

Disclosures. All Authors: No reported Disclosures.

1826. Impact of Rapid Diagnostics and Ceftazidime-Avibactam on Mortality after Bacteremia Caused by Carbapenem-Resistant Enterobacteriaceae

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Friday, October 4, 2019: 2:00 PM

Background. Patients with bloodstream infections (BSIs) due to carbapenem-resistant Enterobacteriaceae (CRE) have long delays until receipt of appropriate antimicrobial therapy and high mortality rates. Rapid molecular diagnostics and novel therapies, such as ceftazidime-avibactam (CAZ-AVI), offer promise to improve outcomes, but their clinical impact is unclear.

Methods. We conducted an observational study of patients with CRE BSI from January 2016 to June 2018 at 8 New York and New Jersey medical centers. Patient demographics, comorbidities, clinical presentations, diagnostic methods, and treatments were compared between patients who died within 30 days of BSI onset and survivors. Independent risk factors for mortality were identified using logistic regression. We then compared time to receipt of active antimicrobial therapy between patients whose positive blood culture bottles underwent testing for the *Klebsiella pneumoniae* carbapenemase gene (*blaKPC* PCR) and patients where this test was not used.

Results. We identified 178 patients with CRE BSI (*K. pneumoniae*: $n = 104$, 58%; *Enterobacter cloacae*: $n = 26$, 15%; *Escherichia coli*: $n = 26$, 15%). The 30-day mortality rate was 38%. An increasing Acute Physiology and Chronic Health Evaluation II score (adjusted odds ratio [aOR] 1.06, $P = 0.005$) was independently associated with increased 30-day mortality; whereas, use of *blaKPC* PCR (aOR 0.31, $P = 0.005$), urinary tract source (aOR 0.12, $P = 0.001$), and source control (aOR 0.25, $P = 0.001$) were independently associated with survival. Initial targeted therapy with CAZ-AVI was associated with a 28% 30-day mortality rate, compared with a 49% 30-day mortality rate among patients who received a polymyxin or aminoglycoside ($P = 0.036$). Patients whose blood culture underwent *blaKPC* PCR were more likely to receive active antimicrobial therapy within 24 hours of BSI onset (42% vs. 28%; $P = 0.07$) and had a decreased median time until receipt of active therapy (25 hours vs. 46 hours; $P = 0.07$), although these differences did not achieve statistical significance.

Conclusion. The use of PCR to rapidly identify blood cultures with *blaKPC* and definitive therapy with CAZ-AVI instead of polymyxins or aminoglycosides were associated with decreased mortality after CRE bacteremia.

Disclosures. All Authors: No reported Disclosures.

1827. Genetic Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates Associated with the Development of Reduced Susceptibility to Vancomycin from Latin America

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Background. Vancomycin (VAN) is a first-line therapeutic option for severe MRSA infections, especially in Latin America where other options are limited. However, reduced susceptibility to VAN may lead to therapeutic failures. The

molecular mechanisms leading the development of VAN-intermediate *S. aureus* (VISA) and heterogeneous-VISA (hVISA) phenotypes are still unclear. We explored genetic signatures associated with hVISA phenotype in MRSA isolates recovered from bacteremic patients in 9 Latin American countries (2011–2014) in order to develop a genomic platform to identify these isolates.

Methods. From 538 VAN-susceptible MRSA (MIC₉₀ = 1 µg/mL) we identified 30 hVISA isolates using GRD and macromethod *E*-tests; from these, 3 were confirmed by PAP-AUC. Whole-genome sequencing was performed in all 30 isolates using Illumina platform. Based on previous studies, we selected 46 genes involved in hVISA development. Multiple Blast alignments were performed using genomes of ATCC29213 and N315 (VAN-susceptible), Mu3 (hVISA) and Mu50 (VISA) as references.

Results. A total of 130 changes in 46 predicted proteins belonging to 8 functional categories were determined: 48 changes related to cell wall biogenesis, 22 to DNA/RNA processing, 17 to regulatory systems, 12 to cofactors and enzymes, 11 to membrane biosynthesis, 9 to virulence, 6 to amino acid metabolism, and 5 to transport of nitrogen and putrescine/spermidine. The most common changes identified in all the hVISA were: Y38H in Atl, N16S in PBP4, S160A in RpoB, L14I in WalK and E156G in YvqF, compared with VSSA strains. The proteins with the highest number of changes detected in the isolates confirmed by PAP-AUC were: CapP, DltA, Pbp4, TcaA, LytM (Cell wall biogenesis); MutL, RpoB (DNA/RNA processing); GraS (Regulatory systems); and PgsA (Membrane biosynthesis).

