling the *MCPH* in infected fetuses at early stages of gestation that results in interference in fetal brain development.

Our next goal is to target the 4 miRNA to further elucidate their biological functions in mice and other animal models.

Author Disclosure Block:

O. Bagasra: None. **R. Bhattarai:** None. **K. Mahalingam:** None.

Session Number:

334

Session Title:

Answering the Threat of Emerging Viruses

Publishing Title:

Epidemiological Analysis of Dengue Fever and Estimate the Potentially Beneficiaries of Newly Developed Vaccine

Author Block:

M. Modi, K. Madhavani, N. Patel, S. Nanda, T. Javadekar; Med. Coll. Baroda, Maharaja Sayajirao Univ., Vadodara, India

Abstract Body:

Background: In recent years, Dengue fever is re-emerged as mosquito borne viral disease across the Globe, which is endemic in Tropics & expanding to the new regions of the world without any hurdle. This study was conducted to estimate the burden of the disease in Vadodara, in western part of India, & to estimate the number of cases where newly developed dengue vaccine might help. Epidemiological analysis was done to characterize the age & gender related prevalence & seasonal variation in infection. **Methods:** Retrospective analysis was done spanning over the period of 5 years and 9 months, from March, 2010 to December, 2015. Samples were received in cold chain from within the hospital and from peripheral centers in rural areas depending on the clinical signs, symptoms, laboratory investigations and condition of the patient. Testing was done by Enzyme Linked Immuno-Sorbent Assay (ELISA) for presence of NS1 antigen and IgM antibody depending on the days of illness. Both, internal and external, positive and negative controls were tested in each run for the validation and quality control. **Results:** Out of total 6,716 patients, 1,749 (26.04%) patients had confirmed dengue infection. 1,151 (65.81%) were males and 598 (34.19%) were females.

Yearly Male-Female & Age Group Distribution									
		Total	Positive	%	Male	%	Female	%	
	2010	483	146	30.23	92	63.01	54	36.99	
	2011	718	58	8.08	38	65.52	20	34.48	
	2012	827	160	19.35	100	62.50	60	37.50	
	2013	1763	497	28.19	315	63.38	182	36.62	

	2014	1222	342	27.99	234	68.42	108	31.58	
	2015	1703	546	32.06	372	68.13	174	31.87	
	TOTAL	6716	1749	26.04	1151	65.81	598	34.19	
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	TOTAL
2010	41	45	32	12	10	2	4	0	146.00
2011	9	19	13	9	4	2	2	0	58.00
2012	38	51	44	9	9	6	2	1	160.00
2013	79	169	141	58	26	19	4	1	497.00
2014	37	122	92	47	23	11	7	3	342.00
2015	63	176	188	63	27	15	13	1	546.00
TOTAL	267	582	510	198	99	55	32	6	1749.00
%	15.27	33.28	29.16	11.32	5.66	3.14	1.83	0.34	100.00

Highest numbers of the infection was noted during September and October in each year, with intermediate number was recorded during November and December. Adolescent (11-20) & young age (21-30) were highly affected as compared to children, middle and older age. Nearly 75% of patients fall in the age group of 9-45 years, which is the recommended age group for the newly developed vaccine. **Conclusion:** Study result shows dengue fever is affecting males more than females. Adolescent as well as young people's are more affected as compared to extremes of ages. Effective preventive measures should be implemented in pre-monsoon season as considerable increase in infection is noted in post-monsoon season. Newly developed vaccine for dengue can able to help tremendously.

Author Disclosure Block:

M. Modi: None. **K. Madhavani:** None. **N. Patel:** None. **S. Nanda:** None. **T. Javadekar:** None.

Session Number:

335

Session Title:**ASM Lecturer's Choice - The Real Function of CRISPR-Cas: Host Defense****Publishing Title:****Inactivation of CRISPR-Cas Systems by Anti-CRISPR Proteins in Diverse Bacteria****Author Block:**

A. Pawluk¹, R. H. J. Staals², C. Taylor², B. N. J. Watson², P. C. Fineran², K. L. Maxwell³, A. R. Davidson¹; ¹Univ. of Toronto, Toronto, ON, Canada, ²Univ. of Otago, Dunedin, New Zealand, ³Donnelly Ctr. for Cellular and Biomolecular Res., Toronto, ON, Canada

Abstract Body:

CRISPR-Cas systems target and destroy foreign DNA in prokaryotes, and therefore pose a significant challenge to bacteriophage replication and horizontal gene transfer. As such, it is likely that mobile genetic elements have evolved mechanisms to circumvent CRISPR-Cas immunity. Our laboratory previously identified the first examples of CRISPR-Cas inhibitor proteins - “anti-CRISPRs” - encoded by phages infecting *Pseudomonas aeruginosa* (Bondy-Denomy et al, *Nature* 2013; Pawluk et al, *mBio* 2014). Here, we conducted bioinformatic analysis of sequence motifs found proximal to known anti-CRISPR genes to discover five novel, distinct gene families that inhibit the activity of the two distantly related type I-F CRISPR-Cas systems of *P. aeruginosa* and *Pectobacterium atrosepticum*. Strikingly, we also discovered the first example of a dual specificity anti-CRISPR that inhibits both type I-F and I-E CRISPR systems using two distinct functional interfaces. Mirroring the distribution of the CRISPR-Cas systems they inactivate, the new anti-CRISPRs were found in species distributed broadly across the phylum Proteobacteria. Importantly, anti-CRISPRs originating from species with very divergent type I-F CRISPR-Cas systems are able to inhibit the two systems we tested, highlighting their broad specificity. These results imply that all type I-F CRISPR-Cas systems in nature are likely vulnerable to inhibition by anti-CRISPRs. The extreme evolutionary pressure for mobile DNA to develop countermeasures to CRISPR-Cas defense, as illustrated by the emergence of at least 14 distinct anti-CRISPR families and a dual specificity anti-CRISPR protein, strongly supports the existence of inhibitors of other types of CRISPR-Cas systems across prokaryotes. We propose that anti-CRISPRs play a broad and influential role in facilitating the movement of DNA between

prokaryotes - and, by extension, the dissemination of antibiotic resistance and virulence genes - by inactivating the powerful barrier imposed by CRISPR-Cas systems.

Author Disclosure Block:

A. Pawluk: None. **R.H.J. Staals:** None. **C. Taylor:** None. **B.N.J. Watson:** None. **P.C. Fineran:** None. **K.L. Maxwell:** None. **A.R. Davidson:** None.

Session Number:

335

Session Title:

ASM Lecturer's Choice - The Real Function of CRISPR-Cas: Host Defense

Publishing Title:

Active and Adaptive Type I-C and I-F Crispr-Cas in *Legionella pneumophila*

Author Block:

C. Rao¹, J. Bondy-Denomy², **A. W. Ensminger¹**; ¹Univ. of Toronto, Toronto, ON, Canada, ²Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

Genomic analysis suggests that over 40% of bacteria possess acquired immunity to foreign DNA through CRISPR-Cas systems, yet the selection that favors the possession of these systems is not always clear. For instance, many isolates of the intracellular pathogen *Legionella pneumophila* possess multiple CRISPR-Cas systems (Type I-C, Type I-F, and Type II-B), yet their targets have remained elusive. Obfuscating matters further, (1) the most commonly studied strain of *L. pneumophila*, isolated during the eponymous 1976 Philadelphia outbreak of Legionnaires' disease, is itself devoid of CRISPR-Cas; and (2) a non-canonical role outside of genome defense has been ascribed to a Type II-B system in *L. pneumophila* str. 130b. Focusing our attention on a collection of *L. pneumophila* clinical isolates from Ontario, we will provide direct evidence of active and adaptive CRISPR-Cas. Through comparative genomics, we observe Type I-C spacer dynamics consistent with acquisition in nature. To monitor for acquisition of new spacers in the laboratory, we have established an automated microbial growth system to sample bacterial populations after defined generations of axenic replication in the presence of foreign DNA. Using this approach, we have observed efficient acquisition of new spacers under "priming" conditions in both Type I-C and Type I-F *L. pneumophila* CRISPR-Cas systems. We propose that *L. pneumophila* CRISPR-Cas may be "tuned" for robust primed spacer acquisition, by tolerating low-level transformation with protospacer-containing sequences. Finally, we have identified the first known target of *L. pneumophila* CRISPR-Cas: a phage-like mobile element that causes condition-specific fitness effects inconsistent with pathogen persistence. We will show that CRISPR-Cas protects against the transfer of this element and provide evidence of an ongoing arms race between it and a diverse set of *Legionella* CRISPR-Cas systems.

Author Disclosure Block:

C. Rao: None. **J. Bondy-Denomy:** None. **A.W. Ensminger:** None.

Session Number:

335

Session Title:

ASM Lecturer's Choice - The Real Function of CRISPR-Cas: Host Defense

Publishing Title:

Genome Defense Systems Compromise for Beneficial Mge Acquisition in "*E. faecalis*"

Author Block:

W. Huo, V. Price, M. Zhang, K. Palmer; Univ. of Texas at Dallas, Richardson, TX

Abstract Body:

Enterococcus faecalis is a Gram-positive bacterium that naturally colonizes humans and opportunistically causes life-threatening infections. Multidrug-resistant (MDR) *E. faecalis* strains have emerged that are replete with mobile genetic elements (MGEs). Considering that bacteria commonly possess two genome defense mechanisms to prevent MGE acquisition, restriction modification (RM, analogous to innate immunity) and CRISPR-Cas (adaptive immunity), we hypothesize that these barriers have been compromised in MDR *E. faecalis* strains. We further hypothesize that RM systems have lineage specificity, and that CRISPR-Cas systems will be prone to mutation due to conflict with beneficial MGEs. We previously identified a Type II RM system, EfaRFI, in *E. faecalis* OG1RF, and an ortholog of the EfaRFI DNA methyltransferase (MTase) was predicted in *E. faecalis* T11. Notably, we observe a thousand-fold stronger defense from plasmid acquisition conferred by the T11 EfaRFI than that from OG1RF EfaRFI. This led us to investigate the distribution of EfaRFI and other RM systems in a larger collection of *E. faecalis* genomes. We predicted DNA MTases in 74 *E. faecalis* draft genomes. We found that the distribution of RM systems is highly variable in *E. faecalis*, and no core systems were identified. However, a predicted RM system is enriched in the MDR ST2 lineage. Towards our hypothesis about the conflict of adaptive immunity with MGEs, we have found that *E. faecalis* T11 with active RM and CRISPR-Cas has a ~6-log lower plasmid acquisition frequency than a derivative with inactive genome defense. However, some plasmid transconjugants still arise. Using serial passage experiments in the presence and absence of antibiotic selection for the plasmid, we found that there is a cost to maintain both the plasmid and a functional CRISPR-Cas system in these transconjugants. CRISPR-Cas mutants arise under antibiotic selection. Based on these results, we conclude that: 1) innate immunity has variable distribution across *E. faecalis* strains, although a lineage-specific MTase was observed; 2) adaptive immunity can be

lost under selection for MGEs. These results are consistent with our overall hypothesis that compromised genome defense contributes to the emergence of MDR *E. faecalis*.

