615. Daptomycin (DAP) Synergy with β-Lactams in DAP-Resistant (DAP-R) *E. faecium* (*Efm*) Is Dependent On PBP5 Sequence and β-Lactam-binding Affinity

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Background. DAP in combination with β-lactams is a viable option to treat recalcitrant DAP-R/tolerant strains of *Efm*. Ampicillin (AMP), ceftaroline (CPT), and ertapenem (ERT) have the best synergism. Using a DAP tolerant strain (503; DAP MIC 2 μg/mL) of *Efm*, we previously showed that AMP, CPT, and ERT combined with DAP were effective in reducing bacterial loads and prevented emergence of resistance in a simulated endocardial vegetation model. However, against a DAP-R *Efm* strain (R497, DAP MIC of 16 μg/mL), CPT, ERT failed to synergize with DAP. Here, we dissect the mechanistic basis of the differing DAP plus β-lactam synergistic effect.

Methods. We performed comparative transcriptional profiling of *pbp* genes in *Efm* 503 vs. R497 using qRT–PCR. PBP5 protein levels were assessed by immunoblotting. The β-lactam-binding affinity of PBPs was quantified with bocillin-FL staining and SDS–PAGE. PBP5 sequences of *Efm* Com15 (AMP and DAP-susceptible strain) and clinical strains S447, 503 and R497 (all with AMP MIC > 256 μg/mL) were compared *in silico* to identify amino acid (AA) differences in key protein sites which were verified with sequencing

Results. *Pbp* gene transcripts and PBP5 amounts were similar between 503 vs. R497. Interestingly, bocillin SDS–PAGE showed increased β-lactam binding affinity in PBP5 of 503 compared with that of R497 and S447. PBP5 sequences of S447 and R497 were identical. All three clinical strains had classic mutations (M485A and 466'S) important for high-level AMP-R. However, 503 had additional substitutions in the transpeptidase domain (H408Q, A462V, T546N, T558A, S582G, V586L) and penicillin-binding domain (Q632K, L642P) compared with R497 and S447. The latter AA sequences in 503 are common to AMP-susceptible *Efm* strains

Conclusion. We uncovered that a "hybrid" *pbp5* allele of 503 (DAP-tolerant) correlated with synergism of DAP plus AMP, CPT or ERT and was associated with increased PBP5 β-lactam binding affinity. Lack of synergism of DAP plus CPT or ERT is associated with specific PBP amino acids in the transpeptidase and penicillin-binding domains. Thus, *pbp5* alleles are major determinants of the DAP plus β-lactam synergistic effect and could be used as a diagnostic tool to guide therapy in recalcitrant *Efm* infections

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616. Evaluation of *In Vitro* Susceptibility to Ceftazidime/Avibactam of Clinical Isolates of Carbapenem Nonsusceptible Gram-Negative Bacilli from Colombia

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Background. Ceftazidime/avibactam (CZA) is a combination of a third-generation cephalosporin and a diazabicyclooctane β-lactamase inhibitor, which is active against a broad range of class A, C and D β-lactamases. In Colombia, high rates of multidrug-resistant *Enterobacteriaceae* (*Ent*)and *P. aeruginosa* (*Pae*) have been reported. Of special concern are KPC enzymes endemic in *Ent* and found in *Pae,* which are associated with higher mortality and healthcare costs, as well as limited therapeutic options. Herein, we evaluate the susceptibility of clinical isolates of carbapenem nonsusceptible *Ent* (CNS-E) and *Pae* (CNS-P) to CZA with the aim of understanding its role as a therapeutic option for these bacteria.

