Technical **BULLETIN**

Phenotypic Versus Genotypic Antimicrobial Resistance Profiles: *Strategies for Detection of Antimicrobial Resistance*

Antimicrobial resistance (AMR) is a worldwide threat in the 21st century. This is due to the overuse and misuse of antibiotics when treating infections in humans. Additionally, the use of antibiotics in livestock to promote growth and prevent disease has further contributed to the spread of resistant strains of bacteria. Resistance occurs as bacteria develop or acquire genetic mechanisms that allow them to survive when challenged with antibiotics designed to kill them. AMR can result from both chromosomal mutations and acquisition of mobile genetic elements that harbor antibiotic resistance genes.

Antimicrobial susceptibility testing (AST) is culture-based *in vitro* testing to measure the growth response of a bacterial isolate in the presence of various concentrations of antimicrobial agents. The goal of AST is to make a **phenotypic prediction** of the in vivo success or failure of antibiotic therapy by determining the susceptibility or resistance to an antibiotic.

Conventional AST methods include Kirby-Bauer (KB) disc diffusion and microbroth dilution tests. These conventional methodologies begin with the isolation of bacterial colonies on selective agar plates, followed by subculture, bacterial identification, and downstream susceptibility testing. The phenotypic approach can take up to 72 hours for results; meanwhile the patient is often treated empirically with broad-spectrum antibiotics. As AST is a predictive model, *in vitro* results cannot be guaranteed to mimic an organism's anticipated *in vivo* response to a prescribed course of antibiotics. Phenotypic AST relates only to the concentration of a specific antimicrobial agent that inhibits bacterial growth with no indication of underlying molecular mechanisms of resistance that may be present but unexpressed.

Genotypic characterization of AMR uses molecular methods to detect specific genes responsible for resistance to different classes of antibiotics. In order to successfully perform genotypic characterization, assays should be designed and validated to detect clinically relevant gene variants directly from clinical samples without the need for time-consuming bacterial isolation and identification.

In collaboration with Thermo Fisher Scientific (South San Francisco, CA), Diatherix has developed a panel of molecular assays for genotypic characterization of AMR directly from clinical samples. The ABRx™ Antibiotic Resistance Panel allows for detection of seventeen gene types within seven gene classes associated with resistance to three major groups of antibiotics: **beta-lactams** (carbapenems, cephalosporins, and monobactams), **quinolones**, and **macrolides**. Results are reported as "Detected/Not Detected," and a recommendation is given to avoid a class of antibiotics associated with the resistance gene detected.

Diatherix performed accuracy studies using fifty-three blinded isolates, comparing the phenotypic versus genotypic detection of antibiotic resistance. The analysis focused on detection of beta-lactam resistance as this is the most utilized family of antibiotics to treat infections caused by *Enterobacteriaceae*. The ABRx Panel was used for genotypic characterization, while KB disc diffusion and the semi-automated Sensititre™ microbroth dilution methods were used to establish the phenotypic characterization of the bacterial isolates. Minimum inhibitory concentration (MIC) results from Sensititre™ testing and zone of inhibition measurements from disc diffusion testing were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI).[1] Results of the ABRx Panel were considered "resistant" when any of the target genes were detected (Table 1). MIC values from Sensititre™ were used as the "gold standard" and compared to the KB method and ABRx genotypic profiles.

Table 1. Comparison of Phenotypic and Genotypic Antimicrobial Susceptibility Profiles

Antibiotic Class	Resistance Genes Detected by ABRx™	Sensititre™ Detection	
		KB (%)	ABRx (%)
Carbapenems	KPC, IMP, VIM, NDM, OXA-48, GES	93	85
Cephalosporins	KPC, IMP, VIM, NDM, OXA-1, OXA-48, FOX, GES	98	94
Monobactams	CTX-M Groups 1, 2, 8/25, 9, KPC, PER, VEB, GES	100	91

Our analysis shows that genotypic AMR profiles can be determined with a high degree of confidence when compared to phenotypic AST profiles, and these genotypic profiles can be used to define an antibiotic avoidance strategy. Rapid prediction of antibiotic resistance mechanisms present in clinical specimens provides useful information to the physician in a timely manner, resulting in improved patient outcomes and reduced healthcare costs.

Reference:

1. Clinical and Laboratory Standards Institute. 2015. Performance Standards for Antimicrobial Susceptibility Testing. Approved guideline, 25th Informational Supplement. CLSI document M100-S25.

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