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-ß-lactamases (MBLs) (n = 24) were resistant to all ß-lactams, including CAZ-AVI and C-T. The only compound inhibiting >50% of the isolates was colistin. ESBL genes (bla<sub>TEM</sub>, or bla<sub>SHV</sub>), some oxacilllines, and PDC variants caused resistance to CAZ-AVI and C-T, but the presence of bla<sub>TEM</sub>, and PDC-97 led to resistance to C-T, but not to CAZ-AVI. Disruptions of ampR (PDC regulator) and armZ (anti-repressor of mecZ) 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 age<sub>4</sub> 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 ß-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 μg/mL.

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 and C-T resistance in PSA were noted among C-T-resistant isolates. However, 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.

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

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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 <i>E. faecium</i>. Importantly, the standard combination of DAP plus ß-lactam is ineffective against strains harboring mutations in <i>yycFG</i> transcriptional profiling identified a cluster of two genes (<i>xpaC</i> and <i>telA</i>) that is upregulated in the presence of a YycG<sup>Δ</sup> substitution. <i>xpaC</i> and <i>telA</i> are annotated as 5-bromo-4-chloroindolyl phosphate hydrolysis and tellurite resistance proteins, respectively. Here, we aimed to determine the contribution of <i>xpaC</i> and <i>telA</i> in DAP resistance.

Methods. Non-polar in-frame deletions of <i>xpaCtelA</i> and complementation of <i>xpaC</i> were performed in clinical strain <i>E. faecium</i> R446<sup>Δ</sup>. All mutants were characterized by broth microdilution 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<sup>®</sup>-labeled DAP (BDP-DAP). Cell membrane phospholipid microdomains were visualized using 16-N-nonyl-acridine orange (NAO) and fluorescence microscopy.

Results. <i>xpaC</i> and <i>telA</i> deletion mutants of R446<sup>Δ</sup> exhibited increased binding of the antibiotic molecule to the cell membrane, similar to DAP MIC 8 fold (to 4 μg/mL). R446<sup>Δ</sup> showed a redistribution of phospholipid anionic phospholipid (CM) at the division septum. DAP resistance (DAP-R) in <i>E. faecium</i> exhibited increased binding of the antibiotic molecule to the cell membrane, similar to DAP MIC 8 fold (to 4 μg/mL). R446<sup>Δ</sup> showed a redistribution of phospholipid anionic phospholipid (CM) at the division septum. DAP resistance (DAP-R) in <i>E. faecium</i> exhibited increased binding of the antibiotic molecule to the cell membrane, similar to DAP MIC 8 fold (to 4 μg/mL).

Conclusion. <i>xpaC</i> is a key contributor to DAP binding and phospholipid microdomain architecture of <i>E. faecium</i> but only in the presence of an intact <i>telA</i>. The <i>xpaC</i> and <i>telA</i> gene cluster is a novel mediator of DAP resistance in <i>E. faecium</i> via the YycFG system and independent of the LiaFSR system.

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

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Background. Daptomycin (DAP) is a lipodeptide antibiotic that targets the cell membrane (CM) at the division septum. DAP resistance (DAP-R) in <i>E. faecalis</i> (Ef) 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 an approach that 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. <i>Ef</i> encodes two cis genes (cis1 and cis2). Deletion mutants of both cis genes were generated using the Cre/rep/cass system in the daptomycin-sensitive strain <i>Ef</i> OG117<sup>Δ</sup> and <i>Ef</i> OG117<sup>Δ</sup>liaX<sub>Δ</sub> (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. Backcross two-hybrid system was used to study interactions between LiaY with Cis1 or Cis2.

Results. Single or double deletion of cis1 or cis2 in <i>Ef</i> OG117 did not affect DAP MIC, and no changes in CM architecture were seen by NAO staining. In contrast, deletion of cis1alone (or in conjunction with a deletion of cis2) in a DAP-R derivative of OG117<sup>Δ</sup>liaX, resulted in a marked decrease in DAP MIC, and NAO staining of <i>Ef</i> OG117<sup>Δ</sup>liaXcis1Δcis2 shows a restoration of septal APL microdomain localization. In the same DAP-R background, deletion of cis2 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 Cis1 but not with Cis2.

Conclusion. We have identified the biochemical basis for DAP-R associated CM remodeling. In a proposed model, the LiaY-mediated activation of the LiaY triggers specific interactions with Cis1 displacing the protein away from the septum, resulting in local generation of APL microdomains that prevents DAP-mediated damage to the CM.

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

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Background. The recent discovery of carbapenemase-producing hypervirulent <i>Klebsiella pneumoniae</i> (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 have sequenced a blaNDM-1-bearing hypervirulent plasmid found in a KPC- and NDM-positive <i>K. pneumoniae</i> 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 MikeSeq and independent of the LiaFSR system.