Author Disclosure Block:

W. Huo: None. **V. Price:** None. **M. Zhang:** None. **K. Palmer:** None.

Session Number:

339

Session Title:

Bacterial Motility

Publishing Title:

The Actin Homologue MreB and Gliding Motors Show Interdependent Movements in the Bacterium *Myxococcus xanthus*

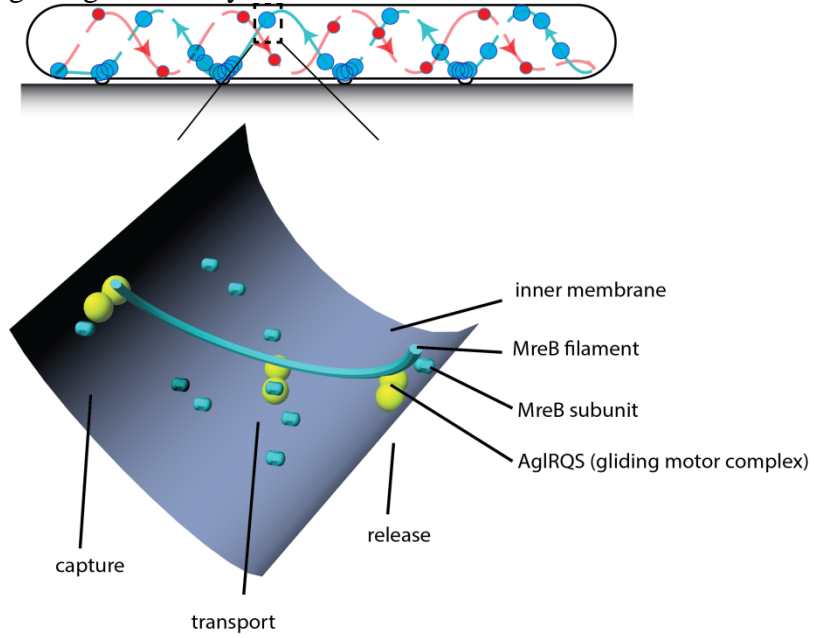
Author Block:

B. Nan; Texas A&M Univ., College Station, TX

Abstract Body:

Background:MreB is a bacterial actin homologue involved in a wide range of functions including cell wall biosynthesis and chromosome segregation. MreB also plays crucial roles in *Myxococcus xanthus* gliding motility, which is impaired when MreB polymerization is inhibited. However, it remains unknown how MreB facilitates and adapts to the fast motion of the gliding machinery.**Methods:**We tracked the dynamics of single MreB molecules in *M. xanthus* using single particle tracking photo-activated localization microscopy (sptPALM).**Results:**We found that a subpopulation of MreB moved actively in helical trajectories, similar to the movement of the gliding machinery. MreB interacts directly with motor protein AglS, a MotB homologue, and MreB movements were lost in the mutants that carried truncated motors, indicating that MreB movement is driven by the gliding complexes. The speed of MreB movement was two orders of magnitude higher than that in *Bacillus subtilis* and *E. coli*. Unlike these organisms, the fast motion of MreB was not blocked by inhibitors of cell wall biosynthesis in *M. xanthus*.**Conclusions:**Our results imply that in *M. xanthus*, the function of MreB is adapted to provide a scaffold for the gliding motors and that the

gliding machinery drives the movement of MreB filaments.



Author Disclosure Block:

B. Nan: None.

Session Number:

339

Session Title:

Bacterial Motility

Publishing Title:

Bacterial Gliding Machinery

Author Block:

A. Shrivastava¹, **T. Roland**¹, **P. Lele**², **H. C. Berg**¹; ¹Harvard Univ., Cambridge, MA, ²Texas A&M Univ., College Station, TX

Abstract Body:

Bacterial gliding, i.e. movement of bacteria over surfaces without the aid of flagella or pili, is a widespread yet barely understood phenomenon. Motile bacteria of the large bacterial phylum bacteroidetes glide over surfaces with speeds reaching ~2 micrometer/s. Such bacteria are found in diverse ecosystems such as human oral biofilms, fish skin, crustacean shells and on organic detritus suspended in aquatic ecosystems. Because of high gliding speed and recently developed genetic tools, *Flavobacterium johnsoniae*, a member of the phylum bacteroidetes, has emerged as a powerful bacterium to investigate gliding. *F. johnsoniae* has cell-surface filaments about 160 nm in length by 6 nm in diameter, composed of a protein SprB. Interaction of SprB with a surface produces gliding. We tethered *F. johnsoniae* cells to glass by adding anti-SprB antibody. Tethered cells spun about fixed points, rotating at speeds of about 1 Hz. The torques required to sustain such speeds were large, comparable to those generated by the flagellar rotary motor. Using a flow cell apparatus, we changed load on the gliding motor by adding the viscous agent Ficoll to tethered cells. We found that a gliding motor runs at constant speed rather than constant torque. We attached gold nanoparticles to the SprB filament and tracked its motion. We fluorescently tagged a bacterial Type IX secretion system (T9SS) protein and imaged its dynamics. Fluorescently tagged T9SS protein localized near the point of tether, indicating that T9SS localizes with the gliding motor. Based on our results, we propose a model to explain bacterial gliding.

Author Disclosure Block:

A. Shrivastava: None. **T. Roland:** None. **P. Lele:** None. **H.C. Berg:** None.

Session Number:

339

Session Title:

Bacterial Motility

Publishing Title:

The Effects of Fungally Produced Ethanol on *Pseudomonas aeruginosa* Motility Regulation

Author Block:

K. A. Lewis¹, C. E. Harty¹, A. I. Chen², D. A. Hogan¹; ¹Geisel Sch. of Med. at Dartmouth, Hanover, NH, ²Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Pseudomonas aeruginosa causes infections in compromised individuals, burn wounds, and the lungs of individuals with cystic fibrosis (CF). The CF lung is often infected with multiple species of bacteria and fungi, namely *P. aeruginosa* and the fungus *Candida albicans*. CF patients with both of these organisms in their lungs are usually linked to a worse prognosis and increased exacerbations than those with *P. aeruginosa* alone. WspR, a diguanylate cyclase (DGC) in the Wsp sensory system, was previously identified in a genetic screen and shown to produce increased c-di-GMP (cdG) levels, in the presence of EtOH, which leads to increased Pel exopolysaccharide production and biofilm formation. Multiple lines of evidence lead us to hypothesize that other DGCs may respond to EtOH to reduce flagellar motility independent of Pel production. HPLC analysis of extracted nucleotides from WT PA14 and $\Delta wspR$, grown in liquid cultures without and with 1% EtOH, showed a 2.6 and 2.4 fold cdG increase ($p < 0.005$) in EtOH samples, respectively. $\Delta gcbA$, a DGC involved in the initial stages of biofilm formation, and $\Delta gcbA wspR$ also had 2.3 and 1.7 fold cdG increase ($p < 0.005$) in EtOH samples, respectively. These data indicate that in EtOH, WspR and GcbA contribute to increased cdG in WT while other DGCs may contribute to a lesser extent. The swim assay, where cells are inoculated into 0.3% agar, incubated and the swim diameter within the agar measured, was used to assess motility. Here, WT, $\Delta pelA$, and $\Delta wspR$ grown without and with 1% EtOH had a 32% decrease in diameter in EtOH ($p < 0.005$). Thus, this indicated that exogenous EtOH represses flagellar motility independent of Pel. This repression is mediated by the MotAB regulatory stator complex whose mutant has the same swim diameter without and with 1% EtOH, indicating a resistance to the swim repression observed in WT EtOH samples. We identified two other DGCs that have swim phenotypes similar to $\Delta motAB$ and they may be important in the MotAB-mediated repression of flagellar motility in response to EtOH. Together, the cdG and swim data point to a complex and possibly redundant DGC network that not only controls cdG levels in response to EtOH but the switch from a

motile to sessile lifestyle. These data may also inform us on how the microbial community of the CF lung leads to increased exacerbations in patients.

Author Disclosure Block:

K.A. Lewis: None. **C.E. Harty:** None. **A.I. Chen:** None. **D.A. Hogan:** None.

Session Number:

339

Session Title:

Bacterial Motility

Publishing Title:

Assembly and Extension Mechanisms of R Bodies, Reversible, pH-Driven, Membrane-Breaking Protein Pistons

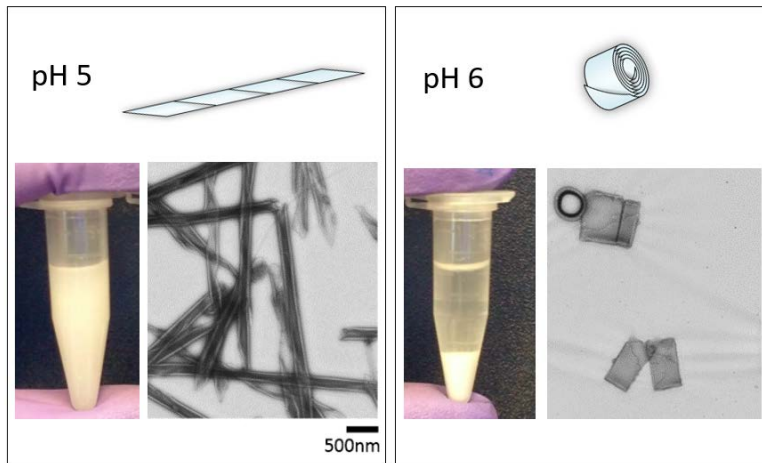
Author Block:

J. Polka, P. Silver; Harvard Med. Sch., Boston, MA

Abstract Body:

Background: R bodies are large polymeric protein structures produced in the cytoplasm of *Caedibacter taeniospiralis*, a bacterial endosymbiont of *Paramecium*. At cytoplasmic pH they resemble a coil of ribbon 500nm in diameter and approximately 400nm deep. At low pH, however, the R body reversibly and rapidly extends to form a hollow needle with pointed ends that can be up to 20um long. R bodies function as toxin-delivery devices that pierce the food vacuole of a predatory eukaryote. **Methods:** We employed a high-throughput visual screen to identify mutants defective in pH response, then characterized these mutants with circular dichroism, phase contrast timelapse microscopy, and negative stain EM to identify the role of specific amino acids in pH-mediated conformational changes. We identified regions involved in covalent R body assembly with Tandem Mass Tag Mass Spec (TMT-MS) and used Structured Illumination Microscopy (SIM) monitor assembly dynamics. **Results:** The R body assembly process requires specific lysine residues, suggesting that the covalent assembly process relies on isopeptide bond formation. These covalently-assembled subunits then arrange themselves into sheets and spools. We also present data suggesting that a protein required for R body assembly - RebC - forms a long-range assembly template. Our mutagenesis implicates a small unstructured region a helical transition that drives R body extension. **Conclusions:** Taken together with structural data, our results lead us to propose a curvature-driven model for R body extension. We also use R bodies as tools to rupture foreign membranes, triggering the release of encapsulated cargo. Further uses of R bodies - as a remodeling scaffold, as a smart material that can cause reversible hemostasis, and as a micron-scale switch - are

also explored.