Methods. Three hundred ninety-nine nonduplicate clinical isolates of carbapenem nonsusceptible Gram-negative bacilli were collected in 13 medical centers from 12 Colombian cities, from January 2016 to October 2017 (137 *K. pneumoniae* [*Kpn*], 76 *E. coli*, 34 *Enterobacter* spp., 21 *S. marcescens* [*Sma*] and 131 *Pae*). CNS-E was defined as minimum inhibitory concentrations (MIC) ≥1 mg/L for ertapenem and CNS-P was defined as MIC ≥4 mg/L for meropenem. MIC were determined by broth microdilution and interpreted according to current CLSI guidelines. CZA MIC were determined using double dilutions of ceftazidime and a fixed concentration of avibactam of 4 mg/L. Comparator agents were ceftazidime, cefepime, piperacillin/tazobactam, imipenem, meropenem, tigecycline (TGC), and fosfomycin (FOS).

Results. Antimicrobial activity of CZA and comparators is shown in Table 1. CZA susceptibility ranged from 69% in *Kpn* to 81% in *Sma,* whereas 49% of CNS-P were susceptible to CZA. In both, CNS-E and CNS-P, CZA was superior to all other tested β-lactam compounds. Notably, in CNS-E CZA susceptibility was comparable to FOS and TGC (except for TGC in *Sma*).

Conclusion. CZA is the most active β-lactam against CNS-E and CNS-P. CZA nonsusceptibility suggests the presence of other resistance mechanisms, such as class B β-lactamases that are not inhibited by avibactam, and which are more frequently reported in CNS-P. Our results highlight the key role of new agents such as CZA in KPC endemic countries and the need for surveillance studies to determine the nature of resistance mechanisms.

Table 1. Antimicrobial activity of ceftazidime/avibactam and comparators against 399 clinical isolates of carbapenem non-susceptible *Enterobacteriaceae* and *P. aeruginosa* from 13 Colombian hospitals

Organism	Isolates	CZA%S	CAZ%S	FEP%S	TZP%S	IMI%S	MER%S	TGC%S	FOS%S
K. pneumoniae	137	69	0	0	0	18	23	73	80[a]
E. coli	76	72	0	0	0	45	47	64	76
Enterobacter spp	34	74	12	12	21	29	38	76	71[a]
S. marcescens	21	81	5	5	19	19	24	52	81[a]
P. aeruginosa	131	45	27	29	30	1	0	-	79[b]

CZA: ceftazidime/avibactam; CAZ: ceftazidime; TZP: piperacillin/tazobactam; FEP: cefepime; IMI: imipenem; MEM: meropenem; TGC: tigecycline; FOS: fosfomycin

[a] Fosfomycin breakpoints for *Enterobacteriaceae* were extrapolated from *E. coli* breakpoint by CLSI (fosfomycin non-susceptible MIC ≥ 128 mg/L).

[b] There are no fosfomycin breakpoint for *P. aeruginosa* by EUCAST or CLSI. ECOFF of ≤ 128 μg/ml by EUCAST for *P. aeruginosa* was applied.

Carbapenem non-susceptibility was defined as MIC ≥ 1 mg/L for ertapenem in *Enterobacteriaceae* and MIC ≥ 4 mg/L for meropenem non-susceptibility in *P. aeruginosa.*

Ceftazidime non-susceptible MIC ≥ 8 mg/L for *Enterobacteriaceae* and ≥ 16 mg/L for *P. aeruginosa*.

Cefepime non-susceptible MIC > 4 mg/L for *Enterobacteriaceae* and ≥ 16 mg/L for *P. aeruginosa*.

Piperacillin/tazobactam non-susceptible MIC ≥ 32/4 mg/L for *Enterobacteriaceae* and *P. aeruginosa*.

Ceftazidime/avibactam susceptible MIC ≤ 8/4 mg/L for *Enterobacteriaceae* and *P. aeruginosa* and ceftazidime/avibactam resistant MIC ≥ 16/4 mg/L for *Enterobacteriaceae* and *P. aeruginosa*.

