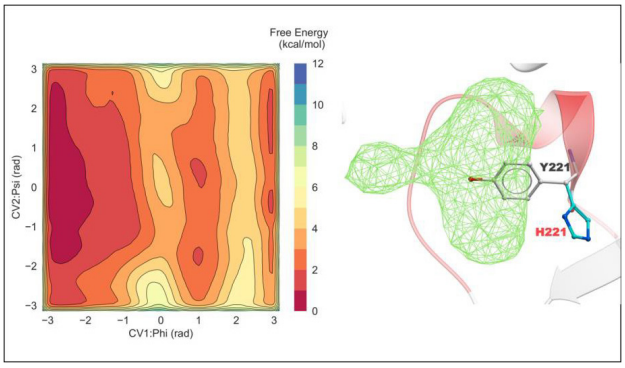
responsible for β-lactam antibiotic resistance in *P. aeruginosa*. Single amino acid substitutions in the essential Ω-loop region (e.g. Y221H by structural alignment-based numbering of class C β-lactamases) have been shown to enhance hydrolysis of ceftazidime (CAZ) and ceftolozane (TOL), limiting therapeutic options for *P. aeruginosa*.

Methods. We undertook detailed studies to explore the mechanisms by which Y221H enhances CAZ and TOL MICs. MIC measurements were performed per CLSI guidelines using MH Agar. Thermal stability was determined by circular dichroism. Enzyme kinetic properties were determined using spectrophotometric techniques. Molecular dynamics techniques were used to predict structural changes.

Results. *E. coli* expressing $bla_{PDC\text{-}3^{-}Y221H}$ is less susceptible to CAZ (MIC 0.5 mg/L WT → 8 mg/L Y221H) and TOL (MIC 2 mg/L WT → 16 mg/L Y221H). Using steady-state kinetic analysis, Y221H was found to hydrolyze CAZ with a K_M = 585 μM, a k_{cat} = 3.4 sec^{-1}, and k_{cat}/K_M = 0.0058 $\mu M^{-1}s^{-1.}$ With cephalothin, a good PDC substrate, we observed K_M = 26.6 μM, k_{cat} = 70.1 s^{-1}, and k_{cat}/K_M = 2.6 μM-1 s^{-1} for Y221H. Using Electrospray ionization mass spectrometry (ESI-MS), CAZ was detected covalently bound to WT, but not Y221H when incubated at 1000-fold molar excess. Avibactam (AVI) inhibited Y223H (K_i = 70 nM vs. 19 nM for WT). Y221H thermal stability decreased by 5°C (Tm = 47°C vs 52°C WT). AVI at 10-fold molar excess does not increase Tm in Y221H or WT. WT-MetaDynamics (WT MDS) predicts the opening of a hidden pocket by repositioning residue 221 (**Figure 1**).).

Figure 1: (Left) We carried out enhanced sampling metadynamics simulations to generate free-energy landscapes as a function of the dihedral angles of residue 221. This identifies the differences in the dynamics of the tyrosyl side chains in the wild type Y221 and the imidazole ring of the H221 variant. (Right) The rotation of the side chain in H221 opens a cryptic pocket (green mesh), which is occluded in the wild type. The Ω-loop is colored red.



Conclusion: PDC-3 Y221H increases CAZ & TOL MICs and alters catalytic activity, primarily by a change in k_{cat}. Our modelling analyses suggest altered conformational flexibility and structure-function relationships in the Ω-loop. These results help to advance our understanding of PDC and will inform development of novel antibiotics and inhibitors.

Disclosures. Robert A. Bonomo, MD, Entasis, Merck, Venatorx (Research Grant or Support)

1446. Dynamics of Enterococcus faecalis Cardiolipin Synthase Gene Expression Reveal Compensatory Roles in Daptomycin Resistance

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Session: P-66. Resistance Mechanisms

Background. Daptomycin (DAP) is a lipopeptide antibiotic targeting membrane anionic phospholipids (APLs) at the division septum, and resistance (DAP-R) has been linked to mutations in genes encoding ***i***) the LiaFSR stress response system or its effector LiaX, and ***ii***) cardiolipin synthase (Cls). Activation of the *E. faecalis (Efs)* LiaFSR response is associated with DAP-R and redistribution of APL microdomains away from the septum, and cardiolipin is predicted to be a major component of these APL microdomains. *Efs* harbors two putative *cls* genes, *cls1* and *cls2*. While changes in Cls1 have been implicated in DAP-R, the exact roles of each enzyme in resistance are unknown. We aim to characterize the contributions of Cls1 and Cls2 in the development of DAP-R.

Methods. *cls1* and *cls2* were deleted individually and in tandem from DAP-S *Efs* OG117 and DAP-R *Efs* OG117Δ*liaX* (a DAP-R derivative strain with an activated LiaFSR response). Mutants were characterized by DAP minimum inhibitory concentration (MIC) using E-test on Mueller-Hinton II agar and localization of APL microdomains with 10-N-nonyl-acridine orange staining. Quantitative PCR (qRT-PCR) was used to study gene expression profiles of *cls1* and *cls2* in *Efs* OG117Δ*liaX* relative to *Efs* OG117 across the cell growth cycle.

