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*

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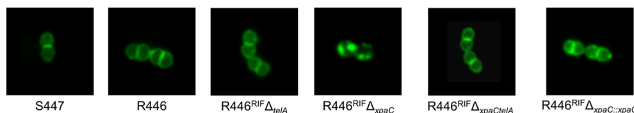
Background. The YycFG system is an essential two-component regulatory system involved in cell wall homeostasis associated with the development of daptomycin (DAP) resistance in *E. faecium*. Importantly, the standard combination of DAP plus β-lactam is ineffective against strains harboring mutations 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*

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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

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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

elements on the same plasmid suggests that the CP-Hv pathotype could spread rapidly through horizontal transfer. This discovery demonstrates the critical role of genomic characterization of emerging resistance and virulence phenotypes by the AR Lab Network as part of US containment efforts.

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604. Gram-Negative Bacilli Carrying Multiple Carbapenemases: the United States, 2012–2018

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Background. Gram-negative bacilli carrying multiple carbapenemase genes (multi-CP-GNB) present an emerging public health threat; to date, most isolates reported in the literature have been from outside the United States. We reviewed multi-CP-GNB reported to CDC.

Methods. Reports of multi-CP-GNB isolates carrying genes encoding >1 targeted carbapenemases (i.e., KPC, NDM, OXA-48-type, VIM, or IMP) were received from healthcare facilities, health departments, and public health laboratories, and included isolates tested through the AR Laboratory Network (ARLN) beginning in 2017 as well as isolates sent to CDC for reference testing. Epidemiologic data were gathered by health departments during public health investigations.

Results. From October 2012 to November 2018, 111 multi-CP-GNB isolates from 71 patients in 20 states were identified. Two patients had three different multi-CP-GNB and one patient had two different multi-CP-GNB. The majority of cases (76%) were reported in 2017 or later, after ARLN testing began. Among patients with multi-CP-GNB, the most common organism-mechanisms combination was *Klebsiella pneumoniae* carrying NDM and OXA-48-type enzymes (table). Urine (44%) and rectal (20%) were the most frequent specimen sources for isolates. The median age of patients was 63 years (range 2–89 years); most had specimens collected at acute care hospitals (87%) or post-acute care facilities (9%). Of 50 patients with information available, 37 traveled internationally in the 12 months prior to culture collection. Among these, 88% were hospitalized for ≥1 night while outside the United States with 10 countries reported, of which India was most common ($n = 18$). All 5 patients with *Pseudomonas aeruginosa* co-carrying carbapenemases reported recent hospitalization outside the United States.

Conclusion. The multi-CP-GNB reported to CDC include diverse organisms and carbapenemase combinations and often harbored carbapenemases from different β-lactamase classes, which may severely limit treatment options. Healthcare exposures outside the United States were common; providers should ask about this exposure at healthcare admission and, when present, institute interventions to stop transmission in order to slow further US emergence.

Table: Unique Patients With Gram Negative Bacilli Producing Multiple Carbapenemases, by Organism and Mechanism, N=76**

Organism	IMP+NDM+ (n=2)	KPC+NDM+ (n=17)	KPC+OXA+ (n=1)	KPC+VIM+ (n=7)	NDM+OXA+ (n=47)	NDM+VIM+ (n=2)
Enterobacteriaceae (n=71)	0	17	1	6	47	0
E. coli (n=10)		2			8	
Enterobacter (n=10)		8		2		
*K. Pneumoniae** (n=48)		7	1	2	38	
K. Oxytoca (n=1)				1		
Citrobacter freundii (n=1)				1		
Providencia rettgeri (n=1)					1	
P. aeruginosa (n=5)	2			1		2

*Includes organisms now classified as *Klebsiella aerogenes*

**71 patients had 76 isolates with unique organism-mechanism combinations

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605. Identification of a Novel CMY-Variant Enzyme in a Clinical *Escherichia coli* Strain with Treatment-Emergent Ceftazidime–Avibactam Resistance

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Background. Ceftazidime–avibactam (CZA) is a novel β-lactam / β-lactamase inhibitor with *in vitro* activity against multidrug-resistant Gram-negatives, including those harboring CMY-2 enzymes. Treatment-emergent resistance to CZA has been described in KPC-producing *Klebsiella pneumoniae* but has not been described in non-carbapenemase-producing, carbapenem-resistant *Enterobacteriaceae* (CRE).