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617. Efficacy of Dimercaptosuccinic Acid (DMSA), a Zinc Chelator, in Combination with Imipenem Against Metallo-β-Lactamase Producing *Escherichia coli* in a Murine Peritonitis Model

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Background. A strategy used by bacterial strains to resist β-lactam antibiotics is the expression of metallo-β-lactamases (MBL) requiring zinc for activity. The use of a zinc chelator may restore carbapenem activity against MBL-producing Enterobacteriaceae. DMSA is a heavy metal chelator approved in humans with a satisfactory safety record. Our objective was to evaluate the activity of DMSA in combination with carbapenems, *in vitro* and in a fatal murine peritonitis model, against MBL-producing *Escherichia coli*.

Methods. Isogenic derivatives of wild-type *E. coli* CFT073 producing the MBL NDM-1, VIM-2, IMP-1, and the serine carbapenemases OXA-48 and KPC-3 were constructed. Minimum inhibitory concentrations (MICs) of imipenem, meropenem, and ertapenem were determined against each strain alone or in combination with DMSA. Mice were infected with *E. coli* CFT073 or NDM-1 and treated intraperitoneally for 24 hours with imipenem 100 mg/kg every 4 hours, DMSA 200 mg/kg every 4 hours, or both. Mice survival rates and bacterial counts in peritoneal fluid (PF) and spleen were assessed at 24 hours.

Results. *In vitro*, DMSA in combination with each carbapenem permitted a significant decrease of the MICs against all MBL-producing strains, in a concentration-dependent manner. The maximum effect was found for the NDM-1 strain with a 6- to 8-fold MIC reduction, depending on the carbapenem used. NDM-1 strain became susceptible to carbapenems with concentrations of DMSA ≥6 mM. Increasing zinc concentrations above 1 mg/L (average human plasma concentration) did not alter this effect. No benefit of DMSA was observed against non-MBL strains. *In vivo*, when used alone, the DMSA regimen was not toxic in uninfected mice and ineffective against NDM-1-infected mice (100% mortality). Combination of imipenem and DMSA significantly reduced bacterial counts in PF and spleen as compared with imipenem alone ($P < 0.001$), and reduced mortality, although not significantly (11% vs. 37%, respectively, $P = 0.12$). No benefit of the combination was observed against CFT073.

Conclusion. DMSA is highly effective *in vitro* in reducing carbapenems MICs against MBL-producing *E. coli* and appears as a promising strategy in combination with carbapenems for the treatment of NDM-1-related infections.

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618. Fluconazole-Resistant *Candida albicans* Vaginitis with Cross-Resistance to Azoles: A Case Report

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Background. Local and systemic use of azole derivatives are common in the treatment of vulvovaginal candidiasis. However, there are cases unresponsive to these agents. Herein, we present treatment and follow-up of a patient with fluconazole–itraconazole and voriconazole-resistant recurrent vaginal candidiasis.

Methods. A 37-year-old woman with no comorbidity used topical and oral antifungal/antibacterial medications (including fluconazole and itraconazole) in the treatment of recurrent vulvovaginitis, was hospitalized due to continuous complaints. Intense, white-colored, odorless vaginal discharge was observed on physical examination. Urine and vaginal swab samples were taken for mycological and bacteriological culture. Metronidazole (500 mg 3x1 i.v.) and high dose fluconazole (600 mg/day i.v.) were initiated empirically for the possibility of dose-dependent resistant Candida infection, but there was no clinical response.

Results. Candida albicans was isolated in vaginal swab culture, but response to systemic fluconazole treatment for one week was inadequate. Antifungal susceptibility test was performed by microdilution method according to CLSI M27A3 guidelines and MIC values were reported respectively; fluconazole 4 μg/mL (SDD), itraconazole 1 μg/mL (R), posaconazole 0.06 μg/mL (WT), voriconazole 0.25 μg/mL (SDD), anidulafungin ≤ 0.015 μg/mL (S), amphotericin B 0.06 μg/mL (WT). For the resistance mechanism, point mutation in the ERG11 gene and MDR1 and MDR2 from efflux pumps were investigated and only the G464S mutation was detected in the ERG11 gene. Treatment was switched to IV anidulafungin (200 mg on day 1 followed by 100 mg/day). Clinical response was achieved in the patient whose complaints were reduced, and there was no Candida in the repeated vaginal swab culture taken on day 3 of treatment. The patient was discharged after 2 weeks of treatment. She had no recurrence after 2 years follow-up.

