243. Transfection Fourier Transform Infrared Spectroscopy as a Real-Time Strain Typing Technique: A Vancomycin-Resistant Enterococcus faecium (VRE) Typing Prospective Study
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Session: 39. Diagnostics: Sequencing and Typing
Thursday, October 3, 2019: 12:15 PM

Background. Rapid bacterial strain typing for nosocomial outbreak surveillance is critical for timely outbreak detection and implementation of appropriate infection control policies, particularly 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. Transfection Fourier transform infrared (FTIR) spectroscopy is a nondestructive and reagent-free technique for rapid microbial identification and subspecies-level discrimination. The potential of employing transfection FTIR spectroscopy as a rapid, real-time typing technique was evaluated in the present study.

Methods. Transfection 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 transfection FTIR spectral database previously developed in our laboratory, isolates were further typed by un-supervised 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 analy- ies 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 transfection 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 transfection 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.

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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, 7E, 9V, 14, 18C, 19A, 19F, 23F covered by the 13-valent (PCV13) 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 pneumo- cocal 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 and 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 preva- lence 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.

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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 offer 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 (mcDNA) sequencing may offer a noninvasive alternative for culture-independent diagnosis and help refine interpretations of respiratory metagenomics.

Methods. We obtained concurrent plasma and endotracheal aspirate (ETA) samples from 29 mechanically-ventilated patients (15 culture-positive, 11 culture-negative pneumonia, 3 uninfected control patients). We performed plasma mcDNA 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 mcDNA in plasma and ETA samples. Plasma 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 path- ogens 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 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 7/11 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 and Karius and Endotracheal aspirate (ETA) sequencing by MiNION) in mechanically-ventilated patients. A. 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 pathogenic causes missed by ETA 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 di- verse, 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 100individual colonies were
chosen for Illumina whole-genome sequencing. Strains from 3 patients were tested for in vitro phenotypes and virulence in mice.

Results. Patients had BSIs due to ST258 K. pneumoniae (Kp; 6), non-ST258 Kp (3), and K. michiganensis (Km; 1). 5 patients were infected with strains that differed by core genome single nucleotide polymorphism: phylotype (2-5 unique genotypes/patient) [figure 1]. 6 patients were infected with strains that differed by gene or plasmid content, and/or gene deletions (table). Differences in individual patients encompassed antibiotic resistance and putative virulence genes (including mixtures of blaKPC and porin mutant strains). In total, BSIs in 8 of 10 patients were caused by a genotypically diverse population. In each of 3 patients, genetically diverse ST258 Kp strains demonstrated significant differences in antibiotic susceptibility. CPS content, mucoviscosity, adherence, resistance to serum killing, and mortality rates and tissue burdens during BSIs of mice. ST258 strains from a pt with and without a KPC-bearing IncFIA plasmid differed in β-lactam susceptibility, but were equally virulent. Progression of loss of CPS in ST258 strains from another patient enhanced serum killing and adherence, and attenuated virulence. Using PCR markers to test 96 colonies per positive BC bottle, we demonstrated that strains selected by the clinical lab were more diverse than genotypically diverse populations. In each of 3 patients, genotypically diverse ST258 Kp strains demonstrated remarkable genotypic diversity, which impacted antibiotic susceptibility, virulence and other phenotypes. Differences were not recognized during hospitalization since clinical labs select single, morphologically distinct colonies for evaluation. Studies are needed to understand the clinical implications of our findings, diversity during other BSIs, and whether clinical lab practices need revision.

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247. Evaluation of the T2Candida Panel as an Antifungal Stewardship Tool in Transplant and Non-Transplant Patients at a Tertiary Care Center
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Session: 40. Fungal Diagnostics

Background. Invasive candidiasis (IC) has a mortality of >30% and likelihood of death increases 50% each day antifungal therapy (AF) is delayed. In patients with suspected IC early empiric AF improves outcomes but has the potential for overuse. Blood cultures (BC) detect ∼50% of candidemia with a turnaround-time (TAT) of 2–5 days. T2Candida Panel (T2) an FDA-approved molecular diagnostic test, detects 5 Candida species directly in blood with specificity 99.4%, sensitivity 91.1%, and TAT of 3–5 hours. Our institutional guidelines permit the use of empiric AF in patients with suspected IC (Figure 1). We evaluated the utility of T2 as an AF stewardship tool to support these guidelines.

Methods. We reviewed patients that had T2 done January 2016 to May 2016 at Henry Ford Hospital, a 900-acute care bed teaching hospital in Detroit, MI. Patients with negative T2 (T2(−)) and negative concurrent BC (BC(−)) were evaluated. The primary endpoint was discontinuation (d/c) of AF after a T2(−) result. Secondary endpoints were candidemia after d/c of AF and all-cause 30-day mortality. Comparative analyses were performed of transplant (txp) vs. non-transplant (non-txp) patients. Univariate analysis was done to determine the association of risk factors and outcomes. Multivariate regression using forward selection was used to model mortality risk. Time to d/c of AF were modeled using Kaplan–Meier estimators.

