Genetic Detection of Antimicrobial Resistance: An Update

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The objectives of the presentation are to 1) review emerging antimicrobial/antiviral resistance determinants, 2) present novel genotypic/phenotypic testing methods for detection of resistance, 3) discuss the advantages/disadvantages of genotypic methods and 4) describe specific genotypic testing applications.

Emerging resistance mechanisms in bacteria include the ominous New Delhi metallo β-lactamase plasmid, which not only contains the gene encoding for broad-spectrum penicillin, cephalosporin and carbepenem hydrolysis, but provides the insertion capacity for multiple other antibiotic resistance genes in gram-negative bacteria. Also alarming is multiple fluoroquinolone resistance determinants now extant in mobile plasmids in *Salmonella enterica* serovar Typhi. Multi-drug resistant tuberculosis continues to be an issue and new technology now permits the identification of low frequency (quantity) HIV antiviral-resistant quasi-species.

Novel testing methods for determining antimicrobial resistance include real-time PCR, mass spectroscopy and next generation sequencing. Many laboratory-developed tests (LDT's, a.k.a., "homebrews") have been reported by a number of investigators for all of these methods. Real-time PCR has been adapted by several commercial providers (Cepheid, BD, and Roche) for direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) directly from nares, infected soft tissue and/or blood culture bottles. Detection of Vancomycin-resistant enterococci (VRE) by real-time PCR from stool samples is commercially available from Cepheid, Roche and BD (non-US). Finally, an assay detecting both *M. tuberculosis* and rifampin has recently been marketed by Cepheid but only for markets outside of the United States.

Matrix-Assisted Laser Disorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry has recently been applied for the detection of micro-organisms based on protein spectra. Another

form of mass spectrometry, PCR plus electron spray ionization mass (PCR/ESI-MS) has been applied for detection of MRSA strains (target genes: *mecA*, *mecR1*, *nuc*; *Wolk et al; JCM 47:3129*) and fluoroquinolone resistant mutations in *Acinetobacter* spp. strains (target genes: *gyrA*, *parC*; *Hujer, JCM 47:1436*). These mass spectrometry methods hold great promise as they are rapid, require minimal sample preparation and are "reagent less" detection methods.

Next-generation nucleic acid sequencing (Next-Gen Sequencing) facilitates comprehensive highly sensitive interrogation of large quantities of nucleic acid. Also referred to as "massive parallel sequencing", these newer sequencing platforms now permit detection of minority drug-resistant HIV-1 variants; i.e., populations <20% of current sequencing detection methods. However, the clinical significance of these low frequency variants remains uncertain (*Johnson and Geretti: J Antimicrob Chemother 65: 1322*). As more antiviral therapies become available for the hepatitis and herpes viruses, determination of low frequency drug-resistant variants may also be useful. Next-Gen sequencing also has the potential for determining the micro-environment of (i.e., microbiome) within normally colonized areas of the human body, e.g., vagina. A change in the microbiome may serve as a sentinel for various disease states, e.g., vaginosis. Also Next-Gen sequencing has the potential to detect and quantitate organisms directly from specimens. Future studies are required to determine the utility of these potential applications.