Conclusion. It should be kept in mind that resistant strains may be responsible for recurrent and unresponsive vulvovaginal candidiasis cases. Although there is no case report in which anidulafungin is used for treatment and it should be kept in mind that the anidulafungin is also in the treatment as it is summarized.

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619. High Multidrug-Resistant due to TEM and CTX-M-1 Types of Extended-Spectrum β-Lactamase and *bla*NDM-1 Type Carbapenemase Genes among Clinical Isolates of Gram-Negative Bacilli in Asella, Central Ethiopia

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Background. Acute infectious diseases and sepsis are among the leading causes of mortality in Ethiopia. The lack of local data concerning causative pathogens and resistance patterns results in suboptimal empirical treatment and unfavorable clinical outcome. The objective of this study was the characterization of bacterial pathogens in hospitalized patients with febrile infections in Central Ethiopia.

Methods. In total, 684 patients ≥1 year of age with fever admitted to the Asella Teaching Hospital from April 2016 to June 2018 were included. Blood and other appropriate clinical specimens were cultured. Susceptibility testing was performed using the Kirby–Bauer method and VITEK2. Confirmation of species identification and identification of resistance genes were conducted using MALDI-ToF and PCR at a microbiology laboratory in Düsseldorf, Germany.

Results. In total, 684 study participants were included; 54% were male and mean age was 26.7 years. Thus, the overall culture positivity rate was 7.5%. Of the 83 cultured organisms, 38(46%) were Gram-negative, 43(52%) Gram-positive, and 2(2%) Candida species. Among the 38 Gram-negative isolates, 16(42%) were *E. coli*, 15(39%) *K. pneumoniae*, and 4(11%) *P. aeruginosa*. Resistance against commonly used antibiotics for Gram-negative at the study site was: piperacillin/tazobactam 48%(13), ampicillin/sulbactam 93% (25), cefotaxime 89%(24), ceftazidime 74%(20), Cefipime 74%(20), meropenem 7%(2), amikacin 4% (1) and gentamicin 56%(15). Of 27 Gram-negative available for resistance-gene detection, blaNDM-1 was detected in one *K. pneumoniae* isolate and blaNDM-1 plus blaOXA-51 in *A. baumannii*. 81%(22/27) of the Gram-negative rods were confirmed to contain ESBL-genes as follows: TEM 17(77%), CTX-M-1-group 15(68%), SHV-6(27%) and CTX-M-9-group 2(9%). Among isolated *S.aureus,* 1(5%) was confirmed to be Methicillin-resistant *S. aureus*.

Conclusion. We found a high prevalence (81%) of ESBL-producing bacteria and 7.4% carbapenem resistance at the study site. More than half of Gram-negative isolates had two or more mobile resistance genes. These findings warrant the need for local national multidrug-resistant surveillance. Strengthening of antimicrobial stewardship programs is needed in order to face the threat of multidrug-resistant bacteria.

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620. Sub-MIC Concentrations of Levofloxacin and Delafloxacin Enhance *Staphylococcus aureus* Biofilm Formation: Significance of Maximizing Exposure

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Background. Fluoroquinolones are utilized in Staphylococcal prosthetic joint infections due to their anti-biofilm activity. When antibiotic dosing is not optimized or antibiotics do not reach the site of infection, additional virulence factors may upregulate. We aimed to determine whether exposure to sub-MIC concentrations of levofloxacin and delafloxacin affect biofilm formation in *Staphylococcus aureus.*

