nonsusceptible. The daptomycin non-susceptible isolate demonstrated regrowth by 72 hours of simulated treatment with vancomycin (2 g Q12H) or daptomycin (10 mg/kg daily). Adding cefazolin (2 g Q8H) to vancomycin or daptomycin prevented regrowth at 72 hours. The daptomycin-resistant isolate was deficient in hemolysin production suggesting *agr* dysfunction. Comparative sequencing identified daptomycin-resistant isolate mutations in *mprF, purR* and *agrA*.

Conclusion. This case underscores the complex dynamics of the emergence of S. aureus resistance to daptomycin *in vivo*. Our pharmacokinetic modeling supports combination therapy in the treatment of endovascular MRSA infection. Reduced hemolytic activity supports the hypothesis that *agr* modulation is associated with persistent infection and/or treatment failure. Ongoing studies will identify features of distinct bacterial populations that promote ecological succession during infection at a sequestered anatomical site.

Disclosures. **All authors:** No reported disclosures.

597. Cross-resistance of Ceftolozane-Tazobactam and Imipenem-Relebactam Against Clinical *P. aeruginosa* Isolates: SMART United States 2016–2018

Sibylle Lob, PhD[1]; Krystyna Kazmierczak, PhD[1];
Daryl DePestel, PharmD, BCPS-ID [2]; Janet Raddatz, PharmD[2];
Katherine Young, MS[2]; Mary Motyl, PhD[3] and Daniel F. Sahm, PhD[1]; [1]IHMA, Inc., Schaumburg, Illinois; [2]Merck & Co, Inc., Kenilworth, New Jersey; [3]Merck & Co., Inc., Kenilworth, New Jersey

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. Ceftolozane-tazobactam (C/T) is an antipseudomonal cephalosporin combined with a β-lactamase inhibitor. The combination was cleared by FDA and EMA and is approved in the United States and over 60 countries worldwide. Relebactam (REL) is an inhibitor of class A and C β-lactamases that is in clinical development in combination with imipenem (IMI). Using clinical isolates collected in the United States as part of the global SMART surveillance program, we compared the activity of C/T and IMI/REL against *P. aeruginosa* (PA) isolates.

Methods. In 2016–2018, 29 clinical laboratories from the United States collected up to 250 consecutive, aerobic or facultatively anaerobic, gram-negative pathogens (GNP) from blood, intra-abdominal, urinary, and lower respiratory tract infections. A total of 14,606 GNP were collected, of which 2,774 were PA. MICs were determined using CLSI broth microdilution and interpreted with CLSI 2019 breakpoints; IMI breakpoints were used for IMI/REL.

Results. The activity of C/T and IMI/REL against 2,774 PA is shown (table). Among all PA, 1.8% of isolates were nonsusceptible (NS) to both agents; 4.4% were susceptible (S) to C/T but not to IMI/REL, and 2.9% were susceptible to IMI/REL but not to C/T. Among the subset of isolates collected from patients in ICUs (*n* = 827), 87.3% were susceptible to both C/T and IMI/REL, 2.7% were nonsusceptible to both agents, 5.8% of isolates were susceptible only to C/T, and 4.2% of isolates were susceptible only to IMI/REL. Among all C/T-NS isolates (all patient locations, *n* = 132), 61.4% were IMI/REL-S and <30% were susceptible to all other studied β-lactams and fluoroquinolones. Among all IMI/REL-NS isolates (*n* = 173), 70.5% were C/T-S and <36% were susceptible to all other studied β-lactams and fluoroquinolones. Of the tested agents, only amikacin and colistin exceeded the activity of C/T or IMI/REL against these NS subsets.

Conclusion. Resistance to C/T or IMI/REL was not common among recent clinical isolates of PA collected in the United States, and both agents promise to be important treatment options. A significant proportion of isolates nonsusceptible to one agent was susceptible to the other, especially among isolates from patients in ICUs. The data suggest that susceptibility to both agents should be tested at hospitals.

		IMI/REL		
		Susceptible	Intermediate	Resistant
C/T	Susceptible	2520 (90.8%)	77 (2.8%)	45 (1.6%)
	Intermediate	40 (1.4%)	7 (0.3%)	9 (0.3%)
	Resistant	41 (1.5%)	12 (0.4%)	23 (0.8%)

Disclosures. **All authors:** No reported disclosures.