Author Disclosure Block:

J. Polka: None. **P. Silver:** None.

Session Number:

340

Session Title:

Cell Architecture and Structure: From Mechanism to Community Structure

Publishing Title:

Coordination Between Cell Division and Chromosome Segregation in *Escherichia coli*

Author Block:

J. Mannik; Univ. of Tennessee, Knoxville, TN

Abstract Body:

Coordination between cell division and chromosome segregation is essential for a cell to produce viable progeny. Several partially redundant molecular systems have been identified in the past that are involved in this coordination in *Escherichia coli*. These systems include the Min system, which inhibits formation of the divisome at cell poles, and nucleoid occlusion mediated by SlmA, which prevents localization of the divisome in the vicinity of unsegregated nucleoid. Both of these systems are based on negative regulation of cell division assembly [1]. Using fluorescence microscopy and genetic tools we have recently discovered that *E. coli* also harbors a positive regulation system, which localizes the cell division plane with respect to the replication terminus region of the chromosome. This system is referred to as the Ter linkage and it involves MatP, ZapA and ZapB proteins. [2]. While all three systems are important in increasing the fitness of the cell, none of them are essential for viability. Also, none of these three systems are conserved among the bacteria. The question then arises: what mediates the essential coordination between cell division proteins and chromosomes that is minimally needed for bacterial cells to propagate? Our most recent research has shown that the essential coordination may be provided by the division septum itself, which closure physically partitions chromosomes into two daughter compartments. Investigating mutant cells we found a fast FtsK-independent translocation of the nucleoid across the division plane as it closes. Analysis of microscope images shows that the translocation can affect as much as half of the chromosome. [1] Männik J, Bailey MW (2015) *Spatial coordination between chromosomes and cell division proteins in Escherichia coli*. *Front. Microbiol.* **6** (2015) 306. doi: 10.3389/fmicb.2015. [2] Bailey MW, Bisicchia P, Warren BT, Sherratt DJ, Männik J (2014) *Evidence for divisome localization mechanisms independent of the Min system and SlmA in Escherichia coli*. *PLOS Genetics* **10** (2014) e1004504. doi:10.1371/journal.pgen.1004504.

Author Disclosure Block:

J. Mannik: None.

Session Number:

340

Session Title:

Cell Architecture and Structure: From Mechanism to Community Structure

Publishing Title:

The Intersection Of Cyclic-Di-Gmp And A Plec/Divj Sensor Kinase Homologue Regulates Adhesion In *agrobacterium Tumefaciens*

Author Block:

J. E. HEINDL¹, C. Fuqua²; ¹Univ. of the Sci., Philadelphia, PA, ²Indiana Univ., Bloomington, IN

Abstract Body:

Prokaryotic developmental processes, including morphological transformations as well as motile-sessile transitions, are tightly regulated. A conserved regulatory pathway directs developmental transitions and asymmetries in *Agrobacterium tumefaciens*. Core components of this pathway include two integrated phosphorelays. One of these phosphorelays includes at least four histidine sensor kinase homologues, DivJ, PleC, PdhS1, and PdhS2, and at least two response regulators, DivK and PleD. Previously we demonstrated that PdhS2 reciprocally regulates biofilm formation and swimming motility. In the current study we further dissect the architecture of this pathway in *A. tumefaciens* with respect to PdhS2. We show that PdhS2-dependent effects on attachment and motility require the response regulator, DivK, but do not require PdhS2 autokinase or phosphotransfer activities. We also demonstrate that PdhS2 regulation of biofilm formation is dependent upon multiple diguanylate cyclases, including PleD, DgcA, and DgcB, implying that PdhS2 regulation of this process intersects with pathways regulating levels of the second messenger cyclic-di-GMP. Finally, we show that upon cell division a GFP fusion to PdhS2 dynamically localizes to the new pole of the bacterium suggesting that PdhS2 controls processes in the daughter cell compartment of predivisional cells. These observations suggest that PdhS2 negatively regulates DivK, and possibly PleD, activity to control developmental processes in the daughter cell compartment of predivisional cells, as well as in newly released motile daughter cells.

Author Disclosure Block:

J.E. Heindl: None. **C. Fuqua:** None.

Session Number:

340

Session Title:**Cell Architecture and Structure: From Mechanism to Community Structure****Publishing Title:****Structural Basis of the Myf and Ph6 Fimbriae Mediated Tropism of Pathogenic Yersinia****Author Block:**

N. Pakharukova¹, S. Roy², M. Tuittila¹, S. Paavilainen¹, A. Zavialov¹; ¹Univ. of Turku, Turku, Finland, ²Swedish Univ. of Agricultural Sci., Uppsala, Sweden

Abstract Body:

Three pathogenic species of genus *Yersinia*, *enterocolitica*, *pseudotuberculosis*, and *pestis* show significant differences in the mode of transmission. *Y. enterocolitica* is a strictly enteric pathogen, whereas *Y. pseudotuberculosis* can also infect via lungs and its close relative *Y. pestis* is either blood- or airborne pathogen. Each of these pathogens assembles host cell adhesion fimbriae via the chaperone/usher (CU) pathway. *Y. enterocolitica* assembles Myf fimbriae from MyfA subunits. *Y. pseudotuberculosis* and *pestis* instead elaborate the pH6 antigen fimbriae consisting of PsaA subunits. To better understand the role of these fimbriae in tissue tropism of pathogenic *Yersinia*, we performed high-resolution structural studies of MyfA and PsaA subunits complexed with binding determinants of their glycolipid receptors. We found that, as PsaA, MyfA specifically binds to galactose, a terminal residue of ganglioside receptors. The galactose-binding pocket is located in the same segment of MyfA and PsaA structure, but is arranged of different binding residues. Co-crystallization experiments of PsaA with choline, the head group of phosphatidyl choline lipid, revealed that it also can bind to three phosphatidyl choline molecules simultaneously, using one previously found and two novel binding sites. The choline binding depends on several tyrosine residues, which are all missing in MyfA. Thereby, Myf fimbriae may facilitate intestine colonization by *Y. enterocolitica* by recognizing ganglioside receptors on enterocytes, whereas PsaA may additionally promote colonization of lung by *Y. pseudotuberculosis* and *Y. pestis* by binding to gangliosides and phosphatidyl choline lipids abundant in alveolar cells.

Author Disclosure Block:

N. Pakharukova: None. **S. Roy:** None. **M. Tuittila:** None. **S. Paavilainen:** None. **A. Zavialov:** None.

Session Number:

340

Session Title:

Cell Architecture and Structure: From Mechanism to Community Structure

Publishing Title:

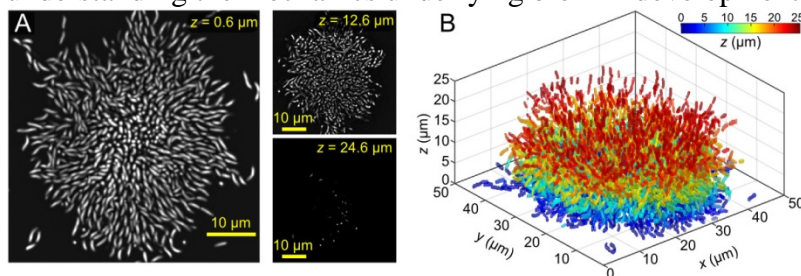
Architectural Transitions During *Vibrio cholerae* Biofilm Development at Single-cell Resolution

Author Block:

K. Drescher¹, J. Dunkel², C. Nadell¹, N. Wingreen³, H. Stone³, B. Bassler⁴; ¹Max Planck Inst. for Terrestrial Microbiol., Marburg, Germany, ²Massachusetts Inst. of Technology, Boston, MA, ³Princeton Univ., Princeton, NJ, ⁴Princeton Univ./HHMI, Princeton, NJ

Abstract Body:

Many bacterial species colonize surfaces and form dense three-dimensional structures, known as biofilms, which are highly tolerant to antibiotics and constitute one of the major forms of bacterial biomass on Earth. Bacterial biofilms display remarkable changes during their development from initial attachment to maturity, yet the cellular architecture that gives rise to collective biofilm morphology during growth is largely unknown. Here, we use high-resolution optical microscopy to image all individual cells in *Vibrio cholerae* biofilms at different stages of development, including colonies that range in size from 2 to 4500 cells (Figure 1). From these data, we extracted the precise three-dimensional cellular arrangements, cell shapes, sizes, and global morphological features during biofilm growth on submerged glass substrates under flow. We discovered several critical transitions of the internal and external biofilm architectures that separate the major phases of *V. cholerae* biofilm growth. Optical imaging of biofilms with single-cell resolution provides a new window into biofilm formation that will prove invaluable to understanding the mechanics underlying biofilm development.



Author Disclosure Block:

K. Drescher: None. **J. Dunkel:** None. **C. Nadell:** None. **N. Wingreen:** None. **H. Stone:** None. **B. Bassler:** None.

