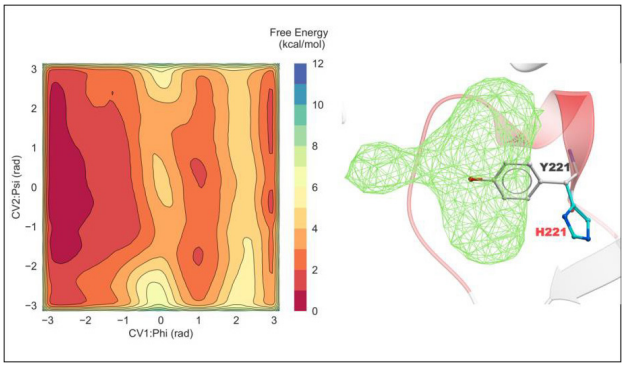
responsible for β-lactam antibiotic resistance in *P. aeruginosa*. Single amino acid substitutions in the essential Ω-loop region (e.g. Y221H by structural alignment-based numbering of class C β-lactamases) have been shown to enhance hydrolysis of ceftazidime (CAZ) and ceftolozane (TOL), limiting therapeutic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

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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.

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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

bictegravir+emtricitabine+tenofovir alafenamide (BIC+FTC+TAF) and with dolutegravir and lamivudine (DTG+3TC). Here, viral breakthrough (VB) and resistance development were evaluated under alternating high and low drug exposures simulating variable adherence levels.

Methods. Wild-type HIV-1 (IIIb)-infected MT-2 cells were exposed to drug combinations and monitored for VB. Experiments alternated between high and low drug concentrations of either BIC+FTC+TAF or DTG+3TC (Table 1). Drug concentrations for each regimen were determined using human plasma-free adjusted clinical trough concentrations (C_{min}), at simulated C_{min} after missing 2 or 4 consecutive doses (C_{min}-2 and C_{min}-4) based on drug half-lives. Emergent HIV-1 were genotyped by deep sequencing and a 2% threshold.

Results. In these experiments, constant drug concentrations corresponding to full adherence (C_{min}) did not lead to VB. Using C_{min} concentrations for one week followed by constant C_{min}-2 exposures for 4 weeks, DTG+3TC had VB and emergence of M184V/I in reverse transcriptase (RT) but there was no VB for BIC+FTC+TAF. Using alternating drug exposures of C_{min} (weeks 1 and 3) and C_{min}-2 or C_{min} -4 (weeks 2, 4, and 5), VB was not observed with BIC+FTC+TAF, and VB was decreased or delayed with DTG+3TC compared to DTG+3TC held at C_{min}-2 or C_{min}-4. Resistance development was observed in some cultures with VB: 1 culture with BIC+FTC+TAF had G163R in IN and 19 cultures with DTG+3TC had INSTI and RT resistance including 10 with M184V/I.

Table 1. Summary of Breakthrough Frequency and Resistance Development

In Vitro Dosing (By Week)					Breakthrough Frequency (Resistance Development)			
					BIC+FTC+TAF		DTG+3TC	
Week 1	Week 2	Week 3	Week 4	Week 5	VB (n/N,%)	With Resistance (n)[c]	VB (n/N,%)	With Resistance (n)[c]
Cmin [a]					0/60; 0	0	9/60; 15	Other (3)
Cmin					0/12; 0	0	0/12; 0	0
Cmin-2 [a]					0/60; 0	0	54/60; 90	M184V/I (4/7) [b]
Cmin	Cmin-2				0/12; 0	0	7/12; 58	M184I (1/2) [b]
Cmin	Cmin-2	Cmin	Cmin-2		0/12; 0	0	0/12; 0	0
Cmin-4					31/36; 86	Other (1/1) [b]	36/36; 100	M184I (1/3) [b]
Cmin	Cmin-4	Cmin	Cmin-4		0/12; 0	0	12/12; 100	M184I (4/4)

[a] Previously determined data; [b] Other mutations were G163R in integrase for BIC+FTC+TAF and V75I, V118I, T215A/I, and K219N/R in reverse transcriptase and M50I, L74M, A128T, G140E, P145S, S153F, E157K, and R263K in integrase for DTG+3TC; [c] (number of cultures with M184V/I/number of cultures with emergent resistance)

Conclusion. BIC+FTC+TAF has high *in vitro* forgiveness and consistent protection against emergence of drug resistance during simulations of short lapses in adherence. Higher DTG+3TC exposure, whether constant or intermittent, was better at preventing or delaying VB than lower DTG+3TC exposures, but DTG+3TC was less forgiving than BIC+FTC+TAF. Prevention of viral replication and resistance development is necessary to maintain lifelong viral suppression, particularly in the real world where drug adherence is often imperfect.

Disclosures. Andrew Mulato, BS, MBA, Gilead Sciences, Inc. (Employee, Shareholder) Rima K. Acosta, BS, Gilead Sciences, Inc. (Employee, Shareholder) Stephen R. Yant, PhD, Gilead Sciences, Inc. (Employee, Shareholder) Tomas Cihlar, PhD, Gilead Sciences, Inc. (Employee, Shareholder) Kirsten L. White, PhD, Gilead Sciences, Inc. (Employee, Shareholder)

1449. Frequency and Antimicrobial Susceptibility of Coagulase-Negative Staphylococcal (CoNS) Species Isolated from Clinical Specimens in United States and European Hospitals

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Session: P-66. Resistance Mechanisms

Background. CoNS represent an important cause of bloodstream infections, osteoarticular infections, foreign-body-associated infections and endocarditis. We evaluated the frequency of CoNS species and the activity of dalbavancin (DALB) in comparison to vancomycin (VAN), daptomycin (DAP) and other agents against a large collection of CoNS isolates.

