Antimicrobial Susceptibility Testing: Improved Detection of Beta-lactam Resistance

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The ever increasing number of β-lactamases affecting carbapenems, extended-spectrum cephalosporins and aztreonam, as well movement of these enzymes into multiple genera of Enterobacteriaceae, necessitated the re-evaluation of antimicrobial susceptibility testing methods and interpretive breakpoints used for accurate, rapid detection and reporting of potential resistance to β-lactam agents. The development of the original breakpoints for most of the β-lactam agents predated the emergence of ESBLs and carbapenemase enzymes, as well as the newer pharmacokinetic/pharmacodynamic (PK/PD) data that are currently used for breakpoint development. Clinical and Laboratory Standards Institute (CLSI) has recently published lower breakpoints for specific 3rd generation cephalosporins, aztreonam, and carbapenems. These revised breakpoints are based on contemporary microbiological and PK/PD data, along with review of limited clinical data available. When the lower breakpoints are used, CLSI recommends that tests to detect ESBLs and carbapenemases are no longer needed for the clinical care of patients so long as specific minimum dosage regimens are used. The sensitivity and specificity of reporting of results based on MIC or zone size for various extended-spectrum cephalosporins and carbapenems without additional specific enzyme testing will be discussed as well as the implications for infection control. Laboratories can implement the new breakpoints immediately if they use disk diffusion or an MIC method with antibiotic concentrations low enough to measure the new breakpoints. For laboratories that use commercial or automated methods that can not test and report the lower breakpoints, the older breakpoints can be used with the continuation of ESBL and carbapenemase confirmatory testing. However, these older methods will inevitably miss gram-negative bacteria with many of the newer, complex mechanisms of resistance and may be inadequate for successful infection control programs.

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 carbapenem 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.

One of the traditional limitations of diagnostic microbiology in the effective management of infection has been the time taken to culture and identify pathogens from clinical specimens. This is particularly true for the management of sepsis, infective endocarditis and meningitis, and in the timely application of infection control measures for patients carrying multi resistant organisms. In addition, some fastidious organisms have been difficult or impossible to culture making diagnosis only possible by serology or histopathology. Advances have been made by use of semi-automated platforms but organism growth and biochemical identification tests remain the basis for these technologies.

Technological developments however have significantly changed with the capability now of applying molecular techniques to provide diagnostic answers in hours rather than days. Also these techniques allow identification of those organisms previously non-cultivable. What has led to this paradigm shift in diagnostic microbiology? Microbial genome sequencing and technologies such as PCR have facilitated this revolution. It is not possible to discuss all the new technologies and the potential afforded by new generation pyrosequencing. An overview of current techniques with established applications will be presented with an in depth look at techniques employed in my own laboratory.

Rapid diagnosis is paramount for certain infections. It is well recognised that the mortality from blood stream infection is dependent on the time to effective therapy. The use of broad spectrum empirical therapy in part overcomes the need for rapid identification however this has the potential to drive antimicrobial resistance selection and no matter how broad the spectrum of a drug not all organisms will be susceptible. There are two strategies employed by new technologies for the identification of pathogens causing blood stream infection. The first aims to detect the causative organism direct from blood or serum. The second identifies