Session Number:

341

Session Title:

Emergent Behaviors from Bacterial Interactions

Publishing Title:

Interspecies Interactions Between the Predator “*Myxococcus xanthus*” and “*Bacillus*” Species

Author Block:

S. MUELLER¹, S. N. Strack¹, S. E. Ryan¹, C. Hoefle², P. D. Straight², D. B. Kearns³, J. R. Kirby¹; ¹Univ. of Iowa, Iowa City, IA, ²Texas A&M Univ., College Station, TX, ³Indiana Univ., Bloomington, IN

Abstract Body:

Myxococcus xanthus and *Bacillus subtilis* are both soil-dwelling bacteria with the ability to produce an array of specialized metabolites, form highly organized biofilms, and sporulate under nutrient limiting conditions. Both organisms are thought to affect the composition and dynamics of microbial communities within the soil. *M. xanthus* however is known to be a predatory bacterium with a broad prey spectrum under low nutrient conditions. Predation is facilitated through the secretion of an array of lytic factors, specialized metabolites and the coordination of both motility systems (1). We hypothesize that *M. xanthus* is a major player in the top down control of bacterial composition in the environment as it is able to consume a large variety of gram-negative, gram-positive bacteria, phage and even yeast (1). However, the ancestral strain of *B. subtilis*, NCIB3610, resists predation by *M. xanthus*. We identified that *B. subtilis* utilizes the secondary metabolite bacillaene encoded by the *pks* gene cluster to protect itself from predation (1). Long-term predation-stress leads to the formation of megastructures by *B. subtilis*, specific biofilms that contain spores embedded into matrix material to guarantee survival. These megastructures are genetically distinguishable from colony biofilm formation on MSgg (matrix-inducing) medium (2). We wondered if the ability to make megastructures is conserved within the Bacilli species as those are all soil bacteria most likely interacting with the predator *M. xanthus*. To assess this question we conducted predation assays using *B. subtilis*, *B. cereus*, *B. pumilis*, *B. licheniformis* and *B. thuringiensis*. However only *B. cereus* was able to make megastructures similar to *B. subtilis* indicating the core elements required for megastructures are conserved between these two species. (1) Müller, S., Sarah N. Strack, Christopher Hoefler, Paul D. Straight, Daniel B. Kearns and Kirby, J.R. (2014). Bacillaene and Sporulation protects *Bacillus*

subtilis from predation by *Myxococcus xanthus*. *AEM* **80**: 5603-10.(2) **Müller, S.**, Sarah N. Strack, Sarah E. Ryan, Daniel B. Kearns and Kirby, J.R. (2014). Predation by *Myxococcus xanthus* induces *Bacillus subtilis* to form spore-filled megastructures. *AEM* accepted.

Author Disclosure Block:

S. Mueller: None. **S.N. Strack:** None. **S.E. Ryan:** None. **C. Hoefle:** None. **P.D. Straight:** None. **D.B. Kearns:** None. **J.R. Kirby:** None.

Session Number:

341

Session Title:

Emergent Behaviors from Bacterial Interactions

Publishing Title:

Controlling Bacterial Weapons: Biosynthesis and Regulation of Antifungal, Antibacterial and Nematicidal Metabolites in *Serratia*

Author Block:

M. A. Matilla¹, J. E. E. U. Hellberg², G. P. C. Salmond³, T. Krell¹; ¹EEZ-CSIC, Granada, Spain, ²Univ. of Cambridge, Cambridge, United Kingdom, ³Univ. of Cambridge, Cambridge, United Kingdom

Abstract Body:

The extended use of antibiotics and chemical pesticides has driven the emergency of multidrug resistance microorganisms. As a consequence, great efforts are being made to isolate and develop new broad-spectrum antibiotics. Soil bacteria are a promising source of secondary metabolites, which are mainly synthesized by large multifunctional enzymes known as polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs). During the screening of a collection of soil and plant-associated bacteria, we found that one of them, the rhizobacterium, *Serratia plymuthica* A153, showed a broad spectrum of biological activities - including antifungal, anti-nematode, and antibacterial. Using genome sequencing, mutagenesis, chemical analyses and *in vivo* antagonistic and virulence assays, we identified three secondary metabolites as responsible for the observed bioactivities. Firstly, we isolated and characterized for the first time the PKS gene cluster responsible for the biosynthesis of the antifungal and anti-oomycete haterumalide, oocydin A. Secondly, we found that A153 produces the hybrid NRPS/PKS antibacterial acetyl-CoA carboxylase inhibitor, andrimid. Finally, we demonstrated that the observed nematicide properties are due to the production of the PKS/NRPS antibiotic, zeamine. Using the nematode worm, *Caenorhabditis elegans* as a model organism, we showed that early larvae are more sensitive to zeamine. We also investigated the genetic regulation of these three secondary metabolites. Our results showed that the expression of their respective gene clusters is highly controlled at both transcriptional and posttranscriptional levels - highlighting the complexity of the regulatory mechanisms controlling secondary metabolism in this enterobacterium.

Author Disclosure Block:

M.A. Matilla: None. **J.E.E.U. Hellberg:** None. **G.P.C. Salmond:** None. **T. Krell:** None.

Session Number:

341

Session Title:

Emergent Behaviors from Bacterial Interactions

Publishing Title:

Variability in Microbial Community Structure in Replicate Nutrient-Cycling Model Ecosystems

Author Block:

A. Free¹, E. Pagaling², K. Vassileva¹, C. G. Mills¹, T. Bush¹, R. A. Blythe¹, J. Schwarz-Linek¹, F. C. Strathdee¹, R. J. Allen¹; ¹Univ. of Edinburgh, Edinburgh, United Kingdom, ²James Hutton Inst., Aberdeen, United Kingdom

Abstract Body:

Understanding, predicting and controlling the development of complex microbial communities is a key goal of microbial ecology. Such predictive understanding is crucial for effective management of natural ecosystems and maintenance of ecosystem services. Yet it remains unclear under what circumstances microbial community development is inherently predictable, being controlled by deterministic processes, or unpredictable, being controlled by stochastic processes. To address this, we studied in detail the reproducibility of community development in replicate nutrient-cycling microbial microcosms that were allowed to develop under the same environmental conditions. Multiple replicate closed microcosms were constructed using pond sediment and water, enriched with cellulose and sulphate, and allowed to develop over several months under constant environmental conditions, after which their microbial communities were characterised using next-generation sequencing. Our results show that microbial communities can follow alternative - yet stable - trajectories, diverging in time in a system size-dependent manner. The divergence between replicate communities increased in time and decreased with larger system size. In particular, notable differences emerged in the communities of heterotrophic degraders in our microcosms; one group of steady state communities was enriched with *Firmicutes*, while the other was enriched with *Bacteroidetes*. Network analysis of these alternative communities showed that *Anaerolineaceae* were co-selected with the *Firmicutes*, while *Spirochaetes* and *Verrucomicrobia* were co-selected with the *Bacteroidetes*. This bimodality in community composition shows an interesting analogy to observed differences in the human gut microbiome between individuals.

Author Disclosure Block:

A. Free: None. **E. Pagaling:** None. **K. Vassileva:** None. **C.G. Mills:** None. **T. Bush:** None. **R.A. Blythe:** None. **J. Schwarz-Linek:** None. **F.C. Strathdee:** None. **R.J. Allen:** None.

Session Number:

341

Session Title:

Emergent Behaviors from Bacterial Interactions

Publishing Title:

Distinct Colonization Sites Promote *Vibrio fischeri* Strain Diversity During Initial Colonization of the Squid Light Organ

Author Block:

Y. Sun¹, E. D. LaSota¹, A. C. Cecere¹, K. LaPenna¹, J. Larios-Valencia¹, M. S. Wollenberg², **T. Miyashiro¹**; ¹Pennsylvania State Univ., University Park, PA, ²Kalamazoo Coll., Kalamazoo, MI

Abstract Body:

Animal physiology depends on beneficial interactions with horizontally-acquired microbes. The light-organ symbiosis established between the Hawaiian squid *Euprymna scolopes* and the bioluminescent bacterium *Vibrio fischeri* is a model system for examining how hosts acquire horizontally-transmitted microbial symbionts. Recent studies have revealed that the light organ of wild-caught *E. scolopes* squid contains polyclonal populations of *V. fischeri* bacteria; however, how such strain diversity is established within the symbiosis is unknown. Here, we report our investigation into the establishment of strain diversity during light organ colonization using two phylogenetically distinct strains. Strains ES114 and FQ-A001 were isolated from the light organs of adult squid collected from Oahu, HI in 1989 and 2015, respectively. Relative to ES114, FQ-A001 is 2.9-fold slower on motility plates and 36-fold brighter in the presence of the autoinducer molecule that stimulates bioluminescence in *V. fischeri*. To determine the crypt colonization profiles of particular strains, we conducted a series of co-colonization experiments with evenly mixed inocula with two strain types labeled with either YFP and CFP. When ES114 was differentially labeled, the frequencies of crypts singly colonized by YFP or CFP ranged from 25-50% and 40-62% across three trials (100 crypts examined per trial). In addition, 10-13% of the resulting crypts were colonized with mixed populations. When YFP-labeled FQ-A001 cells were combined with CFP-labeled ES114 cells as mixed inocula, we found that the majority of crypts (82-96%) were colonized by FQ-A001, suggesting that FQ-A001 was able to out-compete ES114. Strikingly, among over 100 crypts scored in each of three trials, no co-colonized crypts were observed. Our results suggest that the establishment of FQ-A001/ES114 diversity within the light organ requires each strain to colonize separate crypt spaces.

Furthermore, these findings suggest that mechanisms of strain compatibility within crypts impact the overall strain diversity associated with a squid host. Thus, our study provides insight into how strain diversity can influence the assembly of populations and consortia within microbiota.

Author Disclosure Block:

Y. Sun: None. **E.D. LaSota:** None. **A.C. Cecere:** None. **K. LaPenna:** None. **J. Larios-Valencia:** None. **M.S. Wollenberg:** None. **T. Miyashiro:** None.

Session Number:

344

Session Title:

Every Host Is an Island: Eco-evolutionary Dynamics in Host-associated Microbial Communities

Publishing Title:

Intra-Host Symbiont Genetic Diversity Exhibits Evidence Of Mixed Infections And Recombination

Author Block:

S. L. Russell, C. M. Cavanaugh; Harvard Univ., Cambridge, MA

Abstract Body:

Pairing bacterial physiological diversity with eukaryotic structural complexity has been an enormously successful evolutionary strategy for a wide diversity of taxa. These symbiotic associations range from single bacterial species to bacterial communities living with a eukaryotic host. Even in associations with low taxonomic diversity, intra-host symbiont populations can reach 10^6 to 10^9 symbiont cells. Theory and early work have suggested that these populations maintain low genetic diversity as a result of transmission bottlenecks or to avoid competition between symbiont genotypes. However, despite its importance for understanding symbiont population evolution, the diversity of intra-host symbiont populations remains a long-standing question. To address this, we investigated the chemosynthetic symbiosis between the bivalve *Solemya velum* and its gammaproteobacterial symbionts, for which data exhibits evidence of both vertical and horizontal transmission modes. Genomic DNA from symbiont-containing gill tissue from thirteen individual *S. velum* from five populations along the east coast of N. America was sequenced at high depth (200-1000x) on the Illumina HiSeq platform and mapped to the symbiont reference genome. Alignments were analyzed to calculate pairwise nucleotide diversity across the genome, allele frequency spectra, and linkage between variant sites. While much of the genome sequences were homogeneous, particular regions were found to be polymorphic and correlated in allele frequency. Furthermore, nearby variant sites were consistently found to occur on the same read. Plotting these variant sites among intra-host populations revealed common tracts of polymorphism along the genome that likely originated through recombination events. These data reveal that intra-host symbiont populations contain haplotype blocks, which were probably acquired from prior mixed infections. This diversity is expected to have profound influence on symbiont genome evolution as it provides the opportunity for selection to act within intra-host

populations to maintain fitter symbiont genotypes, limiting the extent of reductive genome evolution commonly seen in obligately intracellular symbionts.

