**243. Transflection Fourier Transform Infrared Spectroscopy as a Real-Time Strain Typing Technique: A Vancomycin-Resistant Enterococcus faecium (VRE) Typing Prospective Study**

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***Background.*** Rapid bacterial strain typing for nosocomial outbreak surveillance is critical for timely outbreak detection and implementation of appropriate infection control protocols in hospitals. Pulsed-field gel electrophoresis (PFGE) remains the gold standard for strain typing, but it has the disadvantages of being time-consuming and costly. Transflection Fourier transform infrared (FTIR) spectroscopy is a nondestructive and reagent-free technique for rapid microbial identification and subspecies-level discrimination. The potential of employing transflection FTIR spectroscopy as a rapid, real-time typing technique was evaluated in the present study.

***Methods.*** Transflection FTIR spectra were acquired from vancomycin-resistant *Enterococcus faecium* (VRE) isolates obtained from rectal swabs ($n$ = 36) of patients in 6 units at a Montreal hospital over a 3-month period and from environmental screening samples ($n$ = 2). Upon confirmation as VRE using a transflection FTIR spectral database previously developed in our laboratory, isolates were further typed by unsupervised hierarchical cluster analysis and principal component analysis of the FTIR spectral data with the use of a feature selection algorithm.

***Results.*** Analysis of the FTIR data identified independent cases of VRE outbreak in 2 of 6 units; these outbreaks were confirmed retrospectively by PFGE. Based on the PFGE typing results for all 38 isolates included in this study, FTIR spectral analyses successfully identified 95% ($n$ = 18) of isolates related to the outbreaks and 95% ($n$ = 18) of non-outbreak-related isolates, resulting in a false-positive ($n$ = 1), and a false-negative ($n$ = 1), rate of 5%. Additionally, the two environmental isolates were identified as part of the outbreak from one of the outbreak-positive units.

***Conclusion.*** The results in this study indicate that transflection FTIR spectroscopy-based typing can be considered as an alternative typing technique to PFGE, providing real-time results to track the spread of antibiotic-resistant pathogens within hospitals. Furthermore, when combined with the use of a transflection FTIR spectral database, both identification and typing of an isolate can be achieved from a single spectral measurement, thereby reducing the time and cost required for outbreak investigation.

***Disclosures.* All authors:** No reported disclosures.

**244. Development, Qualification, and Clinical Validation of an Immunodiagnostic assay for the Detection of 11 Additional S. pneumoniae Serotype-Specific Polysaccharides in Human Urine**

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***Background.*** Identifying Streptococcus pneumoniae (Sp) serotypes by urinary antigen detection assay (UAD) is the most sensitive and specific way to evaluate the changing epidemiology of non-bacteremic community-acquired pneumonia (CAP) and efficacy of pneumococcal vaccines. We first described an UAD to detect the Sp serotypes 1,-3,-4,-5,-6A,-6B,-7F,-9V,-14,-18C,-19A,-19F,-23F covered by the 13-valent Sp conjugate vaccine PCV13. To assess the pneumococcal disease burden of additional serotypes, a UAD-2 assay was developed to diagnose 11 additional Sp serotypes (-2,-8,-9N,-10A,-11A,-12F,-15B,-17F,-20,-22F,-33F).

***Methods.*** UAD-2 specificity was achieved by capturing highly purified pneumococcal polysaccharides with serotype-specific monoclonal antibodies using Luminex technology. Assay qualification assessed accuracy, precision, and sample linearity. Serotype positivity was based on cutoffs determined by non-parametric statistical evaluation of urine samples from individuals without pneumococcal disease. Clinical sensitivity and specificity of the positivity cutoffs were assessed in a clinical validation.

***Results.*** The UAD-2 was shown to be specific and reproducible. Clinical validation using urine samples from invasive disease patients demonstrated assay sensitivity and specificity of 92.2% and 95.9%, respectively compared with a gold standard of isolating and typing (by Quellung) Sp bacteria from patient samples. Analysis of 11,087 CAP patients showed a UAD-2 and UAD-1 serotype prevalence of 4.33% and 4.60%, respectively (bacteremic and non-bacteremic CAP combined).