598. *In Vitro* Activity of Aztreonam in Combination with Ceftazidime–Avibactam, Amoxicillin–Clavulanate, and Piperacillin–Tazobactam vs. NDM-Producing *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates

Andrew Walkty, MD and James Karlowsky, PhD; Shared Health, Winnipeg, MB, Canada

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. There are limited options available for the treatment of infections caused by *Enterobacteriaceae* that produce an NDM metallo-β-lactamase. The purpose of this study was to compare the *in vitro* activity of aztreonam in combination with three different β-lactam/β-lactamase inhibitors (ceftazidime–avibactam, amoxicillin-clavulanate, piperacillin–tazobactam) vs. NDM-positive *Enterobacteriaceae* clinical isolates.

Methods. Seven *Escherichia coli* and three *Klebsiella pneumoniae* clinical isolates (all NDM-positive by PCR) were included in this study. The *in vitro* activities of ceftazidime–avibactam, amoxicillin-clavulanate, piperacillin–tazobactam, and aztreonam were determined by disk diffusion as described by CLSI. For synergy testing, disks containing a β-lactamase inhibitor (ceftazidime–avibactam, amoxicillin-clavulanate, piperacillin tazobactam) were applied to Mueller–Hinton agar plates inoculated with the test organisms, and the plates were incubated for 1 hour. The disks were then removed and aztreonam disks were dropped on the previous disk sites. The plates were then incubated as per standard CLSI recommendations for disk diffusion testing.

Results. All ten isolates demonstrated phenotypic resistance to aztreonam, amoxicillin-clavulanate, and piperacillin–tazobactam, and eight were resistant to ceftazidime–avibactam (CLSI breakpoints). The zone diameter observed for aztreonam in combination with ceftazidime–avibactam was greater than for either antimicrobial on its own for nine isolates. Seven isolates (70%) had susceptibility to aztreonam restored (zone diameter ≥21 mm) in the presence of avibactam. Aztreonam in combination with amoxicillin-clavulanate demonstrated in increase in zone diameter for all isolates relative to the zone for each antimicrobial alone, but only two (20%) had aztreonam susceptibility restored. Aztreonam susceptibility was not restored for any of the isolates in combination with piperacillin–tazobactam.

Conclusion. Of the three β-lactam/β-lactamase inhibitor-aztreonam combinations evaluated, ceftazidime–avibactam plus aztreonam demonstrated the greatest *in vitro* activity vs. NDM-producing *Enterobacteriaceae*.

Disclosures. **All authors:** No reported disclosures.

599. LiaF is an Activator of the LiaR-Mediated Response Against Daptomycin and Antimicrobial Peptides in Multidrug-Resistant *Enterococcus faecalis* (*Efs*)

Laura C. Ortiz-Velez, PhD[1]; Sandra L Rincon, PhD[2];
Jesse Degani, Medical student[3]; Yousif Shamoo, PhD [4];
Truc T. Tran, PharmD[5]; Cesar A. Arias, MD, MSc, PhD, FIDSA[6] and
Diana Panesso, PhD [7]; [1]UTHealth McGovern Med School, Houston, Texas; [2]UGRA, Universidad El Bosque, Bogota, Distrito Capital de Bogota, Colombia; [3]UTHealth McGovern Med School, Houston, Texas; [4]Rice University, Houston, Texas; [5]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas; [6]CARMiG, UTHealth and Center for Infectious Diseases, UTHealth School of Public Health, Hou, Texas; Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, BOG, COL, Houston, Texas; [7]UTHealth McGovern Med School, Houston. Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. Daptomycin (DAP) is a key first-line agent for the treatment of vancomycin-resistant enterococcal infections. Resistance to DAP in enterococci is regulated by the *liaFSR* three-component regulatory system that consists of a histidine kinase sensor (LiaS), a response regulator (LiaR) and a transmembrane protein of unknown function (LiaF). Previous studies indicate that deletion of isoleucine in position 177 of LiaF results in DAP tolerance and is sufficient to change membrane architecture. Here, we dissect the role of LiaF in DAP resistance

Methods. We generated three *liaF* mutants in OG1RF, a DAP-susceptible laboratory strain of *Efs* (DAP MIC = 2 μg/mL): (i) a non-polar, C-terminal truncation of *liaF* ($OG1RF_{liaF\Delta152}$), (ii) a null *liaF* mutant with a premature stop-codon ($OG1RF_{liaF*11}$), and (iii) an isoleucine deletion at position 177 ($OG1RF_{liaF177}$). We determined DAP MIC by Etest and characterized the localization of anionic phospholipids microdomains using 10-nonyl-acridine-orange (NAO). The expression of the *liaXYZ* (the main target of LiaR) and *liaFSR* clusters were evaluated by qRT-PCR and relative expression ratios (Log_2 fold change) were calculated by normalizing to *gyrB* expression. We assessed activation of LiaFSR by evaluating surface exposure of LiaX by ELISA. We used the bacterial adenylate cyclase two-hybrid system (BACTH) to evaluate the protein-protein interaction between LiaF and LiaS.

