Molecular Detection of Antimicrobial Susceptibility: Changing Paradigm of Laboratory Testing for Multidrug Resistant Organisms

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Introduction

The increasing threat of antibiotic resistance is of worldwide public health concern. Empirical therapy for treatment of infectious diseases has helped create bacterialวลผลวิจัยวิเคราะห์และมีแนวโน้มว่าจะมีผลทำให้เกิดการเปลี่ยนแปลงในกระบวนการการรักษาของยาฆ่าเชื้อ

Materials & Methods

Samples

- Assays included were verified using 157 clinical isolates with established antibiotic resistance-gene profiles and phenotypic susceptibility profiles from various sources (CDC, and Becton Dickinson, Walkerton, ON; HAM, Schaumburg, IL; and Microbiologics, St. Cloud, MN).
- DNA samples were extracted on the KingFisher™ Fab System (Thermo Fisher Scientific, Waltham, MA).

Test System

- TaqMan® assays were designed based on sequences obtained from the basic local alignment search tool (BLAST) using an algorithm evaluating melting temperature, nucleotide composition of primer-pair combinations, and specificity of genomic sequences with closely related gene subtypes.
- Assays were designed to detect the most clinically relevant gene variants within individual enzyme classes (Table 1).
- Assays were verified on OpenArray® plates for high-throughput testing on the SNP Exfoiler System.
- To increase sensitivity for low concentration samples, a target-specific oligo probe was added to each reaction after initial amplification at the optimal annealing temperature for each reaction (Table 1).

Validation

- Plasmid DNA (pDNA) over a nine-log serial dilution ranging from 100 million copies to 33 nL was verified on clinical isolates, urines, stockbroth swabs, and nasal swabs.

Results

- TaqMan® based, characterized isolates (HMA, Inc.) were used to assess the accuracy of the panel.
- Resistant profiles from the ABCx™ Panel were compared to the molecular profile provided by the vendor, as well as two phenotypic methods (Kirby-Bauer disc and Thermo Fisher Scientific Sensititre™ microbroth dilution).
- Results for phenotypic methods were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI).

Results (continued)

- Antibiotic resistance genotypes were consistent with observed antibiotic susceptibility profiles, demonstrating that a resistance genotype from both the clinical isolate to the KPC disc diffusion method and the Thermo Fisher Scientific Sensititre™ microbroth dilution method as a rapid, accurate, and affordable tool to shift the paradigm of diagnostics for multidrug resistant organisms, advancing antibiotic stewardship programs in healthcare facilities.

Conclusions

- TaqMan® assays were designed to ensure the detection of multiple clinically relevant subtypes within three major antibiotic resistance gene classes and across representative bacterial species. The ABCx™ Panel consisting of seven TaqMan® assays printed on the OpenArray® platform, was developed and validated using 157 genomically and phenotypically characterized clinical isolates.
- Clinical specimens can be used directly for detection of multidrug-resistance gene profiles as no additional bacterial isolation or culture is required; established analytical sensitivity was between 102 and 102 CFU/mL. The ABCx™ Panel demonstrated excellent intra- and inter-assay precision with an R2 of 0.99. The ABCx™ Panel was shown to be a powerful tool to expand the diagnostic information.
- Phenotypes were concordant with resistance gene identification on the ABCx™ Panel.
- Molecular testing for antibiotic resistance genes removes the variability and subjectivity found in current phenotypic test methods, allowing for appropriate therapeutic decision-making.

Materials & Methods (continued)

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References


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