Development of a Rapid Multiplex PCR Assay for Emerging Carbapenemases and CMY-2/22

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Background: Multi-drug resistance in common gram-negative bacterial pathogens has emerged in many parts of the world and represents a growing health threat. Multiple plasmid-mediated beta-lactamases including carbapenemases and plasmid-mediated AmpC such as CMY-2 have spread quickly in the United States. Many strains that produce these newer, broad-spectrum beta-lactamases are often also resistant to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole making infections extremely difficult to treat. For the purposes of infection control, public health epidemiology, and management of individual patients, it is important to detect different specific beta-lactamase resistance quickly and accurately.

Methods: Bacterial DNA was isolated using an automated Maxwell system. Real-time PCR was performed in a 7900 ABI Real-Time machine with SYBR detection of KPCpan, VIMpan, IMPpan, NDMpan, and CMY-2 using custom designed primers.

Results: A total of 29 carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains and 7 CMY-2 or -22 producing *Escherichia coli* resulted in detection of KPC-2, -3, and -7, IMP-4, VIM-1, -2 and-27, NDM-1, CMY-2 and -22 genes in a multiplex assay in a 5 hour period.

Conclusions: Combining detection of carbapenemases and a common cephalosporinase in a single multiplex PCR assay can provide rapid detection of highly resistant pathogens.