Results. Full deletion of *liaF* or the C-terminal truncation of LiaF did not have any effect on DAP MICs, membrane architecture or a significant increase in LiaX surface exposure compared with parental strain OG1RF. In contrast, deletion of the codon encoding isoleucine in position 177 of LiaF caused a major increase (8-fold) in LiaX exposure and redistribution of anionic phospholipid microdomains away from the septum without changes in the actual DAP MIC. Transcriptional analyses indicated upregulation (>2 log_2-fold) in the *liaXYZ* gene cluster indicating activation of the stress response. We also observed a positive interaction between LiaF and LiaS.

Conclusion. LiaF is likely a key activator of the LiaFSR stress response and the critical regulatory domain appears to be located in a stretch of four isoleucines toward the C-terminal of the protein.

Disclosures. **All authors:** No reported disclosures.

600. β-Lactam Resistance Mechanisms in *Pseudomonas aeruginosa* Isolates Analyzed Using Whole-Genome Sequencing (WGS) and Transcriptions Analysis and Their Impact in Resistance to New β-Lactam/β-Lactamase Inhibitors

Mariana Castanheira, PhD; Timothy B. Doyle; Cory Hubler;
Matthew Healy, PhD; Caitlin J Smith, PhD and Rodrigo E. Mendes, PhD; JMI Laboratories, North Liberty, Iowa

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. Ceftazidime–avibactam (CAZ-AVI) and ceftolozane-tazobactam (C-T) display excellent antipseudomonal activity, but *Pseudomonas aeruginosa* (PSA) susceptibility against these agents can be affected by acquired resistance genes and mutations. We evaluated resistance mechanisms against these agents among 109 PSA isolates using WGS and messenger (m)RNA-sequencing.

Methods. PSA clinical isolates from Europe (n = 62), Asia-Pacific (n = 22), and Latin America (n = 25) in 2017 were susceptibility tested using reference methods and 109 were randomly selected for WGS and total mRNA-sequencing. Data were analyzed using custom software and logistic regression.

Results. Isolates carrying metallo-β-lactamases (MBLs) (n = 24) were resistant to all β-lactams, including CAZ-AVI and C-T. The only compound inhibiting >50% of the isolates was colistin. ESBL genes (bla_{VEB-1} or bla_{VEB-9}), some oxacillinases, and PDC variants caused resistance to CAZ-AVI and C-T, but the presence of bla_{PER-1}, bla_{GES-6}, and *PDC-97* led to resistance to C-T, but not to CAZ-AVI. Disruptions of *ampR* (PDC regulator) and *glnD* (nitrogen metabolism) were associated with resistance to CAZ-AVI and C-T, but *armZ* (anti-repressor of *mexZ*) disruption was only associated with C-T resistance. The combination of wild-type sequences of various genes was negatively associated with resistance to CAZ-AVI and C-T, but alterations in *dnaJ* (chaperone) and *oprM* were only related to C-T resistance. mRNA-sequencing data did not show strong correlations with CAZ-AVI or C-T resistance or with expression of genes involved in β-lactam resistance, but further analyses will expand the genes analyzed. Interestingly, among 14 isolates overexpressing MexAB-OprM that extrude CAZ, only 6 had CAZ-AVI MICs >8 mg/L.

Conclusion. Resistance mechanisms against CAZ-AVI and C-T remain poorly understood beyond MBL acquisition. In this study, resistance mechanisms statistically associated with CAZ-AVI resistance in PSA were noted among C-T-resistant isolates, but some mechanisms were only observed among C-T-resistant isolates. The richness of results employing these 2 methodologies requires further investigations that are being performed to evaluate sequences and expression alterations.

Disclosures. **All authors:** No reported disclosures.

