Background. Resistance to CZA is a serious limitation of treatment for KPC bearing Enterobacteriaceae infections. Recently, a single amino acid substitution (D179Y) was described in KPC-2 and KPC-3 bearing CZA-resistant K. pneumoniae recovered from patients failing treatment. In class A β-lactamases the D179 residue is located at the neck of the omega loop and is critical for KPC catalytic activity. In attempts to understand the evolution of substrate specificity in KPC-2, the D179Y variant of KPC-2 was shown to be resistant to CZA (ceftazidime forms a long-lived acyl enzyme with in KPC-2), but susceptible to MEM. A similar observation was made in clinical and laboratory-generated K. pneumoniae and E. coli strains bearing D179Y KPC-3. We were compelled to explore the catalytic mechanisms of susceptibility to MEM of the D179Y variants in KPC-2 vs. KPC-3.

Methods. KPC-2, KPC-3, and D179Y in the respective KPC were cloned into an expression vector and the β-lactamase proteins were purified. 5 mg of each β-lactamase with and without MEM (1:1 molar ratio) was incubated for the time indicated and analyzed using the Quadruple Time-of-Flight (QTOF) timed mass spectrometry for the reaction intermediates. To assess thermal stability, denaturation melting curves were performed using a protein expression vector and the β-lactamase proteins were purified. 5 mg of each β-lactamase protein was incubated with and without MEM (1:1 molar ratio) for 24 hours at 50 °C. Results. The D179Y variant forms a stable acyl-enzyme with the substrate in KPC-3 and KPC-2, which can be detected up to 24 hours (Figure 1). This prolonged trapping of the acyl-enzyme by D179Y variants is not evident with the respective KPCs. Further, the tyrosine substitution at the D179 position (Tm = 48–52°C) destabilizes the KPC β-lactamases (TmKPC-2/3 = 52–56°C).

Conclusion. These data suggest that MEM acts as a covalent β-lactamase inhibitor more than as a substrate for KPC-2 and -3. The mechanistic basis of paradoxical susceptibility to carbapenems provides an impetus to develop better therapeutic approaches to the increasing threat of carbapenem resistance and highlights how the rational design of novel β-lactam-β-lactamase inhibitors must consider mechanistic bases of resistance.

![Figure 1](image1.png)

Disclosures. All Authors: No reported Disclosures.

183. Machine Learning Approaches to Predicting Resistance in Pseudomonas aeruginosa

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Background. Multi-drug-resistant (MDR) P. aeruginosa (PA) infections continue to cause significant morbidity and mortality in various patient groups including those with malignancies. Predicting antimicrobial resistance (AMR) from whole-genome sequencing data if done rapidly, could aid in providing optimal care to patients.

Methods. To better understand the connections between DNA variation and phenotypic AMR in PA, we developed a new algorithm, variant mapping and prediction of antibiotic resistance (VAMP), to build association and machine learning prediction models of AMR based on publicly available whole genome sequencing and antibiotic susceptibility testing (AST) data. A validation cohort of contemporary PA bloodstream isolates was sequenced and AST was performed. Accuracy of predicting AMR for various PA–drug combinations was calculated.

Results. VAMP was built from 3,393 bacterial isolates (83 PA isolates included) from 9 species that contained AST data for 29 antibiotics. 14,615 variant genotypes were identified within the dataset and 93 association and prediction models were built. 120 PA bloodstream isolates from cancer patients were included for analysis in the validation cohort. ~15% of isolates were carbapenem resistant and ~20% were quinolone resistant. For drug-isolate combinations where >100 isolates were available, machine-learning prediction accuracies ranged from 75.6% (PA and cefazidime; 90/119 correctly predicted) to 98.1% (PA and amikacin; 105/107 correctly predicted). Machine learning accurately identified known variants that strongly predicted resistance to various antibiotic classes. Examples included specific gyrA mutations in 78.1% (PA; P < 0.0001) and quinolone resistance.

Conclusion. Machine learning predicted AMR in P. aeruginosa across a number of antibiotics with high accuracy. Given the genomic heterogeneity of PA, increased genomic data for this pathogen will aid in further improving prediction accuracy across all antibiotic classes.

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1832. Development of an Ultrasensitive Field-Applicable Plasmodium falciparum Assay for Malaria Diagnosis and Eradication

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Background. Malaria control and eradication have been hampered by asymptomatic carriage which serves as a parasite reservoir. Low-density infections (< 100 parasites/µl) frequently fall below the limit of detection (LOD) of microscopy and rapid diagnostic tests (RDT) which are antigen-based tests. Molecular methods such as polymerase chain reaction are capable of higher sensitivity yet remain impractical for resource-limited settings. We describe development of an isothermal assay using the nucleic acid detection platform SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing), which may also be increasingly important as there has been