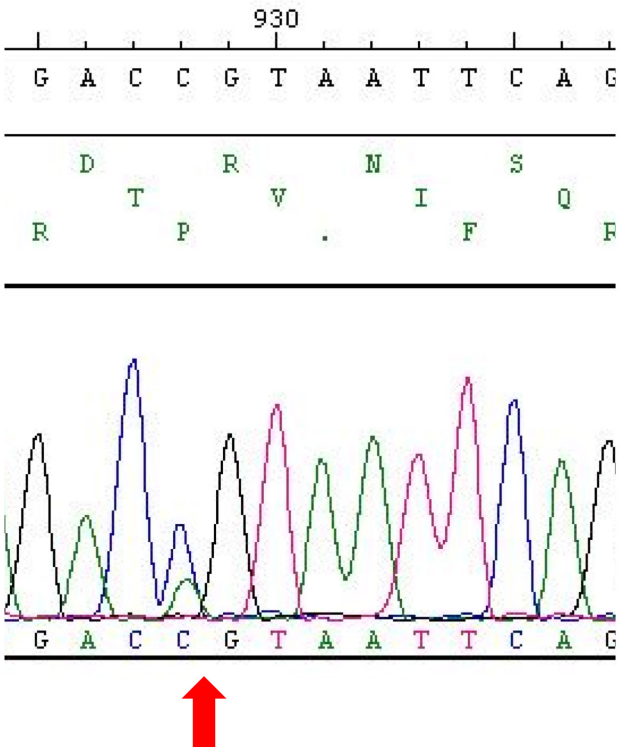
Figure 3: Sanger sequencing of *E. coli* 3p7



Disclosures. **All authors:** No reported disclosures.

610. Meropenem-vaborbactam (MV) *In Vitro* Activity Against Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) Isolates with Outer Membrane Porin Gene Mutations

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Background. Vaborbactam is a cyclic boronic acid β-lactamase inhibitor (BLI) developed to potently inhibit Ambler class A&C enzymes, including KPC carbapenemases. Metallo-β-lactamases (MBL) and some Class D oxacillinases (OXA) are not inactivated by vaborbactam. Meropenem-vaborbactam (MV) was recently approved for the treatment of carbapenem-resistant Enterobacteriaceae complicated urinary tract infections. Recent studies have identified outer membrane porin (Ompk35 and -36) mutations in *Klebsiella pneumoniae* (KP) as a mechanism of decreased susceptibility to MV. We evaluated the activity of MV against a historical cohort of *KP* clinical isolates with these porin gene mutations.

Methods. WGS of carbapenem-resistant *KP* clinical isolates was performed and those harboring mutations in Ompk35 or Ompk36 were selected for testing. Strain *KP* ATCC BAA-1705 was used as a positive control. Meropenem and MV minimum inhibitory concentrations (MIC) were determined by broth microdilution (BMD) in custom 96-well plates (ThermoFisher Scientific) with a constant 8 μg/mL vaborbactam concentration. The MIC of ceftazidime–avibactam (CZA) was determined by standard BMD reference methods and interpreted according to CLSI criteria.

Results. A total of 105 *KP* isolates with either partial or complete mutations in outer membrane porin genes were included in the analysis. All isolates were resistant to Meropenem. The median MV MIC was 0.03 μg/mL (range, 0.015 to >16 μg/mL). Eleven isolates (10.4%) were resistant to MV. Sixteen additional isolates (16.1%) demonstrated higher than expected MV MICs ranging from 1 to 4 μg/mL. Only 1/11 resistant isolates harbored a gene for MBL production. Gene mutations in bla_{KPC} were not detected. See Table 1 for characteristics of resistant isolates.

Conclusion. Resistance and decreased susceptibility to MV is demonstrated in a historical cohort of *KP* clinical isolates dating back to 2013. WGS reliably identifies porin variants secondary to gene mutations in Ompk35 and Ompk36 as the underlying mechanism of decreased susceptibility. CZA appears to retain activity against these isolates. Caution should be exercised regarding the empiric use of MV against increasingly resistant *KP* as a result of non-β-lactamase-mediated mechanisms.