601. TelA and XpaC Are Novel Mediators of Daptomycin Resistance in *Enterococcus faecium*

Truc T. Tran, PharmD[1]; Diana Panesso, PhD [2]; Lorena Diaz, PhD[3];
Rafael Rios, MSc[4] and Cesar A. Arias, MD, MSc, PhD, FIDSA[5]; [1]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas; [2]UTHealth McGovern Med School, Houston, Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, , Houston, Texas; [3]Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics,Universidad El Bosque, BOG, COL; MICROB-R, Bogota, Distrito Capital de Bogota, Colombia; [4]Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, Bogota, Distrito Capital de Bogota, Colombia; [5]CARMiG, UTHealth and Center for Infectious Diseases, UTHealth School of Public Health, HOU, Texas; Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, BOG, COL, Houston, Texas

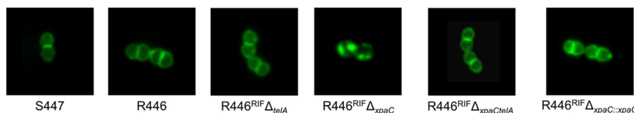
Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. The YycFG system is an essential two-component regulatory system involved in cell wall homeostasis associated with the development of daptomycin (DAP) resistance in *E. faecium*. Importantly, the standard combination of DAP plus β-lactam is ineffective against strains harboring mutations in *yycFG*. Transcriptional profiling identified a cluster of two genes (*xpaC* and *telA*) that is upregulated in the presence of a $YycG_{S333L}$ substitution. *xpaC* and *telA* are annotated as 5-bromo-4-chloroindolyl phosphate hydrolysis and tellurite resistance proteins, respectively. Here, we aimed to determine the contribution of *xpaC* and *telA* in DAP resistance.

Methods. Non-polar in-frame deletions of *xpaC*/*telA* and complementation of *xpaC* were performed in clinical strain *E. faecium* $R446^{RIF}$. All mutants were characterized by PFGE and sequencing of the open reading frames to confirm the deletion. DAP MIC determination was performed by Etest on Mueller–Hinton agar. Binding of DAP was evaluated using BODIPY-labeled DAP (BDP-DAP). Cell membrane phospholipid microdomains were visualized using 10-*N*-nonyl acridine orange. All assays were compared with a DAP-susceptible clinical *E. faecium* strain S447.

Results. $R446^{RIF}$Δ*telA* and $R446^{RIF}$Δ*xpaCtelA* did not alter DAP MICs in $R446^{RIF}$ (24–32 µg/mL). However, deletion of *xpaC* alone ($R446^{RIF}$Δ*xpaC*) markedly decreased DAP MIC 8 fold (to 4 µg/mL). $R446^{RIF}$Δ*telA* and $R446^{RIF}$Δ*xpaCtelA* exhibited similar binding of BDP-DAP compared with parental $R446^{RIF}$. In contrast, $R446^{RIF}$Δ*xpaC* exhibited increased binding of the antibiotic molecule to the cell membrane, similar to that of DAP-susceptible S447. Complementation of *xpaC* restored DAP MIC to 32–48 µg/mL and decrease binding of DAP. NAO staining of S447, $R446^{RIF}$, $R446^{RIF}$Δ*telA*, $R446^{RIF}$Δ*xpaCtelA*, and $R446^{RIF}$Δ*xpaC*::*xpaC* displayed septal and polar distribution. In stark contrast, $R446^{RIF}$Δ*xpaC* showed a redistribution of phospholipid microdomains away from the septa.

Conclusion. XpaC is a key contributor to DAP binding and phospholipid architecture of *E. faecium* but only in the presence of an intact TelA. The *xpaC* and *telA* gene cluster is a novel mediator of DAP-resistance in *E. faecium* via theYycFG system and independent of the LiaFSR system



Disclosures. **All authors:** No reported disclosures.

602. Mechanism of LiaY-Mediated Daptomycin Resistance in *Enterococcus faecalis*

April Nguyen, BSc[1]; Truc T. Tran, PharmD[1]; Diana Panesso, PhD [2];
Ayesha Khan, BSc[3]; Eugenia Mileykovskaya, PhD [4]; Heidi Vitrac, PhD [4] and
Cesar A. Arias, MD, MSc, PhD, FIDSA[5]; [1]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas; [2]UTHealth McGovern Med School, Houston. Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas; [3]UTHealth McGovern Medical School, Houston, Texas; [4]McGovern Medical School, UTHealth, Houston, Texas; [5]CARMiG, UTHealth and Center for Infectious Diseases, UTHealth School of Public Health, HOU, Texas; Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, BOG, COL, Houston, Texas

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. Daptomycin (DAP) is a lipopeptide antibiotic that targets the cell membrane (CM) at the division septum. DAP resistance (DAP-R) in *E. faecalis* (*Efs*) has been linked to mutations in genes encoding the LiaFSR stress response system and lipid biosynthetic enzymes, including cardiolipin synthase (Cls). The signature phenotype of DAP-R is redistribution of CM anionic phospholipid (APL) microdomains. Using a genetic approach, we have identified a transmembrane protein (LiaY) as a major mediator of cell membrane APL redistribution associated with DAP-R. Here, we explore the mechanism of LiaY-mediated changes in the CM under the hypothesis that CM remodeling occurs through interactions with Cls.