Methods. A patient with an intra-abdominal infection due to a carbapenem-resistant *E. coli* (ertapenem MIC 16 μg/mL; meropenem MIC 2 μg/mL; CZA MIC 2 μg/mL; carbapenemase negative) was treated with CZA. On day 48 of therapy, a CZA resistant, carbapenem-sensitive *E. coli* was identified from abdominal drainage (CZA MIC ≥256 μg/mL; meropenem MIC 0.19 μg/mL). Illumina MiSeq whole-genome sequencing (WGS) was performed on both isolates to identify potential resistance mechanisms. The ResFinder database was used to identify known β-lactamase enzymes, and *in silico* modeling of β-lactamase structure was assessed.

Results. WGS revealed that both isolates were ST410 *E. coli*, with the sole difference in β-lactam resistance determinants between the two being a novel CMY β-lactamase harbored on an Inc1-type conjugative plasmid in the second isolate. The novel CMY has 4 amino acid substitutions relative to CMY-2: A134E, Q140K, V231S, and N366Y. The V231S substitution is found in CMY-42 and has previously been associated with increased ceftazidime hydrolysis. The remaining three substitutions have not previously been identified. Previous studies have identified that substitutions at position 366 influence the rate of ceftazidime hydrolysis rate. Preliminary protein structure analysis suggests that positions 140 and 366 are in the active site. No other differences in β-lactam resistance determinants were identified between the first and second isolates.

Conclusion. To our knowledge, we have identified the first case of CMY-associated CZA resistance. Given the widespread and transferrable nature of CMY enzymes, this finding raises concern for additional cases of resistance with increasing usage of CZA. Further analysis is needed to identify the mechanism by which this enzyme confers CZA resistance.

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606. Identification and Characterization of HMB-2, a Novel Metallo-β-Lactamase in a *Pseudomonas aeruginosa* Isolate

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Background. Carbapenemases, a global health threat, are a diverse group of β-lactamases active against cephalosporins and carbapenems, which are often last resort treatments for multidrug-resistant gram-negative infections. The most common carbapenemases reported among *Pseudomonas aeruginosa* are metallo-β-lactamase (MBLs). We describe a novel MBL (designated HMB-2) identified in a *P. aeruginosa* isolate from a urine specimen collected in 2015 as part of CDC's Emerging Infections Program.

Methods. We performed antimicrobial susceptibility testing (AST) by broth microdilution, real-time PCR to screen for common carbapenemases (IMP, KPC, NDM, VIM, and OXA-48), and modified carbapenem inactivation method (mCIM) to test for carbapenemase production. The isolate underwent whole-genome sequencing (WGS) using Illumina MiSeq and PacBio RS II (Pacific Biosciences) platforms. Long read sequences were polished using Quiver and corrected by Pilon utilizing Illumina reads. We further characterized a putative novel MBL identified in WGS data by amplifying and cloning the gene into the pCR2.1-TOPO II vector (Invitrogen), which was then sub-cloned into a pET21 expression vector (Sigma–Aldrich). The resulting *hmb2+* pET21 plasmid was transformed into a susceptible *Escherichia coli* for AST, including the imipenem-EDTA method to confirm MBL activity.

Results. The isolate displayed resistance to carbapenems and demonstrated phenotypic carbapenemase activity (mCIM positive), but was negative for carbapenemase genes by PCR. WGS analyses identified a putative MBL gene located on the chromosome. The gene shared 98% DNA and protein sequence identity with an MBL reported in 2016 in a *P. aeruginosa* isolate from Germany (HMB-1) and thus was named *hmb-2*. The cloned *hmb-2* gene conferred resistance to carbapenems (meropenem and ertapenem) and third-generation cephalosporins (cefotaxime and ceftazidime) in transformed *E. coli*. The Minimum Inhibitory Concentrationratio for the imipenem-EDTA method was ≥4.

Conclusion. A putative, novel β-lactamase gene, bla_{HMB-2}, was identified and cloned. The imipenem-EDTA results indicated that HMB-2 is an MBL. This discovery