Conclusion. Changes in genes associated with cell wall biogenesis, DNA/RNA processing, regulatory systems, and membrane biosynthesis were the most prevalent in Latin American hVISA strains. Genetic signatures in genes encoding GraR (N197S), RpoB (H481Y, H481N), VraS (I5N), WalK (L14F, R222K) and MrsR (E146K) are potentially associated with this phenotype. These changes could be used to develop a platform for possible identification of hVISA isolates.

Disclosures. All Authors: No reported Disclosures.

1828. Bedaquiline Resistance in *Mycobacterium intracellulare* Is Mediated by the Transcriptional Repressor *MmpT5*

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Background. Bedaquiline (BDQ) is an FDA approved antibiotic with antimycobacterial activity. BDQ resistance has been observed in several *Mycobacterium* species. High-level resistance is due to mutations in ATP synthase. Low-level resistance is attributed to drug efflux. Previously, we suggested that the *MmpSL5* efflux system mediates BDQ resistance in *M. intracellulare*. Here, we examine the role of *MmpT5* in transcriptional regulation of *mmpSL5* and BDQ resistance.

Methods. In this study, *mmpSL5-mmpT5* genes were cloned from 2 pre-treatment (wild-type *mmpT5*) and 2 relapse (mutant *mmpT5*) isolates of *M. intracellulare* and transformed into *M. smegmatis*. BDQ MICs were determined as well as cell survival after 24 hours exposure to an inhibitory concentration (0.07 µg/mL) of BDQ. Transcription of the *M. intracellulare mmpT5* and *mmpSL5* promoters was monitored with luciferase reporter gene fusions in the presence of wild-type and mutant alleles of *mmpT5*. Single and multigene constructs were created using the MoClo system, and transformed into *E. coli* DH5a. Constructs containing the *M. tuberculosis rv0678* gene, which mediates low-level BDQ resistance in *M. tuberculosis*, were also examined.

Results. The BDQ MIC for the *M. smegmatis* control strain, and all strains containing *mmpSL5-mmpT5* constructs, was 0.007 µg/mL. Even so, strains containing mutant *mmpT5* alleles showed enhanced survival after 24 hours exposure to 0.007 µg/mL BDQ. Bacterial colonies associated with mutant *mmpT5* alleles exhibited altered morphology relative to wild-type strains. Transcription of *mmpSL5* was repressed by wild-type *mmpT5*, but neither mutant *mmpT5* nor *rv0678* repressed transcription. The *mmpT5* luciferase reporter was not active.

Conclusion. *MmpT5* represses transcription of *mmpSL5* whereas the operon is dysregulated by *mmpT5* mutations. Although *Rv0678* regulates *mmpSL* expression in *M. tuberculosis*, it cannot repress the *M. intracellulare mmpSL5* genes. The *mmpSL5-mmpT5* genes have no impact on the BDQ MIC for *M. smegmatis*, but constructs containing mutant *mmpT5* alleles do enhance bacterial survival. The altered morphology of these colonies suggests that BDQ resistance is mediated by cell wall changes in combination with drug efflux.

Disclosures. All Authors: No reported Disclosures.

1829. The Paradox of KPC Bearing Strains of *Klebsiella pneumoniae* with the D179Y Substitution: Resistance to Ceftazidime/Avibactam (CZA) and Susceptibility to Meropenem (MEM)

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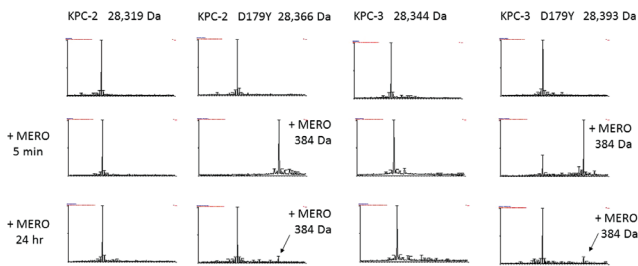
Background. Resistance to CZA is a serious limitation of treatment for KPC bearing Enterobacteriaceae infections. Recently, a single amino acid substitution (D179Y) was described in KPC-2 and KPC-3 bearing CZA-resistant *K. pneumoniae* recovered from patients failing treatment. In class A β -lactamases 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 μ M 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 ($T_m = 48-52^\circ\text{C}$) destabilizes the KPC β -lactamases ($T_m\text{KPC-2/3} = 52-56^\circ\text{C}$).