Methods. This study utilized 50 diverse methicillin-susceptible *S. aureus* (MSSA) clinical isolates collected between 2004 and 2018. Sources included blood, skin/tissue, bone, and joint fluid. Minimum inhibitory concentrations and minimum bactericidal concentrations were identified according to CLSI. Biofilm assays were conducted as previously described by our program. Biofilm quantification was categorized as strong (OD570 ≥ 2), moderate (OD570 ≥ 1 and < 2), or weak (OD_{570} < 1). Prevention assays were conducted with the addition of increasing concentrations of delafloxacin or levofloxacin. We evaluated the amount of isolates that demonstrated increased biofilm formation in the presence of sub-MIC concentrations and extent of biofilm enhancement. Percent change was calculated between OD570 of the isolate growth control without antibiotic exposure and peak biofilm OD570 when exposed to the antibiotic.

Results. Of the 50 MSSA isolates, 14 (28%) exhibited moderate/strong formation and 36 (32%) exhibited weak biofilm formation. 52% and 58% of the isolates demonstrated a ≥50% increase in formation when exposed to sub-MIC concentrations of delafloxacin and levofloxacin, respectively. None of the strong biofilm formers demonstrated a ≥50% peak increase in formation when exposed to the antibiotics. Of the isolates that demonstrated a ≥50% peak increase, the average percent change was 267% (±29) with levofloxacin and 258% (±33) with delafloxacin.

Conclusion. Sub-MIC concentrations of delafloxacin and levofloxacin increased biofilm formation in *S. aureus* isolates that normally exhibit weak or moderate biofilm formation when not in the presence of antibiotics. Maintaining appropriate fluoroquinolone concentrations at the site of action is critical in preventing enhancement of biofilm formation. Further research is needed to identify the mechanism behind this increase.

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621. *In vitro* Ceftazidime: Avibactam Resistance in Carbapenem-Resistant Enterobacteriaceae Isolates

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Background. Ceftazidime–avibactam (CAZ-AVI) is a new antibiotic with activity against many Carbapenem-resistant Enterobacteriaceae (CRE). Although CAZ-AVI resistance in CRE has been reported, it is not consistently assessed. Our study aimed to assess the prevalence of CAZ-AVI resistance in CRE isolated from patients with and without prior exposure to CAZ-AVI.

Methods. We tested 116 CRE isolates for CAZ-AVI resistance by Kirby–Bauer (KB) disk diffusion susceptibility. Resistant isolates were verified by repeat KB and E-test performed by the Stony Brook Hospital laboratory. The bla_{KPC} gene of resistant strains was amplified by PCR and sequenced. Patient data were used to determine whether patients were colonized or infected, and whether they were exposed to CAZ-AVI.

Results. Of the 116 CRE isolates from 86 patients (96 encounters), 50% were *Klebsiella* species, 23.2% were *Enterobacter* species, 10.3% *Escherichia coli* and 16.5% other CRE. They were recovered from colonized (37%) and infected (63%) patients of which 18% were treated with CAZ-AVI during their hospitalizations (median duration of therapy, 6 days). Two CRE isolates (1.7%) were found to be resistant on repeated testing. One isolate was *K. pneumoniae* derived from the sputum of a patient diagnosed with ventilator-associated pneumonia who received 40 days of CAZ-AVI therapy prior to isolation of the resistant isolate (KB diameter 20 mm, MIC > 512 μg/mL by E-Test). Sequencing of the strain's bla_{KPC3} gene revealed a previously described Ambler-position D179Y mutation that has been shown to convey resistance. The second CAZ-AVI-resistant *K. pneumoniae* (KB diameter 19 mm, MIC 64 μg/mL by *E*-test) was isolated from the urine of a colonized patient naïve to CAZ-AVI therapy. The strain's bla_{KPC10} gene had no mutations.

Conclusion. In our strain collection, the rate of resistance to CAZ-AVI remains low <2%. Although we found one mutation (D179Y) previously linked to CAZ-AVI resistance we also discovered one *K. pneumoniae* isolate with *in vitro* resistance to CAZ-AVI that did not exhibit any bla_{KPC} mutations conveying CAZ-AVI resistance. Interestingly, this strain