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.

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

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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%)

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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

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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*.

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

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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* ($OG1RF_{liaFΔ152}$), (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.

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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

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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.