Table 1. Whole genome sequencing and MICs of MV resistant isolates

		MIC (μg/ml)			Typing	Enzymes	Outer membrane porin variant	
Strain	Date	MV	MEM	CZA	MLST	β-lactamase	OmpK35	OmpK36
1	2012	>16/8	>8	2/4	ST-258	KPC-2; SHV-160	FS 121insG	ins Gly134-Asp135
2	2015	>16/8	>8	2/4	ST-258	KPC-2; SHV-160	FS 121insG	WT
3	2017	>16/8	>8	>64	ST-147	NDM-5; OXA-181; CTXM-15; SHV-11	Partial FS	ins Asp135, Thr136
4	2014	16/8	>8	1/4	ST258	KPC-2; SHV-160	FS 121insG	ins Gly134-Asp135
5	2013	16/8	>8	1/4	ST-258	KPC-2, SHV-160	FS 121insG	ins Gly134-Asp135
6	2014	4/8	>8	1/4	ST-258	KPC-2; SHV-160	FS 121insG	ins Gly134-Asp135
7	2013	16/8	>8	1/4	ST-258	KPC-2, SHV-11	FS 121insG	ins Gly134-Asp135
8	2013	>16/8	>8	0.5/4	ST-258	KPC-2; SHV-160	FS 121insG	WT
9	2013	8/8	>8	1/4	ST-258	KPC-2, SHV-160	FS 121insG	ins Gly134-Asp135
10	2013	8/8	>8	4/4	ST-258	KPC-2; SHV-160	FS 121insG	ins Gly134-Asp135
11	2017	>16/8	>8	8/4	ST-258	KPC-2; SHV-11; SHV-12	FS stop aa89	ins Gly134-Asp135

Disclosures. **All authors:** No reported disclosures.

611. Fosfomycin Resistance of Multidrug-Resistant *Escherichia coli* and Mechanisms of Fosfomycin Resistance

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Background. Fosfomycin is one of the antibiotics that may be a candidate for the next-generation antimicrobial agents againt multidrug-resistant bacteria. To date, it is known that the resistance rate is not high for *Escherichia coli*. However, it is necessary to update the fosfomycin resistance rates in *E. coli* according to the studies that extended spectrum β-lactamase (ESBL) producing *E. coli* strains are highly resistance to fosfomycin. We evaluated the resistance rate of fosfomycin, the resistant mechanism of fosfomycin in *E. coli*, and the activity of fosfomycin against susceptible and resistant strains of *E. coli*.

Methods. A total of 283 clinical isolates was collected from patients with *Escherichia coli* species during the period of January 2018 to June 2018, in three tertiary hospitals of Republic of Korea. *In vitro* antimicrobial susceptibility tests were performed in all E. coli isolates using the broth microdilution method according to the Clinical and Laboratory Standard Institute (CLSI). Multilocus sequence typing (MLST) of the Oxford scheme was conducted to determine the genotypes of *E. coli* isolated. Fosfomycin genes were investigated for all fosfomycin-resistant *E. coli* strains.

Results. The overall resistance rate to fosfomycin was 10.2%, compared with 53.4%, 46.3%, 41.3%, 31.1%, 10.6%, 2.5%, and 2.1% for ciprofloxacin, cefixime, cefepime, piperacillin/tazobactam, colistin, ertapenem, and amikacin, respectively. The 29 fosfomycin-resistant isolates did not show a clonal pattern on the phylogenetic tree. *MurA* and *glp* genes were identified in all strains. *FosA3* were identified in two strains and *uhp* gene were identified in 4 strains. In time-kill curve studies, fosfomycin was more bactericidal than cefixime against all sensitive *E. coli* strain. Morever, fosfomycin was more bactericidal than piperacillin/tazobactam against ESBL-producing *E. coli* strain.

Conclusion. The resistant rate of fosfomycin to *E. coli* is still low. Fosfomycin was active against *E. coli* including ESBL producing strains.

Disclosures. **All authors:** No reported disclosures.

612. Molecular Mechanisms Leading to Ceftolozane–Tazobactam Resistance in Clinical Isolates of *Pseudomonas aeruginosa* from Five Latin American Countries

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Background. Ceftolozane–tazobactam (C/T) is a combination of an antipseudomonal cephalosporin with a known β-lactamase inhibitor, with a potent in vitro activity against P. aeruginosa (Pae), without activity against carbapenemases. Among the mechanisms of resistance to C/T that have emerged, substitutions in the Pseudomonal-derived cephalosporinase (PDC), in AmpR, and in some ESBLs, are the most commonly described. The aim of this study was to identify the molecular mechanisms responsible for the in vitro non-susceptibility (NS) to C/T in a group of clinical Pae strains from Latin America.

Methods. Clinical Pae isolates (n = 508) were collected between January 2016 and October 2017 from 20 hospitals located in Argentina, Brazil, Chile, Colombia, and Mexico. Minimum inhibitory concentrations (MICs) to C/T were determined by standard broth microdilution and interpreted according to CLSI M100 S28 breakpoints. Production of carbapenemases in Pae isolates displaying NS to C/T was assessed by carbaNP* followed by PCR to detect blaKPC, blaNDM-1, blaVIM and blaIMP. Illumina whole-genome sequencing (WGS) was performed for isolates in which NS to C/T was not mediated by carbapenemases. The presence of mutations in PDC, AmpR, oprD and dacB as compared with PAO1, was evaluated.

