Daptomycin (DAP) Synergy with β-Lactams in DAP-Resistant (DAP-R) E. faecium (EfM) Is Dependent On PBPS 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. Amoxicillin (AMP), ceftriaxone (CPT), and ertapenem (ERT) have the best synergy. 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-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 pfl genes in EfM less than 50 vs. R497 using qRT-PCR. PBPS protein levels were assessed by immunoblotting. The β-lactam-binding affinity of PBPs was quantified with bovine FL staining and SDS-PAGE. PBPs sequences of EbCom1 (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.** Pfl gene transcripts and PBPS amounts were similar between 503 vs. R497. In R497, the SDS-PAGE showed increased β-lactam binding affinity in PBPs 503 compared with that of R497 and S447. PBPS sequences of S447 and R497 were identical. All three clinical strains had classic mutations (M485A and 466S) important for high-level AMP-R. However, 503 had additional substitutions in the transpeptidase domain (H406Q, A407V, T546N, T588A, P582G, V5860L) 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” pfl5 allele of 503 (DAP-tolerant) correlated with synergy of DAP plus AMP, CPT or ERT and was associated with increased PBPS β-lactam binding affinity. Lack of synergy of DAP plus CPT or ERT is associated with specific PBP amino acids in the transpeptidase and penicillin-binding domains. Thus, pfl5 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.

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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.** Cefazidime/avibactam (CZA) is a combination of a third-generation cephalosporin and a novel dazabactan/cyclobatam β-lactam inhibitor, which inhibits a broad range of β-lactamases. In Colombia, high rates of multidrug-resistant Enterobacteriaceae (End) and P. aeruginosa (Pae) have been reported. Of special concern are KPC enzymes endemic in End 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 End (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) ≥4 mg/L for imipenem, meropenem, andertapenem were determined against each strain alone or in combination with DMSA.

**Results.** In reducing carbapenems MICs against MBL-producing strains, in vitro and in a fatal murine peritonitis model, against MBL-producing Enterobacteriaceae.

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617. Efficacy of Dimercapto succinic Acid (DMSA), a Zinc Chelator, in Combination with Imipenem Against Metallo-β-Lactamase Producing Enterococci (CVA) 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 Enterococchi.

**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, andertapenem 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 be-

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618. Flucloxacillin-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 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 3x.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 of recurrent vaginalvaginitis, 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 3x.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.

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 ß-Lactamase and blaNDM-1 Type Carbapenemase Genes among Clinical Isolates of Acinetobacter baumannii 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 21 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 resistant strains 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 species, 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-negatives at the study site was: piperacillin/tazobactam 48%(13), ampicillin/ sulbactam 93%(25), cefotaxime 89%(24), ceftazidime 74%(20), Cephalin 74%(20), meropenem 77%(2), amikacin 4%(1) and gentamicin 56%(15). Of 27 Gram-negative isolates available for resistance-gene detection, blaNDM-1 was detected in one K. pneumoniae isolate and blaNDM-1 plus blaoXA-51 in A. baumammii. 81%(22/27) of the Gram-negative rods were confirmed to contain ESBL genes as follows: TEM (1777%), CTX-M-1 group15%(19), SHV-6(27%) and CTX-M-9 group 29%(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 Emily C. Bodo, PharmD; Kathryn E. Daifinee, BS; Kerry LaPlante, PharmD; Rhode Island Infectious Diseases Research Program, Worcester, Massachusetts

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Background. Fluoroquinolones are utilized in Staphylococcal prosthetic joint infection treatment, but their antibiotic combination 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 (OD570 < 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 2(4%) exhibited weak biofilm formation. 52% and 58% of the isolates demonstrated a ≥250% increase in formation when exposed to sub-MIC concentrations of delafloxacin and levofloxacin, respectively. None of the strong biofilm former demonstrated a ≥250% peak increase in formation when exposed to the antibiotics. Of the isolates that demonstrated a ≥250% 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 Cefazidime: Avibactam Resistance in Carbapenem-Resistant Enterobacteriaceae Isolates Maymonah Belal, MD; Lori Villasis, MS, SM; Elizabeth Diago-Navarro, PhD, MPH; Michael Motley, BS; Allen Young, Eric Spitzer, MD, PhD; Bettina C. Fries, MD, FIDSA; Melinda Monteforte, BS, PharmD, BCPS AQ; Stony Brook University Hospital, East Setauket, New York

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Background. Cefazidime–avibactam (CAZ-AVI) is a new antibiotic with activity against many Carbapenem-resistant Enterobacteriaceae (CRE). Although CAZ-AVI treatment has been shown to be effective in CRE, it has not been 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 blabla_3 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 blabla_3 gene revealed a previously described Ambler-classification D1’79Y 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 naive to CAZ-AVI therapy. The strain blabla_3 gene contained KPC-2.

Conclusion. In our strain collection, the rate of resistance to CAZ-AVI remains low <2%. Although we found one mutation (D1’79Y) 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 blabla_3 mutations conveying CAZ-AVI resistance. Interestingly, this strain