Methods. *Efs* encodes two *cls* genes (*cls1* and *cls2*). Deletion mutants of both *cls* genes were generated using the Crispr/cas9 system in the daptomycin-sensitive strain *Efs* OG117 and *Efs* OG117Δ*liaX* (a DAP-R derivative of OG117). DAP minimum inhibitory concentration (MIC) was determined using E-test on Mueller–Hinton II agar. Visualization of APL microdomains was performed by staining mid-logarithmic phase cells with 1 µM of 10-N-nonyl-acridine orange (NAO) and fluorescence microscopy. Bacterial two-hybrid system was used to study interactions between LiaY with Cls1 or Cls2.

Results. Single or double deletion of *cls1* or *cls2* in *Efs* OG117 did not affect DAP MIC, and no changes in CM architecture were seen by NAO staining. In contrast,deletion of *cls1* (alone or in conjunction with a deletion of *cls2)* in a DAP-R derivative of OG117 OG117Δ*liaX*, resulted in a marked decrease in DAP MIC, and NAO staining of *Efs* OG117Δ*liaX*Δ*cls1*Δ*cls2* shows a restoration of septal APL microdomain localization.In the same DAP-R background, deletion of *cls2* alone did not have any effect on DAP MIC or APL microdomain distribution. Additionally, bacterial two-hybrid assays showed a positive interaction of LiaY with Cls1 but not with Cls2.

Conclusion. We have identified the biochemical basis for DAP-R associated CM remodeling. In a proposed model, the LiaR-mediated activation of the LiaY triggers specific interactions with Cls1 displacing the protein away from the septum, resulting in local generation of APL microdomains that prevents DAP-mediated damage to the CM.

Disclosures. **All authors:** No reported disclosures.

603. Identification of a Carbapenemase-Producing, Extensively Drug-Resistant *Klebsiella pneumoniae* Isolate Carrying a *blaNDM-1*-Bearing, Hypervirulent Plasmid, United States 2017

Richard A. Stanton, PhD[1]; Gillian A. McAllister, BS[2]; Amelia Bhatnagar, BS[3];
Maria Karlsson, PhD[2]; Allison C. Brown, PhD MPH[1];
James Rasheed, PhD[1]; Christopher Elkins, PhD[1] and Alison L. Halpin, PhD[2]; [1]CDC, Atlanta, Georgia; [2]Centers for Disease Control and Prevention, Atlanta, Georgia; [3]Eagle Medical Services, Atlanta, Georgia

Session: 65. Mechanisms of Antimicrobial Resistance
Thursday, October 3, 2019: 12:15 PM

Background. The recent discovery of carbapenemase-producing hypervirulent *Klebsiella pneumoniae* (CP-HvKP) has signaled the convergence of multidrug resistance and pathogenicity, with the potential for increased mortality. While previous studies of CP-HvKP isolates revealed that most carried carbapenemase genes and hypervirulence elements on separate plasmids, a 2018 report from China confirmed that both could be harbored on a single, hybrid carbapenemase-hypervirulent plasmid. As part of a project sequencing isolates carrying multiple carbapenemase genes identified through CDC's Antibiotic Resistance Laboratory Network (AR Lab Network), we discovered a *blaNDM-1*-bearing hypervirulent plasmid found in a KPC- and NDM-positive *K. pneumoniae* from the United States.

Methods. Antimicrobial susceptibility testing (AST) was performed by reference broth microdilution against 23 agents. Whole-genome sequencing (WGS) was performed on Illumina MiSeq and PacBio RS II platforms.

Results. AST results indicated the isolate was extensively drug-resistant, as it was non-susceptible to at least one agent in all but two drug classes; it was susceptible to only tigecycline and tetracycline. Analysis of WGS data showed the isolate was ST11, the same sequence type that caused a fatal outbreak of CP-HvKP in China in 2016. The genome included two plasmids. The smaller one (129kbp) carried seven antibiotic resistance (AR) genes, including the carbapenemase gene *blaKPC-2*. The larger plasmid (354kbp) harbored 11 AR genes, including the metallo-β-lactamase gene *blaNDM-1*, as well as virulence factors *iucABCD/iutA*, *peg-344*, *rmpA*, and *rmpA2*, which comprise four of the five genes previously identified as predictors of hypervirulence in *K. pneumoniae*.

Conclusion. This is the first report of a hybrid carbapenemase-hypervirulent plasmid in the United States. The presence of both blaNDM-1 and hypervirulence