Author Disclosure Block:

S.L. Russell: None. **C.M. Cavanaugh:** None.

Session Number:

344

Session Title:

Every Host Is an Island: Eco-evolutionary Dynamics in Host-associated Microbial Communities

Publishing Title:

Ecological and Evolutionary Drivers of Antibiotic Resistance in Non-Vaccine Type Pneumococcus

Author Block:

C. P. Andam¹, P. Mitchell¹, A. Callendrello¹, Q. Chang¹, J. Corander², L. McGee³, K. Lindsay³, B. Beall³, M. Moore³, W. Hanage¹; ¹Harvard T. H. Chan Sch. of Publ. Hlth., Boston, MA, ²Univ. of Helsinki, Helsinki, Finland, ³CDC, Atlanta, GA

Abstract Body:

A common resident of the upper respiratory tract, the bacterium *Streptococcus pneumoniae* (pneumococcus) is a major cause of pneumonia, bacteremia and meningitis, accounting for high morbidity and mortality worldwide. Recombination and selection play an important role in driving the population dynamics and evolution of different pneumococcal lineages, allowing them to successfully evade the impacts of selective pressures such as vaccination and antibiotic treatment. However, it remains unclear how vaccines, which target only a small subset of the 94 pneumococcal serotypes, affect the evolution of the non-vaccine serotypes that occupy the niche left behind by the vaccine serotypes. We completed whole-genome sequencing of 881 penicillin-resistant, non-vaccine serotype pneumococcal isolates from invasive disease cases across the United States sampled before and after 13-valent conjugate vaccine introduction in 2010. Recombination rates and patterns are highly variable across the population with recombination to mutation parameter per genome ranging from <1-101 and 10-273 recombination events within a single sequence type. At least 10 novel genetic variants have emerged post-vaccine, many of which possess recombinant genomic regions. Evidence of at least nine incidents of serotype switching, where genes coding for a specific capsule are altered or exchanged with other types through recombination, has been identified in the post vaccine sample. We conclude that selective pressures due to vaccination can alter recombination dynamics and the evolution of penicillin resistance of pneumococcal lineages not specifically targeted by the vaccine.

Author Disclosure Block:

C.P. Andam: None. **P. Mitchell:** None. **A. Callendrello:** None. **Q. Chang:** None. **J. Corander:** None. **L. McGee:** None. **K. Lindsay:** None. **B. Beall:** None. **M. Moore:** None. **W. Hanage:** None.

Session Number:

344

Session Title:

Every Host Is an Island: Eco-evolutionary Dynamics in Host-associated Microbial Communities

Publishing Title:

Stochastic and Deterministic Bacterial Community Assembly in a *C. elegans* Host Model

Author Block:

N. Vega¹, A. Ortiz Lopez², J. Gore¹; ¹Massachusetts Inst. of Technology, Cambridge, MA, ²Natl. Autonomous Univ. of Mexico, Mexico City, Del. Coyoacán, Mexico

Abstract Body:

Background: Despite decades of theoretical and observational investigation, the factors directing community assembly in real ecosystems are still poorly understood. The problem is particularly acute in host-associated systems such as the intestinal microbiota. Furthermore, while these communities are known to vary between hosts, the causes and consequences of this heterogeneity are not well understood. **Methods:** Here we use *C. elegans* as a simple host model to determine general rules for community assembly in a biotic environment. Gut-associated communities are assembled *de novo* from a set of bacterial species selected to cover a broad taxonomic range and to include different host interactions, including both probiotics and pathogens of the worm. Worm mutants with and without responsive immune function are used to describe the effects of immunity on bacterial community establishment. Stochastic models and simulations are parameterized from experimental results, and the resulting models are used to illuminate the stochastic and deterministic forces directing the dynamics of these host-associated communities. **Results:** We describe colonization as an interaction between bacterial competition in a spatially structured environment and host effects mediated in part by innate immunity. Migration rate into the intestine is used to move between drift-dominated and deterministic regimes, and the resulting heterogeneity between individual communities is described. **Conclusions:** Our results indicate that host immune response, interspecies competition, and stochastic migration interact non-trivially to determine final community composition in the host environment.

Author Disclosure Block:

N. Vega: None. **A. Ortiz Lopez:** None. **J. Gore:** None.

Session Number:

344

Session Title:

Every Host Is an Island: Eco-evolutionary Dynamics in Host-associated Microbial Communities

Publishing Title:

Systems Analysis of Mutation Impact in Evolving Lung Pathogens

Author Block:

J. A. Bartell, H. K. Johansen, S. Molin; Technical Univ. of Denmark, Hørsholm, Denmark

Abstract Body:

During pathogen evolution in chronic host infections, strains may persist because they converge to a beneficial phenotype, but we have not defined the ideal endpoint phenotype(s) for most infections. Here, we use the chronic lung infections by *Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF) patients as a model of evolution. Using computational modeling, we predict isolate-specific phenotypes and map them to associated systems of mutations, probing functional interrelationships to identify the critical mutations that enable convergence. We have conducted genomic analyses of 457 longitudinal isolates of PA from 34 CF patients (Marvig et al. 2015.). Here, we focus on metabolic mutations and their systemic repercussions. To predict the functional impact of mutations on relevant 'tasks' that may contribute to an ideal CF phenotype, we utilize constraint-based modeling with a genome-scale network model of PA metabolism. Computational estimates of protein dysfunction from isolate-specific mutations are converted into activity constraints of network components using a novel assessment of gene contribution to protein function. The resulting isolate-specific models allow us to evaluate mutation impact on systemic functions. The 457 models were phenotyped by mutation patterns, predicted capacity for growth and production of biofilm and secondary metabolites, and flux variability patterns. Substantial metabolic adaptation occurs in the models of 24 out of 36 clone types. *In silico* growth is reduced in 46% and prevented in a further 11% of isolates. Mutations in the 116 genes essential to growth in *in silico* CF sputum contribute to this growth reduction; 70% of these genes are targeted by a mutation in at least one isolate, but only 2 have nonsense mutations. By iteratively reversing one mutation from within the isolate-specific set of mutations integrated into each model, we identified 8 different genes that contain the single critical mutation impacting growth despite other present mutations in a subset of isolate models. Mutations

identified by these complementary analyses serve as potential biomarkers of specific phenotypes and will guide *in vitro* validation studies. Adding patient treatment history to further phenotypic clustering may link early biomarkers to mutational trajectories that correlate with specific therapeutic outcomes.

Author Disclosure Block:

J.A. Bartell: None. **H.K. Johansen:** None. **S. Molin:** None.

Session Number:

346

Session Title:

Influence of Microbial Products on the Gut Microbiota or Host

Publishing Title:

Bacteroides Secreted Antimicrobial Proteins Mediate Competition in the Mammalian Gut

Author Block:

K. G. Roelofs, R. Gentayala, M. J. Coyne, L. E. Comstock; Brigham and Women's Hosp., Boston, MA

Abstract Body:

The human gut is colonized by a complex microbial community in which bacteria compete for resources and niche occupation. Within this ecosystem, *Bacteroides* spp. are abundant and remarkably stable, with specific strains maintained for years within individuals. Competitive interactions have been proposed to stabilize microbial communities, and we find that many *Bacteroides* secrete antimicrobial proteins called BSAPs. We previously characterized the first described BSAP (BSAP-1) which is produced by *B. fragilis*. BSAP-1 targets a subset of *B. fragilis* strains that lack BSAP-1 and produces transmembrane pores via a membrane attack/perforin (MACPF) domain causing cell death. Here we report the identification of a second MACPF domain protein (BSAP-2) in *B. uniformis* that kills a subset of *B. uniformis* strains lacking BSAP-2. We hypothesized that strain-specific surface molecules serve as receptors for these MACPF BSAPs and determine specificity of killing. Transposon mutagenesis of a BSAP-2 sensitive strain and subsequent phenotypic analyses identified an O-antigen of LPS required for BSAP-2 sensitivity, suggesting that a specific O-antigen serves as the receptor for BSAP-2. Interestingly, BSAP-2 producing strains encode an alternative O-antigen locus directly adjacent to the BSAP-2 gene, suggesting that BSAP-2 and a resistant O-antigen allele were acquired together. We used this insight to predict the receptor of BSAP-1 in *B. fragilis*, and identified a predicted outer membrane protein (OMP) adjacent to the BSAP-1 gene. BSAP-1 sensitive strains encode an alternative OMP allele, and expression of the OMP from a BSAP-1 sensitive strain, but not a BSAP-1 producer, conferred sensitivity to previously resistant *Bacteroides*. Using gnotobiotic mouse competitive colonization assays, we found that BSAP receptors or their orthologous molecules in BSAP producers are necessary for host colonization, explaining why these molecules are replaced upon acquisition of the BSAP encoding gene.

Importantly, we found that BSAP producers outcompete isogenic BSAP sensitive strains in the mammalian gut. In total, these studies reveal the genetic basis of BSAP sensitivity and demonstrate that BSAPs are a potent mechanism of direct competition among gut *Bacteroides* that may enable strain-specific therapeutic remodeling of our gut microbiota.

Author Disclosure Block:

K.G. Roelofs: None. **R. Gentayala:** None. **M.J. Coyne:** None. **L.E. Comstock:** None.

Session Number:

346

Session Title:

Influence of Microbial Products on the Gut Microbiota or Host

Publishing Title:

Antibiotic Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production Allowing for *C. difficile* Colonization

Author Block:

C. M. Theriot, R. Thanissery; NCSU CVM, Raleigh, NC

Abstract Body:

Background: It is hypothesized that the depletion of microbial members responsible for converting primary bile acids into secondary bile acids reduces resistance to *C. difficile* colonization. To date, inhibition of *C. difficile* growth by secondary bile acids has only been shown *in vitro*. **Methods:** Using targeted bile acid metabolomics, we sought to define the physiologically relevant concentrations of primary and secondary bile acids present in the murine small and large intestinal tract and how these impact *C. difficile* dynamics. We treated mice with a variety of antibiotics to create distinct microbial and metabolic (bile acids) environments, and directly tested their ability to support or inhibit *C. difficile* spore germination and outgrowth *ex vivo*. **Results:** Susceptibility to *C. difficile* in the large intestine was observed only after specific broad-spectrum antibiotic treatment (cefoperazone, clindamycin and vancomycin) and was accompanied by a significant loss of secondary bile acids (DCA, LCA, UDCA, HDCA, ω MCA and iLCA). These changes were correlated to the loss of specific microbiota community members, the Lachnospiraceae and Ruminococcaceae families. Additionally, physiological concentrations of secondary bile acids present during *C. difficile* resistance were able to inhibit spore germination and outgrowth of many clinically relevant *C. difficile* strains *in vitro*. **Conclusion:** Interestingly, we observed that *C. difficile* spore germination and outgrowth was supported constantly in the murine small intestine regardless of antibiotic perturbation, suggesting that targeting growth of *C. difficile* will prove most important for future therapeutics and antibiotic-related changes are organ-specific. Understanding how the gut microbiota regulates bile acids throughout the intestine will aid the development of future therapies for *C. difficile* infection and other metabolically relevant disorders such as obesity and diabetes.

