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 Quadrupole Time-of-Flight (QTOF) timed mass spectrometry for the reaction intermediates. To assess thermal stability, denaturation melting curves were run for 2 hours using 12 µM β-lactamase.

Results. The D179Y variant forms prolonged acyl-complexes with meropenem in KPC-3 and KPC-2, which can be detected up to 24 hours (Figure 1). This prolonged trapping of meropenem 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.

1831. 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 ceftazidime; 90/119 correctly predicted) to 98.1% (PA and amikacin; 105/107 correctly predicted). Machine learning accurately identifies known variants that strongly predicted resistance to various antibiotic classes. Examples included specific gyrA mutations (TRH; P < 0.00001) 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/microliter) 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...
rising detection of histidine-rich protein 2 (HRP2) gene deletions in *Plasmodium* spp. HRP2 is the most commonly used antigen in RDTs and deletion of this gene would render many RDTs obsolete.

**Methods.** SHERLOCK leverages the endonucleases of CRISPR-associated microbial adaptive immunity. Cas12a is an RNA-guided, DNA-cleaving enzyme, which can be programmed with guide RNAs to cleave nontarget reporter ssDNA. We exploit the nonspecific degradation of labeled ssDNA to detect the presence of the dsDNA target that activated Cas12a (Figure 1). Recombinase polymerase amplification (RPA) coupled with Cas12a detection enables a lower LOD. *Plasmodium falciparum* whole genomic DNA was compared with parasites cultured in red blood cells (RBCs) with known parasitemia and boiled at 95°C for 5 minutes for lysis of RBCs/parasites then diluted 1:2.5 to prevent solidification.

**Results.** This SHERLOCK assay detected simulated *Plasmodium falciparum* infection at attomolar LODs when applied to whole genomic DNA and simulated samples of infected RBCs spiked into whole blood. The genomic assay detected down to 0.2 parasites/microliter and the simulated sample detected to 10 parasites/microliter in the final reaction volume. In comparison, LODs from the initial input volume was 5aM and 250aM, respectively (Figure 2).

**Conclusion.** We demonstrate an isothermal nucleic acid detection platform capable of diagnosis in 60 minutes in a one-pot assay requiring minimal sample preparation and reaching an LOD recommended by the WHO for malaria eradication. In summary, we illustrate the utility of the SHERLOCK platform in application to malaria and global health.

**Disclosures.** All Authors: No reported Disclosures.

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**Results.** The initial screening revealed 82% (73/90) seroprevalence of anti-HA (H1) stem epitope antibodies, as determined by the differential binding to HA SS and it corresponding epitope-mutant probe. Using equimolar amounts, the multivalent presentation of HA SS on np probes induced significantly higher ADCC activity compared with the monovalent SS probes (2- to 6-fold increase). Further, ADCC activity was similarly reported against different influenza group 1 subtypes: H1, H2, and H5. Importantly, ADCC was mediated mainly by antibodies targeting the bNAb-epitope on the HA stem. In conclusion, we developed an assay to measure stem-specific ADCC activity using SS np probes.

**Conclusion.** Our results indicate a high prevalence of HA-stem antibodies with cross-reactive ADCC activity. Such assay could be utilized in the assessment of next-generation influenza vaccines.

**Disclosures.** All Authors: No reported Disclosures.

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**Background.** Generating a vaccine that confers complete protection and overcomes the high variability among influenza viruses is the major goal in designing a universal influenza vaccine. Currently, there is considerable interest in the broadly neutralizing antibodies (bNAb) targeting the conserved HA stem region. These antibodies have been shown to activate cellular immune responses, such as antibody-dependent cellular cytotoxicity (ADCC), in addition to their neutralization activity. We had previously demonstrated that immunization with H1-based stabilized stem (SS) nanoparticles (np) protects against heterosubtypic lethal challenge with H5N1 influenza virus, despite the absence of detectable H5N1 neutralizing activity. Here, we utilized these novel SS np probes to develop a new protocol to assess the ADCC activity mediated by stem-directed antibodies in human sera.

**Methods.** Human sera samples were initially screened for binding reactivity to H1 SS trimer using ELISA procedure. Of these, selected samples representing high, moderate, and low binders were further characterized for binding to H1 and H5 SS and their corresponding Astem (Ile45Arg/Thr49Arg) probes, and were analyzed for ADCC activity using a reporter bioassay.

**Disclosures.** All Authors: No reported Disclosures.