Results. 500 consecutive patients with T2(−) and BC(−) results were identified. Patients on AF for prophylaxis or treatment of fungal infection were excluded. 472 patients (93 txp patients) were included in the analyses. Characteristics of the txp and non-txp patients are in Table 1. Median TAT in hours (hr) of T2 was 6 (±2) vs. 123 (±25) for final BC(−) result. 264/472 (56%) patients demonstrated significant differences in antibiotic susceptibility, CPS content, mucoviscosity, adherence, resistance to serum killing, and mortality rates and tissue burdens during BSIs of mice. ST258 strains from a pt with and without a KPC-bearing IncFIA plasmid differed in β-lactam susceptibility, but were equally virulent. Progression of loss of CPS in ST258 strains from another patient enhanced serum killing and adherence, and attenuated virulence. Using PCR markers to test 96 colonies per positive BC bottle, we demonstrated that strains selected by the clinical lab were more diverse than genotypically diverse populations. In each of 3 patients, genotypically diverse ST258 Kp strains demonstrated remarkable genotypic diversity, which impacted antibiotic susceptibility, virulence and other phenotypes. Differences were not recognized during hospitalization since clinical labs select single, morphologically distinct colonies for evaluation. Studies are needed to understand the clinical implications of our findings, diversity during other BSIs, and whether clinical lab practices need revision.

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Figure 1

Algorithm for use of empiric antifungal therapy for patients with suspected invasive candidiasis and candidemia

Patient with suspected candidemia
(Persistent fever/critical deterioration in a patient on adequate antibiotic therapy for ≥72 hours and risk factors for invasive candidiasis)

Obtain Blood cultures and T2

Initiate empiric antifungal therapy if severe sepsis
-ICU-ankidulafungin; G/P-flucanazole

Blood culture or T2 positive
-Continue antifungal therapy
-Ophthalmology examination
-Infectious Diseases consultation
-Monitor based on species and susceptibility tests

Blood culture and T2 negative
-Stop antifungal therapy

Table 1: Characteristics of Study Population

| Variables | Non-Transplant (N=379) | Transplant (N=93) | P value |
|-----------|-------------------------|------------------|---------|
| Demographics |
| Age in Years (Mean, SD) | 54.14 | 54.14 | 0.9002 |
| Male (%) | 49.3% | 54.8% | 0.3418 |
| Race |
| White | 56% | 75.3% | 0.0068 |
| Black | 32.3% | 20.4% | 0.1150 |
| Asian | 2.1% | 1.1% | 0.6316 |
| Other | 9.6% | 3.2% | 0.4931 |
| Clinical characteristics |
| Renal Failure | 56.5% | 67.7% | 0.0478 |
| HIV | 2.1% | 3.2% | 0.2894 |
| Malignancy or chemotherapy | 11.2% | 28% | <0.0001 |
| Antibiotics ≤48hs preceding test | 97.87% | 96.77% | 0.4532 |
| Fever/hypothermia (Tmax >38 or <36°C) | 41.87% | 35.48% | 0.2619 |
| Neutropenia (ANC <1000 cells/cu) | 5.9% | 16.1% | 0.0001 |
| Severe Sepsis | 22.3% | 20.4% | 0.6424 |
| Vasopressor Required | 27.5% | 16.1% | 0.0242 |
| Ventilator Required | 69.7% | 30.1% | <0.0001 |
| Presence of CVC | 49.4% | 35% | 0.0146 |
| Presence of prosthesis or ICD | 5.8% | 7.5% | 0.5354 |
| TPN | 24% | 19.8% | 0.3921 |
| Tube Feeding | 51.5% | 30.8% | 0.0004 |
| Surgery | 46.3% | 38.5% | 0.1866 |
| Abdominal infection | 8% | 0% | 0.0053 |
| GI perforation | 8.5% | 3.3% | 0.0891 |
| Abscesses | 4.5% | 1.08% | 0.1839 |
| Candida Score (Mean, SD) | 1.14, 1.09 | 0.98, 0.59 | 0.1736 |
| Candida Risk High (Score ≥3) | 10% | 4.3% | 0.0823 |
| ICU location | 88% | 52.2% | <0.001 |
| ID consults | 67.50% | 76.34% | 0.0994 |
| Outcomes |
| Antifungal started | 53.8% | 66.7% | 0.0200 |
| Antifungal discontinuation after negative T2 | 52.5% | 60% | 0.0370 |
| All-cause 30 day mortality | 34.4% | 14% | 0.0002 |

1p≤0.05 significant; 2severe sepsis: at least 2/4 SIRS criteria with multi-organ dysfunction or vasopressor support; 3Candida Score: Severe sepsis point ≥2 points, TPN, surgery, multifocal Candida colonisation each ≥1 point (Lenoe, et al. 2006)