Author Disclosure Block:

C.M. Theriot: None. **R. Thanissery:** None.

Session Number:

346

Session Title:

Influence of Microbial Products on the Gut Microbiota or Host

Publishing Title:

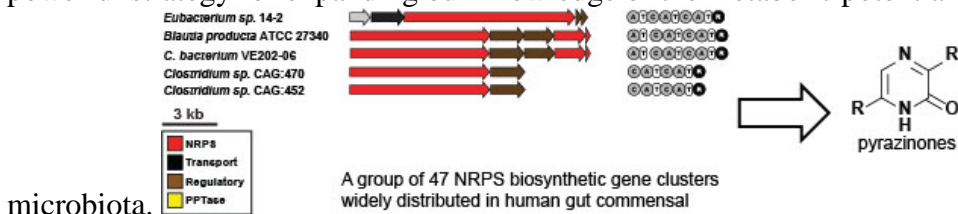
A Family of Nitrogen-containing Heterocycles from Nonribosomal Peptide Synthetases in Gut Bacteria

Author Block:

C. Guo; UCSF, San Francisco, CA

Abstract Body:

Background: The gut microbiota modulate host biology in numerous ways, but little is known about the molecular mediators of these interactions. In a previous systematic analysis of biosynthetic gene clusters from the microbiome, we revealed the presence of a widely distributed family of nonribosomal peptide synthetase clusters from gut bacteria. (Donia et al.) **Methods and Results:** Herein we presented our efforts to systematically characterize this group of NRPSs within this family. We applied various genetic, chemical, and biochemical approaches to decipher these NRP molecules and revealed that NRPSs within this family are capable of synthesizing a total of 32 pyrazinone (PZN) or related natural products, of which 28 are new. An updated bioinformatic analyses suggested that more gut commensals are harboring BGCs in this family. Metatranscriptomic analyses reveal that Clusters from the family are heavily transcribed under native conditions of host colonization **Conclusions:** Our study reveals a large family of microbiota-derived metabolites that accumulate in the host, and it shows that systematically expressing microbiome-derived gene clusters in laboratory hosts is a powerful strategy for expanding our knowledge of the metabolic potential of the



Author Disclosure Block:

C. Guo: None.

Session Number:

346

Session Title:**Influence of Microbial Products on the Gut Microbiota or Host****Publishing Title:****A Chemogenomics Approach to Identify Bacterial Metabolites with Immune-Modulatory Effects****Author Block:**

S. Saha¹, C. K. Jayawickreme¹, J. P. Marino, Jr.¹, A. B. Benowitz¹, D. K. Rajpal, 19406², J. R. Brown¹; ¹GlaxoSmithKline, Collegeville, PA, ²GlaxoSmithKline, King of Prussia, PA

Abstract Body:

Recent metagenomics studies suggest that shifts in the gastrointestinal tract microbiome are associated with human disease incidences. To better understand the microbial basis of several gut-related immune-inflammatory disorders, we focused on metabolites secreted by the microbiome which are potential ligands of human host receptors. From a drug discovery perspective, microbial metabolites or their mimics can be explored as a novel drug class because of their high affinity and selectivity for human receptors. Being endogenous in origin, they also presumably mitigate safety and tolerance issues of drugs in human system. Despite progress in the field, few specific metabolite-human ligand interactions are known and there are lack of systematic screens. Here we discuss the identification of potentially bioactive bacterial metabolites using a combination of computational chemistry, bioinformatics and functional screening using *in vitro* assays representing different disease phenotypes. We searched GlaxoSmithKline's (GSK) pharmaceutical collection of ~4.5 million compounds for structural homologs of 10 known bacterial metabolites as the starting point. Significant biological activities associated with these analogs were systematically mined against human receptors from historical in-house assays. Over 421 compounds were found with a significantly high similarity to the parent metabolites. For these compounds, 101 putative human protein target associations were found in historical GSK assays. The metabolite-mimics revealed novel mechanistic insight for microbial metabolites and show measurable activities against plethora of human targets including G-coupled protein receptors (GPCRs), transporters, kinases, hormone receptors and cytokines. A subset of these metabolites and their corresponding structural analogs were then tested in 12 different human primary cell assay systems which mimic various disease phenotypes (BioMAP^R assay systems,

DiscoverX). From these screens, several novel immune-modulatory compounds were identified which warrant further investigation.

Author Disclosure Block:

S. Saha: None. **C.K. Jayawickreme:** None. **J.P. Marino:** None. **A.B. Benowitz:** None. **D.K. Rajpal:** None. **J.R. Brown:** None.

Session Number:

347

Session Title:

Microbial Mind Control

Publishing Title:

Gaba Modulating Bacteria of the Human Gut Microbiome

Author Block:

P. Strandwitz¹, **K. Kim**², **D. Dietrich**², **D. McDonald**³, **T. Ramadhar**², **E. J. Stewart**¹, **R. Knight**³, **J. Clardy**², **K. Lewis**¹; ¹Northeastern Univ., Boston, MA, ²Harvard Med. Sch., Boston, MA, ³Univ. of California, San Diego, La Jolla, CA

Abstract Body:

The gut microbiome affects many different diseases, and has been recently linked to human mental health. The microbiome community is diverse, but 50-80% of its diversity remains uncultured. We previously reported that uncultured bacteria from the marine environment require growth factors from neighboring species, and by using co-culture, we could cultivate novel diversity. In the present study, we used a similar co-culture approach to grow bacteria from humans stool samples. KLE1738, a “Most-Wanted” member of the human gut microbiome only known by its 16S rDNA signature, was found to require the presence of *Bacteroides fragilis* KLE1758 for growth. Using bio-assay driven purification of *B. fragilis* KLE1758 supernatant, γ -aminobutyric acid (GABA), the major inhibitory neurotransmitter of the central nervous system, was identified as the growth factor for KLE1738. We found no other tested compound but GABA supported the growth of KLE1738, and genomic analysis suggests an unusual metabolism focused on consuming GABA. Due to this unique growth requirement, we provisionally name KLE1738 *Eytepia gabavorous*. Using growth of *E. gabalyticus* as an indicator, we then identified novel GABA producing bacteria from the gut microbiome. Reduced levels of GABA are associated with depression, and we found fewer GABA producers in a human cohort of depressed individuals. By modulating the level of GABA, microbial producers and consumers of this neurotransmitter may be influencing host behavior.

Author Disclosure Block:

P. Strandwitz: None. **K. Kim:** None. **D. Dietrich:** None. **D. McDonald:** None. **T. Ramadhar:** None. **E.J. Stewart:** None. **R. Knight:** None. **J. Clardy:** None. **K. Lewis:** None.

Session Number:

347

Session Title:

Microbial Mind Control

Publishing Title:

Free Fungi Vs Trojan Horse: Breaching The Blood-Brain Barrier In Cryptococcal Pathogenesis

Author Block:

F. H. Santiago-Tirado, M. D. Onken, R. S. Klein, J. A. Cooper, T. L. Doering;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract Body:

Of all the infections of the central nervous system (CNS), the ones caused by fungi have the highest morbidity and mortality worldwide, responsible for about 1.5 million deaths each year (Brown GD *et al.*, 2012). Of these, more than one-third are caused by *Cryptococcus neoformans* (*Cn*), an encapsulated yeast that causes lethal meningitis (Park BJ *et al.*, 2009). Several mechanisms by which *Cn* crosses the blood-brain barrier (BBB) have been proposed, but they have never been compared under similar conditions and the actual mechanism(s) used *in vivo* remains unclear. It has been reported that *Cn* can cross the BBB transcellularly or paracellularly, as free cells or inside phagocytes acting as ‘Trojan horses’, although evidence for the latter is indirect (Tseng HK *et al.*, 2015). Here we sought to fill these gaps in knowledge by directly comparing the transmigration efficiency of free fungi and fungi-loaded phagocytes, and to visualize Trojan horse crossing *in vitro*. We used a flow cytometry strategy to isolate human phagocytes containing single internalized yeast cells with no yeast adherent to the outer surface of the host cell. We assayed this population and the same number of free *Cn* in a human-derived BBB *in vitro* model and found that all transit occurred without disruption of the barrier, consistent with a transcellular pathway. Under normal conditions, free fungi crossed more efficiently than fungi traveling within host cells, although Trojan horse transit still contributed significantly to overall transmigration. Importantly, while the presence of cytokines and chemokines did not influence the transit of free fungi, they significantly stimulated Trojan horse transit such that the two modes reached comparable levels. We also visually confirmed that *Cn* remains inside the host cell while it traverses the BBB. Finally, our imaging analysis provided evidence for additional interactions between infected phagocytes and the brain endothelia that might contribute to brain infection. Our work will allow further dissection of the mechanisms used by this versatile pathogen to invade the CNS, and offers experimental approaches that can be applied to other neuropathogens.

Author Disclosure Block:

F.H. Santiago-Tirado: None. **M.D. Onken:** None. **R.S. Klein:** None. **J.A. Cooper:** None. **T.L. Doering:** None.

Session Number:

347

Session Title:

Microbial Mind Control

Publishing Title:

Effects of Intermittent Fasting on the Gut Microbiome in Multiple Sclerosis

Author Block:

y. zhou¹, F. Cignarella², C. Cantoni³, Y. Dorsett¹, G. Weinstock¹, L. Piccio⁴; ¹The Jackson Lab. for Genomic Med., Farmington, CT, ²Washington Univ. in St. Louis, st louis, MO, ³Washington Univ. in St. Louis, st louis, CT, ⁴Washington Univ. in St. Louis, St. Louis, MO

Abstract Body:

BACKGROUND: Multiple sclerosis (MS) is a chronic autoimmune disease, driven by autoimmune injury to myelin sheaths of the white matter of the brain and spinal cord. Causes of MS have traditionally been postulated to be a combination of genetic predisposition and environmental factors, including diet, pathogens, and diminished sunlight exposure. We have previously shown that a regimen of calorie restriction (CR) is able to ameliorate experimental autoimmune encephalomyelitis (EAE), the main MS animal model. However, the exact mechanism by which CR affects the EAE course is unknown. A growing body of evidence has suggested that the microbiome, as an environmental factor, is strongly influenced by diet. Gut microbiome also plays an important role in regulating host immunity. Here, we aim to test the effects of intermittent fasting (IF) on clinical course, immune responses and gut microbiome during EAE. **METHODS:** C57BL/6 mice were kept on a regimen of IF or ad libitum (controls) for a month prior to immunization with MOG₃₅₋₅₅ and during EAE. Immune cell number and function in peripheral and gut associated lymphoid organs were analyzed by flow cytometry. Stool microbiome was analyzed by 16S rRNA gene sequencing. Fecal transplant from IF to control group was performed to test the protective role of microbiome against EAE. **RESULTS:** The IF group displayed a less severe EAE clinical course with a reduction of inflammatory infiltrates and demyelination in the spinal cord compared to controls. Production of IL17, IFN γ and GM-CSF by T cells in the draining lymph nodes of the site of immunization was attenuated in IF mice. IF was also associated with an increased number of regulatory T cells in mesenteric lymph nodes, changes in the proportion of T cell subsets in the gut lamina propria. IF significantly altered the diversity and the composition of gut microbiome. Potentially protective

bacteria in EAE model were identified.CONCLUSIONS: This study demonstrates that IF can ameliorate EAE clinical course by modulating immune response, affecting the composition of immune cells in gut associated lymphoid tissue and drastically changing the gut microbiome.