***Conclusion.*** The qualified/clinically validated UAD-2 method has applicability in understanding the epidemiology of nonbacteremic Sp CAP as well as assessing vaccine efficacy of future pneumococcal conjugate vaccines.

***Disclosures.* All authors:** No reported disclosures.

**245. Plasma and Respiratory Specimen Metagenomic Sequencing for the Diagnosis of Severe Pneumonia in Mechanically-Ventilated Patients**

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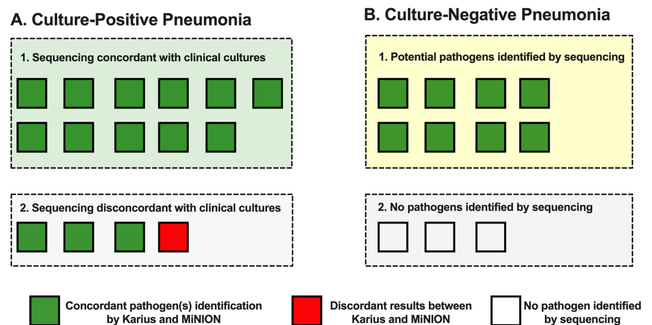
***Background.*** Metagenomic sequencing of respiratory microbial communities may overcome the limitations of culture-based pneumonia diagnostics. Nonetheless, respiratory metagenomics requires high-quality specimens, may miss deep-seated infections and cannot distinguish colonization from infection. Plasma microbial cell-free DNA (mcfDNA) sequencing may offer a noninvasive alternative for culture-independent diagnosis and help refine interpretations of respiratory metagenomics.

***Methods.*** We obtained concurrent plasma and endo-tracheal aspirate (ETA) samples from 29 mechanically-ventilated patients (15 culture-positive, 11 culture-negative pneumonia, 3 uninfected control patients). We performed plasma mcfDNA sequencing (Karius®Test, Redwood City, CA) and ETA metagenomics (MiNION, Oxford Nanopore Technologies). We compared sequencing results with clinical microbiologic cultures for identified DNA pathogens.

***Results.*** Uninfected control patients had a negative signal for mcfDNA in plasma and oral bacteria in ETA specimens. In culture-positive pneumonia samples, Karius testing identified a median of 2 pathogens per sample (range 0–10), which were concordant with clinical isolates in 11/15 (73%) cases (figure). In 5/11 (45%) of concordant cases, Karius and MiNION suggested polymicrobial infections with additional pathogens not identified by cultures. In culture-negative cases, Karius detected potential pathogens in 8/11 (73% cases), which matched the species identified in ETA specimens by MiNION. In cases of clinical aspiration, Karius detected more organisms (median 8, range 0–14) per sample mainly consisting of oral origin bacteria compared with cases without history of aspiration (median 1, range 0–6, $P$ = 0.04).

***Conclusion.*** Metagenomic sequencing in plasma and ETA samples showed good concordance between the blood and lung compartments as well as with culture results in pneumonia patients. Metagenomics revealed potential pathogens missed by cultures in ~75% of culture-negative pneumonias and suggested polymicrobial infections especially in cases with aspiration. Further research is needed to evaluate the clinical utility of real-time metagenomics for pneumonia diagnosis in mechanically ventilated patients.

**Figure: Concordance assessment of sequencing technologies (microbial cell-free DNA sequencing in plasma by Karius and Endotracheal Aspirate metagenomic sequencing by MiNION Nanopore) and clinical microbiologic culture results in culture-positive and negative cases of pneumonia.** A. Concordance of Karius and MiNION ETA metagenomics with clinical microbiologic cultures in 11/15 culture-positive cases. B. Karius and MiNION metagenomics identified potential pathogens missed by cultures in 8/11 cases of culture-negative pneumonia.

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**246. Carbapenem-resistant Klebsiella (CRK) Bloodstream Infections (BSIs) Are Caused by Bacterial Populations That Are Genotypically and Phenotypically Diverse**

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***Background.*** The majority of bacterial BSIs are believed to stem from a single, clonal organism. We hypothesized that most CRK BSIs are caused by genetically diverse, clonal strains that exhibit different phenotypes.

***Methods.*** Blood cultures (BCs) that were positive for CRK from each of 10 patients (patients) were streaked onto agar plates, and 100 individual colonies were