Results. According to the CLSI breakpoints, 162/508 (32%) Pae isolates demonstrated NS to C/T. Due to the absence of growth, only 151/162 were further processed. Table 1 summarizes the results obtained by carbaNP*, PCR and WGS performed on these isolates. In 53% of the isolates, NS to C/T was explained by the production of at least one carbapenemase, KPC or VIM. WGS revealed that in addition to substitutions in PDC and AmpR, some isolates carried mutations in oprD and dacB (encoding PBP4) genes. The molecular mechanism of resistance in 4/56 isolates is yet to be determined.

Conclusion. Carbapenemase production is the most common mechanism of resistance to C/T detected in this study. VIM and KPC were detected in equal proportions, while none of the isolates was found to carry IMP or NDM. Further studies are warranted to establish the role of the novel substitutions found in PDC and AmpR, as well as the degree to which the mutations found in oprD and dacB contribute to the NS phenotype in some isolates.

Table 1. Summary of the molecular mechanisms of resistance to C/T detected.

Country	Isolates	C/T NS (%)	CarbaNP (+) [a]	PCR (+)		WGS Genotypes		
				bla KPC	*bla* VIM	QC [b]	Other β-lactamases (n)	Other mechanisms (n)
Argentina	30	9 (30%)	2/9	0	0	7/7	*bla* PER-1 (1)	PDC-1 + *oprD* mut + *dacB* mut (3) PDC-1 + *dacB* mut (2) PDC-8 (1) , PDC-33 (1)
Brazil	41	13 (31,7%)	8/13	1	0	4/5	None	PDC-3 + *oprD* mut + *dacB* mut (1) PDC-16 + AmpR mut (1) PDC-5 (1) ND [c] (1)
Chile	63	12 (19,45)	7/10	2	4	3/3	None	PDC-3 + *oprD* mut + *dacB* mut (1) PDC-11 (1) ND [c] (1)
Colombia	248	84 (33,9%)	55/80	30	26	22/22	*bla* OXA-2 (5)	PDC-1 / -3 + *oprD* mut + *dacB* mut (4) PDC-1 + *oprD* mut + *dacB* mut + AmpR mut (1) Other PDC [d] + AmpR mut (15) ND (2)
Mexico	127	44 (35,6%)	8/39	0	3	20/21	*bla* IMP-13 (1) *bla* OXA-2 (6)	PDC-3 + *oprD* mut + *dacB* mut (7) PDC-3 + AmpR mut (3) Other PDC [d] + AmpR mut (9) PDC-80 (1)

[a] Two (2) strains from Chile, four (4) strains from Colombia, and five (5) strains from Mexico could not be further processed due to absence of growth. [b] Quality control of the sequences included coverage > 25. [c] ND: Not Determined. [d] Other PDCs identified include PDC-11-like, -12-like, -19-like, -24, -22-like, -37, -64, and -67. Some substitutions found are R53Q, L150I, V213A, E221G (in PDC-80), T267S, and H297Q.

Disclosures. **All authors:** No reported disclosures.

613. Suppression of Daptomycin Resistance Development in *Staphylococcus aureus* Is a Class Effect of β-Lactams and Is Independent of Daptomycin–β Lactam Synergy

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Background. Previous studies demonstrate that adding oxacillin during daptomycin (DAP) exposure can prevent DAP resistance development in community-acquired (ST8/USA300) MRSA, presumably by preventing *mprF* mutation. Hospital-acquired strains, such as MRSA sequence types 5 and 239, typically have higher β-lactam (BL) minimum inhibitory concentrations (MICs) than their community-acquired counterparts and are often less toxigenic, more multidrug-resistant and more refractory to primary antistaphylococcal therapies. It is unknown whether DAP resistance prevention occurs in hospital-acquired MRSA lineages or if augmenting DAP therapy with BL antibiotics other than oxacillin would prevent DAP resistance development.

Methods. MRSA ST5/USA100 (D592) and ST239 (JKD6004) differ in the degree to which BL enhances DAP activity. D592 and JKD6004 were passaged in escalating concentrations of DAP in a stepwise fashion *in vitro* as described previously. Following 28 days of serial passage all replicates were passaged twice on mannitol-salt agar and tested for DAP MIC by Etest. Parallel passages were performed in media supplemented with BL antibiotics. Between-group differences in DAP MIC suppression effectiveness among individual BLs compared with nafcillin was evaluated using Kruskal–Wallis rank-sum testing with Holm-adjusted post-hoc Dunn testing.

