Molecular Detection of Resistance Genes

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

Recently, strains of *Neisseria gonorrhoeae,* with high-level resistance to ceftriazone, have been reported. The molecular mechanisms for this resistance appear to be multi-factorial, related to penicillin binding proteins and outer membrane proteins, but not cephalosporinases. Enterobacteriaceae strains carrying the ominous New Delhi metallo β-lactamase plasmid continue to spread globally. This plasmid not only contains the genes encoding for broad-spectrum penicillin, cephalosporin and carbepenem hydrolysis, but provides the insertion capacity for multiple other antibiotic resistance genes. Also of concern 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 for direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from nares, infected soft tissue and/or blood culture bottles. Commercial real-time PCR assays are also available for the detection of Vancomycin-resistant enterococci (VRE) from stool samples and the detection of both *M. tuberculosis* and rifampin from respiratory secretions.

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 some resistance determinants. 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. 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.