Results. qRT-PCR revealed differential expression profiles of *cls1* and *cls2* associated with DAP-R. *cls1* was highly upregulated in stationary phase concurrent with a decrease in *cls2* expression. However, independent deletion of *cls1* or *cls2* in the DAP-R background resulted in no significant changes in DAP MICs or localization of APL microdomains (remaining non-septal). Further studies revealed that *cls2* expression is upregulated upon deletion of *cls1* in both the DAP-S and DAP-R background, suggesting a potential compensatory role for Cls2. Double deletion of both *cls* genes in the DAP-R strain decreased DAP MIC and restored the septal localization of APL microdomains.

Conclusion. Cls1 is the major and predominant enzyme involved in cell membrane adaptation associated with the development of DAP-R in *E. faecalis*. However, we describe a novel compensatory and overlapping role for cardiolipin synthases to ensure bacterial survival upon attack from antimicrobial peptides and related antibiotics.

Disclosures. **Cesar A. Arias, MD, MSc, PhD, FIDSA**, **Entasis Therapeutics** (Scientific Research Study Investigator)**MeMed** (Scientific Research Study Investigator)**Merck** (Grant/Research Support)

1447. Emergence of Avibactam Resistance in Multidrug-Resistant Enterobacteriaceae

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Session: P-66. Resistance Mechanisms

Background. Avibactam (AVI) is a non-β-lactam β-lactamase inhibitor used clinically to inhibit bacterial β-lactamase activity against the β-lactam antibiotic ceftazidime. We previously observed intrinsic *in vitro* antibacterial activity of AVI against multidrug-resistant

Enterobacteriaceae. Here we characterize the rapid emergence of AVI resistance following AVI exposure.

Methods. We grew two carbapenem- and colistin-resistant isolates (*E. coli* ARLG 2829/MCR1_NJ and *Klebsiella pneumoniae* AR-0636) in liquid culture containing 16x the AVI minimum inhibitory concentration (MIC) for 24 hours. We then tested the AVI MIC of each strain daily for 17 days following serial passage on antibiotic-free media. We also tested MICs of AVI and 6 β-lactam antibiotics against broadly susceptible *E. coli* and *K. pneumoniae* isolates following growth with AVI. Finally, we tested *in vivo* activity of AVI using a mouse thigh infection model in which groups of 5 mice infected with $1x10^8$ CFU/thigh of AR-0636 were treated with AVI 250 mg/kg or saline every 8 hours for 24 hours.

Results. Following growth in AVI 128 μg/mL, the AVI MIC of both strains increased from 8 to > 256 μg/mL and remained ≥ 256 μg/mL for 17 days of serial passage on antibiotic-free media. Following AVI treatment, MICs were also elevated for mecillinam, which, like AVI, targets penicillin-binding protein 2 (PBP2), but not for drugs with different PBP affinities. In a mouse thigh infection model, AVI treatment resulted in an average 1.4 $\log_{10}$ decrease in CFU/thigh compared to placebo. AVI MICs in bacteria recovered from treated mouse thighs were unchanged from initial MIC.

Conclusion. AVI resistance emerged rapidly *in vitro* and persisted for over two weeks in the absence of selective pressure. The co-emergence of mecillinam resistance suggests that AVI resistance may reflect PBP2 alterations. Development of resistance was not observed in a mouse model. These results have important implications for new non-β-lactam β-lactamase inhibitors (nacubactam, zidebactam) with structural similarities to AVI and known intrinsic antibacterial activity that have recently completed Phase I trials in combination with β-lactam drugs and are likely to play an important future role in CRE treatment.

Disclosures. **Thea Brennan-Krohn, MD, D(ABMM)**, **Tecan** (Other Financial or Material Support, HP D300 digital dispenser and its consumables were provided by Tecan. Tecan had no role in study design, data collection/interpretation, or poster preparation.) **Shade Rodriguez, BA**, **Tecan** (Other Financial or Material Support, HP D300 digital dispenser and its consumables were provided by Tecan. Tecan had no role in study design, data collection/interpretation, or poster preparation.) **James Kirby, MD, D(ABMM)**, **AstraDx** (Advisor or Review Panel member, Other Financial or Material Support, Co-founder)**First Light Biosciences** (Advisor or Review Panel member)**Tecan** (Other Financial or Material Support, HP D300 digital dispenser and its consumables were provided by Tecan. Tecan had no role in study design, data collection/interpretation, or poster preparation.)

1448. Forgiveness of BIC/FTC/TAF: In Vitro Simulations of Intermittent Poor Adherence Find Limited HIV-1 Breakthrough and High Barrier to Resistance

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Session: P-66. Resistance Mechanisms

Background. Short lapses in adherence to ARVs can lead to virologic failure and emergence of resistance. Previous *in vitro* studies of regimen "forgiveness" simulated drug exposures of perfect adherence or short-term suboptimal adherence with