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.

Fig. 1



Disclosures. All Authors: No reported Disclosures.

1830. Single-cell Transcriptional Profiling Reveals an Immune Cell State Signature of Bacterial Sepsis

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Background. Despite intense efforts to understand the immunopathology of sepsis, no clinically reliable diagnostic biomarkers exist. Multiple whole-blood gene expression studies have sought sepsis-associated molecular signatures, but these have not yet resolved immune phenomena at the cellular level. Using single-cell RNA sequencing (scRNA-Seq) to profile peripheral blood mononuclear cells (PBMCs), we identified a novel cellular state enriched in patients with sepsis.

Methods. We performed scRNA-Seq on PBMCs from 26 patients with sepsis and 47 controls at two hospitals (mean age 57.5 years, SD 16.6; 54% male; 82% white), analyzing >200,000 single cells in total on a 10x Genomics platform. We identified immune cell states by stepwise clustering, first to identify the major immune cell types, then clustering each cell type into substates. Substate abundances were compared between cases and controls using the Wilcoxon rank-sum test.

Results. We identified 18 immune cell substates (Figure 1a), including a novel CD14+ monocyte substate (MS1) that is enriched in patients with sepsis (Figure 1b). The fractional abundance of the MS1 substate alone (ROC AUC 0.88) outperformed published bulk transcriptional signatures in identifying sepsis (AUC 0.68–0.82) across our clinical cohorts. Deconvolution of publicly available bulk transcriptional data to infer the abundance of the MS1 substate externally validated its accuracy in predicting sepsis of various etiologies across diverse geographic locations (Figure 1c), matching the best previously identified bulk signatures. Flow cytometry using cell surface markers unique to MS1 confirmed its marked expansion in sepsis, facilitating quantitation and isolation of this substate for further study.

Conclusion. This study demonstrates the utility of scRNA-Seq in discovering disease-associated cytologic signatures in blood and identifies a cell state signature for sepsis in patients with bacterial infections. This novel monocyte substate matched the performance of the best bulk transcriptional signatures in classifying patients as septic, and pointed to a specific cell state for further molecular and functional characterization of sepsis immunopathogenesis.

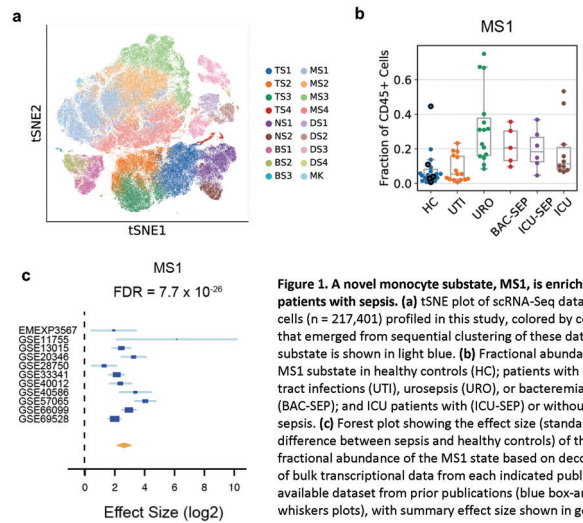


Figure 1. A novel monocyte substate, MS1, is enriched in patients with sepsis. (a) tSNE plot of scRNA-Seq data from all cells ($n = 217,401$) profiled in this study, colored by cell states that emerged from sequential clustering of these data. The MS1 substate is shown in light blue. (b) Fractional abundance of the MS1 substate in healthy controls (HC); patients with urinary tract infections (UTI), urosepsis (URO), or bacteremia and sepsis (BAC-SEP); and ICU patients with (ICU-SEP) or without (ICU) sepsis. (c) Forest plot showing the effect size (standardized difference between sepsis and healthy controls) of the inferred fractional abundance of the MS1 state based on deconvolution of bulk transcriptional data from each indicated publicly available dataset from prior publications (blue box-and-whiskers plots), with summary effect size shown in gold below.

Disclosures. All Authors: No reported Disclosures.

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 (VAMPr), 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. VAMPr 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 identified known variants that strongly predicted resistance to various antibiotic classes. Examples included specific *gyrA* mutations (T83I; $P < 0.00001$) 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.

Disclosures. Samuel L. Aitken, PharmD, Melinta Therapeutics: Grant/Research Support, Research Grant; Merck, Sharpe, and Dohme: Advisory Board; Shionogi: Advisory Board

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