Methods. 5,088 CoNS isolates causing clinically significant infection were consecutively collected from 122 medical centers located in the United States (79 centers) and Europe (43 centers in 21 nations) over 6 years (2014-2019) and susceptibility tested by CLSI broth microdilution methods against DALB and comparators. Species identification was confirmed by MALDI-TOF.

Results. Most isolates were from bloodstream (BSI; 53.5%) or skin/skin structure infections (28.5%). *S. epidermidis* was the most common species overall (54.6%; Table) and for BSI (61.3%). The second most common species were *S. lugdunensis* overall (12.3%) and *S. hominis* for BSI (14.7%). DALB ($MIC_{50/90}$, 0.03/0.06 mg/L) inhibited > 99.9% of CoNS isolates at the susceptible (S) breakpoint established by CLSI for *S. aureus* (≤ 0.25 mg/L) and was 8-fold more active than DAP ($MIC_{50/90}$, 0.25/0.5 mg/L; 99.9% S) and 32-fold more active than VAN ($MIC_{50/90}$, 1/2 mg/L; > 99.9% S). Linezolid was active against 98.7% of isolates ($MIC_{50/90}$, 0.5/1 mg/L). All species were inhibited at ≤0.25 mg/L of DALB, except *S. epidermidis* (> 99.9%) and *S. warneri* (98.9%; Table). The most DALB-S species were *S. capitis* and *S. simulans* ($MIC_{50/90}$, 0.015/0.03 mg/L for both species), whereas the highest DALB $MIC_{50/90}$ values were observed with *S. haemolyticus* and *S. saprophyticus* ($MIC_{50/90}$, 0.06/0.12 mg/L and highest MIC of 0.25 mg/L for both species). In contrast, 47.8% of *S. epidermidis* and 34.7% *S. haemolyticus* exhibited decreased susceptibility to VAN (MIC ≥ 2 mg/L), and 23.2% of *S. capitis* and 28.4% of *S. warneri* showed decreased susceptibility to DAP (MIC ≥ 1 mg/L). Overall oxacillin-S rate was 39.3%, varying from 3.0% for *S. saprophyticus* to 95.4% for *S. lugdunensis*. In general, BSI isolates were slightly less S than non-BSI isolates.

Conclusion. Antimicrobial susceptibility varied widely among CoNS species. DALB exhibited potent *in vitro* activity against all CoNS species.

Table 1

Species / no. tested overall	Cumulative % at DALB MIC of: 0.015	0.03	0.06	0.12	0.25[a]	DALB $MIC_{50/90}$	% at VAN MIC ≥2 mg/L	% at DAP MIC ≥1 mg/L
S. epidermidis (2,777)	21.1	76.2	96.1	99.5	>99.9	0.03/0.06	47.8	2.5
S. lugdenensis (625)	34.9	95.5	99.8	100.0		0.03/0.03	0.5	0.6
S. haemolyticus (449)	4.2	17.6	61.5	95.8	100.0	0.06/0.12	34.7	3.6
S. hominis (462)	33.3	86.6	98.3	100.0		0.03/0.06	9.6	0.6
S. capitis (267)	68.2	93.6	99.3	99.6	100.0	0.015/0.03	8.2	23.2
S. saprophyticus (169)	1.2	10.7	50.3	95.9	100.0	0.06/0.12	13.6	5.3
S. warneri (88)	35.2	70.5	90.9	97.7	98.9	0.03/0.06	8.0	28.4
S. simulans (65)	56.6	96.1	100.0			0.015/0.03	0.0	0.0
Other species (175)	40.6	77.1	94.3	98.9	100.0	0.03/0.06	6.9	11.4
All CoNS (5,088)	25.7	73.3	92.3	99.1	>99.9	0.03/0.06	33.9	4.8

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1450. Frequency of Carbapenem-resistant *Pseudomonas aeruginosa* Among Respiratory Pathogens Impacts First-Line Beta-Lactam Susceptibility: Potential Role for Ceftolozane/Tazobactam (C/T) and/or Imipenem/Relebactam (I/R)

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Session: P-66. Resistance Mechanisms

Background. Carbapenem-resistant *P. aeruginosa* (CRPA) are associated with increased mortality and impose significant treatment challenges for clinicians. Among CRPA, co-resistance to 1st line antipseudomonal agents piperacillin/tazobactam (TZP) and cefepime (FEP) is common and often results in delays to timely effective therapy. A simple strategy for identifying patients at risk for suboptimal therapy is evaluation of institutional or unit specific frequency of CRPA. The purpose of this analysis is to identify beta-lactam (BL) susceptibility trends based on CRPA frequency observed in intensive care units (ICU).

Methods. In 2016-2019, ~20 US institutions per year submitted up to 250 consecutive, aerobic or facultatively anaerobic, gram-negative pathogens from blood, intra-abdominal, urinary, and lower respiratory tract infections as part of the Study for Monitoring Antimicrobial Resistance Trends. A total of 871 *P. aeruginosa* (PA) isolates were collected from lower respiratory tract specimens obtained from ICU patients. MICs were determined using CLSI broth microdilution method and interpreted with CLSI 2020 or FDA breakpoints. Institutions were then stratified into one of three categories based on CRPA frequency: CRPA rates ≤20% (CR1), 21 – 40% (CR2), and ≥41% (CR3). BL susceptibility was then evaluated relative to CRPA frequency.

Results. Thirty-seven (46%), 25 (31%), and 18 (23%) institutions were stratified into CR1, CR2, and CR3, respectively. Overall, CRPA was identified in 28.4% of all