Author Disclosure Block:

Y. zhou: None. **F. Cignarella:** None. **C. Cantoni:** None. **Y. Dorsett:** None. **G. Weinstock:** None. **L. Piccio:** None.

Session Number:

347

Session Title:

Microbial Mind Control

Publishing Title:

Characterization of Microbial Communities in Schizophrenia and Mood Disorders

Author Block:

D. Simone¹, E. Castro-Nallar², M. L. Bendall¹, M. Pérez-Losada¹, R. H. Yolken³, K. A. Crandall¹; ¹George Washington Univ., Ashburn, VA, ²Univ. Andrés Bello, Santiago, Chile, ³Johns Hopkins Sch. of Med., Baltimore, MD

Abstract Body:

Background: Mental and neurological disorders like mood disorders (MD) and schizophrenia constitute a worldwide health issue. Standard treatments often provide inadequate responses. Among factors involved in resistance to psychotropic drugs, microbiota has an underestimated influence on host behavior and in drug metabolism, as suggested by previous findings in humans, mainly based on inflammatory bowel disorders, which are associated with microbiota dysbiosis and are highly comorbid with psychiatric disorders. In this study we aim to characterize the gut microbiome of schizophrenia and MD patients. **Methods:** Gut microbiome from 30 individuals (8 controls, 7 MD patients, 15 schizophrenia patients) was surveyed with shotgun metagenomic sequencing. Exploratory and differential species abundance analyses were performed on read datasets. **Results:** Differential taxon abundance analysis revealed that samples did not differ at the phylum level; genera *Lactobacillus* (\log_2 fold change [LFC] = 8) and *Peptoclostridium* (LFC = 4) were more abundant in schizophrenia patients than in controls. In MD patients, genera *Lactobacillus* and *Bifidobacterium* were relatively more abundant (LFC > 2), while genera *Streptococcus* and *Enterococcus* showed a decreased abundance (LFC < -1). Differentially abundant taxa are involved in metabolism of key neurotransmitters (GABA, serotonin) and brain development at different levels. Alpha-diversity measures showed a reduced species richness in schizophrenia and MD patients, further suggesting a link between the explored diseases and the microbial communities inhabiting the gut. **Conclusion:** Our findings show that gut microbiota is different in schizophrenia and MD patients compared to controls, suggesting that microbiota profiling can constitute a valuable tool for the management and the treatment of psychiatric disorders. Further studies will focus on the metabolic features of microbial communities in different diagnoses.

Author Disclosure Block:

D. Simone: None. **E. Castro-Nallar:** None. **M.L. Bendall:** None. **M. Pérez-Losada:** None. **R.H. Yolken:** None. **K.A. Crandall:** None.

Session Number:

349

Session Title:

New Approaches to Microbial Cultivation: A Growth Industry

Publishing Title:

Unraveling Community Assembly and Organism Interactions with Massively Parallel Enrichment Culturing

Author Block:

N. B. Justice¹, A. Sczesnak², T. Hazen³, A. Arkin¹; ¹Lawrence Berkeley Natl. Lab, Berkeley, CA, ²Univ. of California, Berkeley, Berkeley, CA, ³Univ. of Tennessee, Knoxville, TN

Abstract Body:

Bacterial population structures are central to explaining microbial ecosystem function and properties. However, the ecological forces that shape community structures—including species interactions—are myriad and complex, leaving gaps in our ability to understand and predict microbial community structure and functioning. Here we examine microbial community assembly, uncover species interactions, and examine the influence of abiotic factors in microbial community structure by systematically varying the number of organisms founding each of ~1,000 enrichment cultures started from a single groundwater inoculum. We inoculated the groundwater (containing ~37,000 cells mL⁻¹) into both aerobic and anaerobic nitrate-reducing cultures that spanned five dilutions (10⁻¹-10⁻⁵) and, following incubation, community structures were evaluated with 16S gene amplicon sequencing. As expected, species richness decreased with increasing inoculum dilution as low abundance individuals were removed. Aerobic and anaerobic communities varied in community composition and taxonomic membership, especially at high inoculum concentrations. Using a most probable number technique, we estimated the community structure (as cultivable units/mL of each taxon) of the initial sample in aerobic and anaerobic enrichment conditions, and approximated ~5-7% of cells from the initial inoculum were cultured. The initial estimated abundances of each OTU were used to develop a null model of community assembly which, compared to measured data, was used to estimate the relative fitness of each organism within each condition. In general, major differences in community composition were driven by only a few taxa. Finally, we calculated co-occurrence probabilities for abundant taxa to infer putative positive or negative interspecific interactions amongst organisms. Nearly twice as many interactions were detected in anaerobic samples as aerobic samples, with many of the negative

interactions pointing to antagonistic relationships between species of the Bacillaceae with species of Oxalobacteraceae, Paneibacillaceae, and Pseudomonadaceae. Together, this novel approach allows us to show how abiotic and biotic factors interact to structure microbial communities.

Author Disclosure Block:

N.B. Justice: None. **A. Sczesnak:** None. **T. Hazen:** None. **A. Arkin:** None.

Session Number:

349

Session Title:

New Approaches to Microbial Cultivation: A Growth Industry

Publishing Title:

Top-down Design of a Microbial Consortia by Systematically Exploring the Community Structure-function Landscape

Author Block:

M. Osborne¹, J. F. Poyatos², A. Sanchez¹; ¹Rowland Inst. at Harvard, Cambridge, MA, ²CNB, Madrid, Spain

Abstract Body:

There is growing interest in engineering microbial consortia for biotechnology and health applications. Most approaches have followed a synthetic “bottom-up” strategy, where the function of the community is modularly distributed amongst the different species. An implicit assumption of this bottom-up approach is that the contribution of a microbe to the function of the consortium is not affected by its ecological context. However, complex ecological interactions within a multispecies community may easily lead to behavior that differs from that observed in monoculture. In addition, non-trivial functional interactions are also possible, making the relationship between a community structure and its function a complex one. Here we propose that by systematically exploring this “Structure-Function Landscape”, we can find optimally functional and ecologically stable communities whose functions are larger than one would have predicted naively by assuming additivity between their component species. To demonstrate this idea, we have mapped out the space of all of the possible consortia that can be formed with four different *Bacillus* species that are often used for the industrial production of α -amylase. Species were grown in every possible combination from a fixed initial cell density, and the hydrolytic activity of their combined secreted enzymes was determined. Additionally, species were grown in monoculture and their enzymes were combinatorially combined and their activity then measured. Despite the apparent simplicity of this biochemical network, consisting of a small, redundant set of enzymes acting via first order Michaelis-Menten kinetics, our experiment reveals complex functional interactions that exceed predictions from simple models of independent enzymatic activity. Ecological interactions increase this activity even further. We then explored this Structure-Function Landscape using a genetic algorithm at the level of whole communities, and found a maximally functional, ecologically stable consortium.

Our results indicate that a top-down approach based on the exploration of ecological landscapes may greatly aid in the design of ecologically robust and functionally optimal microbial consortia.

Author Disclosure Block:

M. Osborne: None. **J.F. Poyatos:** None. **A. Sanchez:** None.

Session Number:

349

Session Title:

New Approaches to Microbial Cultivation: A Growth Industry

Publishing Title:

Growth Factors for Uncultured Bacteria

Author Block:

M. N. I. Bhuiyan¹, R. Takai², S. Mitsuhashi², K. Shigetomi², Y. Tanaka³, Y. Kamagata², M. Ubukata²; ¹Bangladesh Council of Scientific and Industrial Res., Chittagong, Bangladesh, ²Hokkaido Univ., Sapporo, Japan, ³Univ. of Yamanashi, Kofu, Japan

Abstract Body:

Background: The number of existing microbial species is estimated at 100000 to 1000000 but only several thousand have been isolated in pure culture. Uncultured microorganisms might grow in pure culture if provided with the growth factors of their natural environment. Research is proposed to identify specific growth factor from *Sphingopyxis* sp (GF9) to enhance vigorous growth of previously uncultured bacterial strain ASN212 in laboratory conditions. **Methods:** Production of growth factors for strain ASN212 was achieved by a 72-h culture of strain GF9 in a 100 L stirred tank bioreactor (three batches). The active MeOH soluble fraction obtained from 210 l of culture broth was subjected to Diaion HP20 column chromatography to give a MeOH eluting fraction, which induced growth of strain ASN212 at equivalent concentration (EC). Subsequent preparative ODS HPLC gave six different growth factors **A-F**. **Results:** The growth of strain ASN212 related to *Leucobacter* sp. was stimulated by the supernatant of strain GF9. In this study, novel porphyrin type growth factors produced by strain GF9 were identified to induce vigorous proliferation of a previously uncultured bacterial strain ASN212 at pico- to nanomolar levels. Even more surprisingly, the growth factors showed self-toxicities against the growth factor producing bacterium, strain GF9 itself at the concentrations of pico- to nanomolar levels. Six different growth factors **A-F** were identified as zinc coproporphyrin I, coproporphyrin I, zincphyrin, coproporphyrin III, zincmethylphyrin I, zincmethylphyrin III. The growth-stimulating activity of zincphyrin (**C**) was most evident with a MEC value of 14 pM. A panel of commercial and synthetic porphyrins was tested to explore the generality of porphyrin as growth factor for strain ASN212, while coproporphyrin I dihydrochloride, coproporphyrin III dihydrochloride, coproporphyrin III tetramethyl ester, hemin and hematin showed significant growth stimulation of strain ASN212. **Conclusion:** This research has broken new ground by

demonstrating that coproporphyrins produced by strain GF9 or commercially available porphyrins could have an impact on the growth of previously uncultured bacterial strain in laboratory conditions.

