Molecular Detection of Antimicrobial Resistance in the Routine Laboratory

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Until recently, molecular methods used to detect antimicrobial resistance have been primarily limited to reference laboratories or laboratories serving tertiary referral centers. This is because many of these methods, which rely on nucleic acid amplification and detection, require development in house, are not automated, and require significant skill and expertise to perform. Recent improvements in nucleic acid amplification-based technology, especially real-time polymerase chain reaction (PCR) now make it possible for routine use of these molecular detection methods in most laboratories.[1]

The first commercially available nucleic acid-based method for detecting antimicrobial resistance was developed for detection of mutations in the HIV genome associated with resistance to antiretroviral drugs. Additionally, commercial tests have been available for some time for typing (determination of genetic variants) of hepatitis C virus (HCV). Typing of HCV has been useful for predicting therapeutic response to interferon and antiviral therapy. These testing methods, which involve amplification of nucleic acid and interrogation of nucleic acid sequences, have been automated to some extent, but still require significant hands-on time and skill in molecular testing methods. Because the capital investment for instrumentation is significant and the results do not have to be available on an urgent basis for patient care, molecular detection of antimicrobial susceptibility for HIV and HCV will likely be performed for some time at higher level reference or tertiary care center laboratories.

Several studies have shown that infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) are associated with higher mortality rates and costs than those associated with methicillin-susceptible *S. aureus* and vancomycin-susceptible enterococci.[2] In May 2003, the Society for Healthcare Epidemiologists of America (SHEA) provided a guideline advocating increased surveillance for carriers of MRSA and VRE among patients admitted to healthcare institutions.[3] These guidelines were based on numerous clinical studies which have demonstrated that identification and isolation of patients with MRSA or VRE carriers decreases nosocomial rates of these infections.[2,3]

The conventional approach for identifying carriers of MRSA is culture of nasal swabs and for VRE, culture of perianal swabs. Culture methods for VRE surveillance from perianal swabs are difficult to perform, lack sensitivity and frequently require $\geq$72h for a definitive result. Culture methods are more sensitive for MRSA from nasal swabs, but the time for a final result is frequently $\geq$48h. In order to make large-scale surveillance programs for MRSA and VRE feasible from the perspective of workload

in the laboratory and from the perspective of prevention of nosocomial VRE and MRSA outbreaks, rapid and sensitive methods are desirable. Recently, two commercial detection systems have become available for direct detection of VRE and MRSA from perianal and nasal swabs respectively. These test methods use real-time PCR for amplification and detection of nucleic acid.[1]

Our research group reported on the value of a commercially available real-time PCR assay for direct detection of VRE from perianal swabs.[4] This assay is marketed by Roche Diagnostics Corporation and uses the LightCycler real-time PCR instrument. We determined that this assay was over 120% more sensitive than culture and required considerably less time for results (3.5 h vs. ≥72 h).

Warren and colleagues evaluated a commercially available FDA-approved real-time PCR assay designed to detect MRSA directly from nasal swabs (IDI-MRSA using the SmartCycler, Cepheid, Sunnyvale, CA). These investigators showed this assay to be as sensitive as a direct plate culture method and final results were available within 2 h vs. 48-72 h for culture.[5]

What about future possibilities for nucleic acid-based testing of antimicrobial resistance?
Recent studies have highlighted rapid, comprehensive testing methods which rely on molecular growth detection of bacteria incubated in broth containing antibiotics. Using real-time PCR targeting highly conserved 16S rDNA or *rpoB* sequences, Rolain and colleagues showed that susceptibility results could be obtained on average within 2h for Gram-negative bacteria and within 4h for Gram-positive bacteria.[6] This compares to the time requirement of usually 18h or greater for conventional culture-based methods. Further refinements in these molecular techniques will likely revolutionize the way bacterial susceptibility testing is performed in the clinical microbiology laboratory.

References

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