Results. Passage of D592 or JKD6004 in DAP resulted in highly DAP-resistant isolates (median ≥256 mg/L, IQR [96,256]). In contrast, when passages were performed in the presence of DAP+BL, DAP resistance development was suppressed. No between-group differences in DAP MIC suppression effectiveness were observed among individual BLs compared with nafcillin. Highly DAP-resistant isolates demonstrated variable collateral susceptibility to BL monotherapy but were frequently susceptible to combination antibiotic exposure.

Conclusion. Addition of β-lactams to DAP can prevent DAP resistance development *in vitro* in ST5/USA100 and ST239 MRSA, consistent with findings in ST8/USA300 lineages. Furthermore, this ability appears to be a class effect of β-lactam antibiotics and is independent of the extent of DAP-BL synergy. This provides evidence to support the use of BL combination therapy without regard to staphylococcal lineage or specific BL used.

Disclosures. **All authors:** No reported disclosures.

614. Metabolic Interventions for the Resensitization of Daptomycin-Resistant (DAP-R) *Streptococcus mitis-oralis* Strains to DAP *In vitro* and *Ex Vivo* in a Simulated Model of Experimental Endocarditis (IE)

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Background. *Streptococcus mitis-oralis* is a leading cause of IE. Treatment of this pathogen is limited by frequent high-level β-lactam resistance and the propensity to develop high-level DAP-R during DAP exposure. The current study elucidated key metabolic perturbations associated with high-level DAP-R in prototype *S. mitis-oralis* strain 351, following *in vitro* selection of DAP-R by 10-day serial passage in sub-inhibitory DAP. Furthermore, to test translatability of such metabolic changes (see below), the synergistic activity of combinations of DAP plus a strategic metabolic inhibitor (i.e., fosfomycin) vs. DAP or fosfomycin alone was assessed, using DAP-R *S. mitis-oralis* strain 351-D10 (MIC >256 μg/mL) *in vitro* and in an *ex vivo* IE model.

Methods. MICs. E test Growth Curve: Optical density (OD600) determined spectrophotometrically at 0–8 hours glyceraldehyde-3-phosphatedehydrogenase (GAPDH) activity. Kit from BioVision®. Metabolomics: one-dimensional ^{1}H NMR-MS and two-dimensional ^{1}H-^{13}C HSQC *in vitro* time-kill assay: Using sub-MIC/MIC drug concentrations (initial inoculum ~1 × 10^{5} CFU/mL) for 0, 2, 4, 6, and 8 hours. *Ex vivo* IE model: Simulated endocardial vegetations (SEVs) quantitatively cultured at 0, 4, 8, 24, 32, 48, and 72 hours with DAP or fosfomycin alone or in combination.

Results. NMR metabolomics analysis identified a number of metabolite differences in the 351 D10 DAP-R vs. 351 DAP-S strain (Figure 1). These data are consistent with a significant reduction in GAPDH activity (a glycolytic enzyme) in 351-D10 vs. 351 strain. Based on these metabolic changes, fosfomycin (a phosphoenolpyruvate analog) was chosen as a strategic metabolic inhibitor to attempt to "resensitize" our DAP-R *S. mitis-oralis* strain to DAP. The combination of DAP + fosfomycin demonstrated synergistic killing of the DAP-R strain vs. DAP or fosfomycin alone in the *in vitro* time-killing assays. Moreover, the DAP-R strain was synergistically cleared from SEVs by DAP + fosfomycin in the *ex vivo* IE model.

Conclusion. Taken together, these data indicate there are unique metabolome signatures associated with the DAP-R phenotype in *S. mitis-oralis*. In addition, these data provide support for further studying the use of strategic *S. mitis-oralis* metabolic inhibitors in additional strain-sets to resensitize DAP-R strains to DAP, using *in vitro*, *ex vivo* and *in vivo* models.

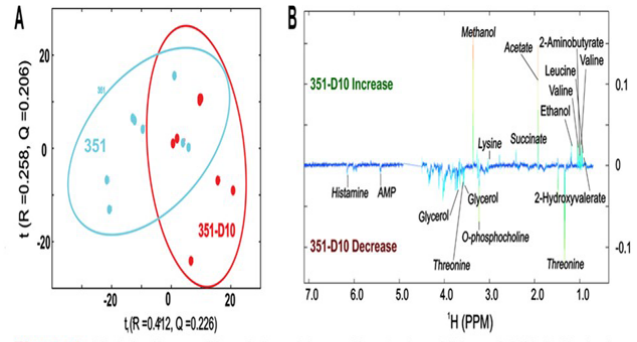

Figure 1. Metabolic profile of *S. mitis-oralis* strains 351 and 351-D10 during exponential growth. (A) PCA scores plot (R^2 0.88, Q^2 0.50) generated from the 1D ^{1}H NMR spectra of *S. mitis-oralis* cell lysates for the 351 DAP-S (blue) and the DAP-R strain 351-D10 (red).

Disclosures. **All authors:** No reported disclosures.