Author Disclosure Block:

M.N.I. Bhuiyan: None. **R. Takai:** None. **S. Mitsuhashi:** None. **K. Shigetomi:** None. **Y. Tanaka:** None. **Y. Kamagata:** None. **M. Ubukata:** None.

Session Number:

349

Session Title:

New Approaches to Microbial Cultivation: A Growth Industry

Publishing Title:

Analysis of the Growth Rate of *Lactobacillus crispatus* and *Gardnerella vaginalis* in a Vaginal Epithelial Cell Co-Culture System

Author Block:

E. Mawel, K. K. Jefferson, A. Amr Abdell Maksoud; Virginia Commonwealth Univ., Richmond, VA

Abstract Body:

Background: Bacterial vaginosis, (BV) is a condition that occurs due to a disturbance in the vaginal ecosystem which results in an overgrowth of vaginal microbiota. The vaginal microbiome can be composed of beneficial bacteria such as *Lactobacillus* species that contribute to health or potentially harmful bacteria perhaps of *Gardnerella vaginalis*. Healthy vaginal lactobacilli can inhibit the growth of harmful bacteria through the production of lactic acid and bacteriocins. However, even women who are colonized by healthy lactobacilli, can sometimes develop BV, and some women experience recurrent BV every month concurrent with the initiation of menses (1). We hypothesized that condition in the vagina during menses favor the growth of BV-associated bacteria. We also hypothesized that certain strains of *lactobacillus crispatus* are more capable of coping with changing vaginal conditions and thus suppress the growth of BV- associated species, even during menses, whereas other strains of *L. crispatus* are less capable of coping with these changes, leading to the development of BV.**Methods:** We created two different co-culture systems that stimulate the vaginal environment during the follicular phase (mid-cycle) and during menstruation phase as closely as possible. Our experimental approach consisted of co-culturing a vaginal keratinocyte cell monolayer and a bacterial strain in media that contains human serum, mucin, glycogen, estrogen, lactic acid, and other components of vaginal fluid. In the follicular phase system, glycogen production by the vaginal epithelial cell line is stimulated by estrogen similar to a real vaginal epithelium and the pH of the media is reduced (2). Media for the menstruation system is adjusted to a neutral pH, is devoid of estrogen, and contains human blood (2).**Results:** In the follicular phase, unlimited colonies count were recorded for *L. crispatus* than *G. vaginalis* when isolated individually and together. However, in the menstruation phase, few colonies counts were recorded for *L. crispatus* while an

increase amount of growth was recorded for *G. vaginalis*. **Conclusions:** Our first objective is to shed light on the development of recurrent BV by analyzing the rate of growth of the two species alone and in competition under the two sets of conditions.

Author Disclosure Block:

E. Mawel: None. **K.K. Jefferson:** None. **A. Amr Abdell Maksoud:** None.

Session Number:

357

Session Title:

Sucking It up and Squirting It out: Novel Findings in Bacterial Secretion Systems

Publishing Title:

Antagonistic Interactions between Different Bacterial Species in the Small Intestine - Role of Type VI Secretion

Author Block:

W. Zhao; Harvard Med. Sch., Boston, MA

Abstract Body:

Vibrio cholerae is the causative agent of the severe diarrheal disease cholera. The type VI secretion system (T6SS), which is evolutionarily related to contractile bacteriophage tails, has been shown to be a conserved protein delivery machine encoded within the genomes of many Gram-negative bacteria. The *V. cholerae* T6SS is able to translocate toxic effectors into both eukaryotic and prokaryotic cells. Previous work from our lab has demonstrated that *V. cholerae* T6SS is actively expressed in infant rabbit model and was able to attack sister-cells that lacked effector immunity; however, interspecies interaction mediated by *V. cholerae* T6SS in vivo has not been well studied. Here we investigated whether the well-established infant mouse model of cholera can be used to observe T6SS killing activity of *V. cholerae* against commensal bacterial species. Gram-negative commensal bacteria from infant mice small intestines (SI) were isolated and their susceptibility against *V. cholerae* T6SS were determined. All of isolates from mice SI were sensitive to *V. cholerae* T6SS. Co-inoculation of two *Escherichia* isolates resulted in more than 2 days stable colonization in SI of mice. Challenge of *V. cholerae* following *Escherichia*-inoculation revealed deficient colonization ability of *V. cholerae* T6SS mutant compared to wild type strain. Furthermore, *Escherichia* strains could not be detected when mice were challenged with wild type *V. cholerae* after 18hr post inoculation; while *Escherichia* isolates remained $\sim 10^5$ - 10^6 CFU/SI when followed by *V. cholerae* T6SS mutant challenge throughout the entire experiment (24hr). Collectively, our data show that interspecies interactions mediated by *V. cholerae* T6SS occur during infection and that these might influence the virulence and evolution of the cholera vibrio.

Author Disclosure Block:

W. Zhao: None.

Session Number:

357

Session Title:

Sucking It up and Squirting It out: Novel Findings in Bacterial Secretion Systems

Publishing Title:

Contact-Dependent Interbacterial Signal Transduction Coordinates Competitive and Cooperative Behavior

Author Block:

E. C. Garcia, S. A. Marlatt, P. A. Cotter; Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract Body:

In prokaryotes and eukaryotes, cell-cell communication and recognition of self are critical to coordinate multicellular functions. While kin and kind discrimination are increasingly appreciated to shape naturally occurring microbe populations, the underlying mechanisms that govern these interbacterial interactions are insufficiently understood. Here we identify a previously unknown mechanism of interbacterial signal transduction that is mediated by contact-dependent growth inhibition (CDI) system proteins. CDI systems are composed of proteins secreted to the bacterial surface via type V secretion and have been characterized by their ability to mediate interbacterial competition. These systems deliver a polymorphic protein toxin into the cytoplasm of a neighboring bacterium, resulting in growth inhibition or death unless the recipient bacterium produces a corresponding immunity protein. Using the model organism *Burkholderia thailandensis* E264 together with RNA-seq and gene expression reporters, we show that delivery of a catalytically active CDI system toxin to immune (self) bacteria results not only in protection from growth inhibition, but also in gene expression and phenotypic changes within the recipient cells. Termed contact-dependent signaling (CDS), this signal transduction pathway promoted biofilm formation and other community-associated traits. Moreover, engineered strains that are isogenic with *B. thailandensis* except within the DNA region encoding the toxin and immunity proteins were unable to cause CDS in *B. thailandensis*, while the human pathogen *Burkholderia dolosa* producing a nearly identical CDI system did induce CDS in *B. thailandensis*. These data indicate that CDI system proteins facilitate both interbacterial antagonism and cooperation, decreasing the fitness of non-self bacteria (via CDI) and increasing the fitness of self bacteria (via CDS), with self being defined by the CDI system allele and not by genealogic relatedness.

Author Disclosure Block:

E.C. Garcia: None. **S.A. Marlatt:** None. **P.A. Cotter:** None.

Session Number:

357

Session Title:

Sucking It up and Squirting It out: Novel Findings in Bacterial Secretion Systems

Publishing Title:

Exploring the Role of SipD in *Salmonella typhimurium* Type III Secretion

Author Block:

H. Wong, A. Azam, D. Tullman-Ercek; Univ. of California at Berkeley, Berkeley, CA

Abstract Body:

Type III secretion systems (T3SS) are complex macromolecular machines utilized by Gram-negative bacteria to facilitate microbe-host interactions by translocating effector proteins into the host cells. Our lab makes use of the SPI-1 T3SS in *Salmonella typhimurium* to secrete proteins of commercial and biotechnological relevance. Bacterial fermentations are fast and cheap, but the current state-of-the-art does not include a secretion strategy, limiting their utility for proteins such as antibody fragments. The *Salmonella* SPI-1 is an attractive candidate for such a secretion platform because it is not essential and therefore can be manipulated and used without affecting cell viability. One of the hurdles for biotechnological applications is the lack of understanding regarding the signal that initiates secretion. With this presentation, we will describe our findings that SipD, the needle capping protein, influences this process via a previously unknown bifunctional regulatory role in addition to its structural role. The intracellular expression of *sipD* inhibits the secretion of heterologous proteins. Conversely, the exogenous addition of SipD increases secretion of both heterologous and native proteins in T3SS-induced cultures. Changes in secretion phenotype were observed using coomassie blue stained SDS gels and semi-quantitative western blotting. Intriguingly, exogenously added SipD also results in changes the temporal expression patterns of SPI-1. Furthermore, each role can be attributed to different structural domains of SipD. We hypothesize that extracellular SipD interacts with components of the cellular envelope and drives SPI-1 secretion by modifying the stability of SPI-1 RNA transcripts and changing the *S. typhimurium* transcriptome. Capitalizing on the SipD effects enables us to secrete >400 mg/L of protein, a 1000-fold improvement that is within reach of the 1 g/L required for commercialization.

Author Disclosure Block:

H. Wong: None. **A. Azam:** None. **D. Tullman-Ercek:** None.

Session Number:

357

Session Title:

Sucking It up and Squirting It out: Novel Findings in Bacterial Secretion Systems

Publishing Title:

Adaptation Of The Type Vii Secretion System In Mycobacteria

Author Block:

T. D. Mortimer, A. M. Weber, C. S. Pepperell; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Introduction:The Type VII secretion system (T7SS), also called ESX, has been shown to secrete important virulence factors in *Mycobacterium tuberculosis*. ESX loci form a paralagous group, members of which are shared among many species of Actinobacteria. Previous studies of ESX loci in *M. tuberculosis* suggest that they are under several distinct forms of selection. Broader patterns of diversity at these loci have not been characterized.**Methods:**Analyses of gene content and organization among Actinobacterial ESX loci, phylogenetic inference, and characterization of selective influences at these loci.**Results:**Our analyses of gene content were in agreement with previous studies suggesting that ESX-4 emerged first, followed by ESX-1, ESX-3, and finally ESX-2 & 5. We identified a novel ESX-1 locus in two species of *Nocardia*, which appears to have been laterally transferred. There was evidence of directional selection at each gene duplication event, suggesting a specific adaptive role for each of these loci. Gene content and order was conserved across bacterial species, except at the ESX-1 locus. Our analyses suggest that this is due to niche adaptation, rather than relaxed purifying selection.**Conclusion:**Patterns of diversity at ESX loci suggest that they diverged functionally following gene duplication events. With the exception of ESX-1, these loci are highly conserved across a wide range of Actinobacterial species. ESX-1, which is known to be involved in *M. tuberculosis* virulence, appears to play a role in niche adaptation for a range of Mycobacterial species.

Author Disclosure Block:

T.D. Mortimer: None. **A.M. Weber:** None. **C.S. Pepperell:** None.