SATURDAY-396

A Molecular Multidrug Resistance Gene Panel as an Innovative and Accurate Alternative to Antibiotic Susceptibility Testing

Paul Dawson, MS; Megan Stonebraker; Don Stalons, PhD, D(ABMM), MPH; Leslie L. Malone, MS, MB(ASCP)^{CM}; Elena Grigorenko, PhD
Diatherix Laboratories, LLC, Huntsville, AL, USA

Paul Dawson, MS
Diatherix Laboratories, LLC
paul.dawson@diatherix.com
256.327.0511

Abstract

Background: Antibiotic susceptibility testing (AST) is one of the most important tests in a clinical microbiology laboratory. Accurate and rapid detection of antibiotic resistance patterns is crucial for all aspects of antimicrobial stewardship, including resistance surveillance and effective patient treatment. Existing traditional culture-based ASTs are time-consuming and often lack specificity for new generations of antimicrobials. In this study, we compare the performance of a rapid molecular multidrug resistance gene panel, ABRx™, to gold-standard Kirby-Bauer and microbroth dilution AST methodology.

Methods: Fifty-three isolates were obtained from IHMA, Inc., with all information about them withheld. Isolates were cultured on a non-selective medium, followed by nucleic acid extraction and testing on custom OpenArray® qPCR plates with seventeen ABRx™ Panel targets. Simultaneously, an antibiotic resistance profile (ARP) was determined using the Kirby-Bauer method of AST. Corresponding resistance phenotypes of detected genes were assigned from published literature and compared with the Kirby-Bauer and microbroth dilution methods' ARPs.

Results: The ABRx™ Panel was highly accurate in detecting the correct resistance gene(s) in the blinded isolates. There was excellent agreement between the ARPs generated through the phenotypic methods and those generated from the detected antibiotic resistance genes. There was a 92% correlation in detection for resistance to carbapenems; 98% to cephalosporins; and 68% to the monobactam, aztreonam. Corresponding genes were not detected for carbapenem phenotypes. This could be due to the absence of corresponding genes on the panel. The low agreement of monobactam resistance between the two methods seemed largely due to the presence of CTX-M genes in the strains. This can be explained from the variability in antibiotic resistance expression among the different CTX-M gene classes reported in published literature.[1]

Conclusions: Our findings indicate that the accuracy of the ABRx™ Panel is comparable to the Kirby-Bauer and the microbroth dilution methods in predicting antibiotic class resistance phenotypes and is an innovative and rapid alternative to conventional AST methods.

*Abstract has been amended to include additional data collected after submission.

Introduction

- Antibiotics have been the mainstay of treating infectious diseases for well over half a century. However, their efficacy is diminishing by the day.
- A major factor in the spread of antibiotic resistance is the prevalence of empirical treatment, leading to the evolution of multidrug resistant bacterial strains with the potential dissemination of associated plasmid-borne antibiotic resistance genes.
- Traditionally, detection of antibiotic resistance included phenotypic testing such as Kirby-Bauer disk diffusion and microbroth dilution methods. These methods require several days to obtain results and are not conducive for making appropriate therapeutic decisions in a timely manner.
- Phenotypic methods fail to identify the underlying genetic mechanisms leading to the proliferation of resistant bacteria in the community, as opposed to molecular methods which can play a large role in surveillance and antibiotic stewardship.
- A molecular diagnostic Antibiotic Resistance (ABRx™) Panel was developed that can screen for seventeen multidrug resistance genes encoding the most clinically prevalent mechanisms of resistance to three major classes of antibiotics: β-lactams/carbapenems, macrolides, and fluoroquinolones. The panel allows for direct detection of resistance genes in clinical specimens without the need for bacterial isolation.
- The objective of the study was to compare antibiotic resistance detection by the ABRx™ Panel to the Kirby-Bauer disk diffusion method and the Sensititre™ microbroth dilution method.

Materials & Methods

Bacterial Strains

- Fifty-three blinded isolates were obtained from International Health Management Associates, Inc. (IHMA, Schaumburg, Illinois). Isolates were cultured on Tryptic Soy Agar (TSA) plates (Teknova, Hollister, CA) at 37˚C for 16-18 hours. Post-incubation, 0.5 McFarland suspensions were immediately prepared for each isolate.
- DNA from bacterial suspensions was extracted using a magnetic particle-based, in-house protocol utilizing extraction reagents from Omega Bio-Tek (Norcross, GA) on a KingFisher™ Flex instrument (Thermo Fisher Scientific, Waltham, MA).

Materials & Methods *(continued)*

Susceptibility Testing

Antibiotic Resistance (ABRx™) Panel

- TaqMan® assays to detect the most clinically relevant gene variants within individual enzyme classes were designed based on sequences obtained from public databases.
- Assays were printed on OpenArray® plates for high-throughput testing on the QuantStudio™ 12K Flex instrument (Thermo Fisher Scientific).
- A target-specific preamplification step was performed prior to real-time PCR amplification on OpenArray® plates.

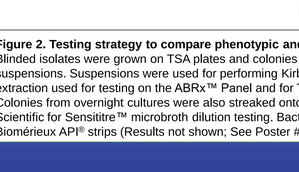


Figure 1. The QuantStudio™ 12K Flex can accommodate up to four OpenArray® Plates for simultaneous real-time PCR detection. The ABRx™ Panel contains seventeen unique TaqMan® assays printed in triplicate. Forty-eight samples can be tested on an individual OpenArray® plate.

Table 1. ABRx™ Panel Content.

Enzyme Class	Panel Target Abbreviation
Class A β-lactamase	CTX-M Group 1
	CTX-M Group 2
	CTX-M Group 8/25
	CTX-M Group 9
	KPC
Class B metallo-β-lactamase	IMP-1
	VIM
	NDM
AmpC β-lactamase	FOX
Class D OXAcillinase	OXA-1
	OXA-48
Minor ESBL	PER
	VEB
	GES
Macrolide	ERM-B
Fluoroquinolone	QNR-A
	QNR-S

Kirby-Bauer Disk Diffusion

- Bacterial lawns were streaked from a freshly prepared 0.5 McFarland on Mueller-Hinton agar (MHA) plates and were incubated at 37°C for 16-18 hours prior to determination of results.
- Antibiotics disks (Becton Dickinson, USA) used: Doripenem (10µg), Meropenem (10µg), Ertapenem (10µg), Imipenem (10µg), Cefazolin (30µg), Cephalothin (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Aztreonam (30µg), Moxifloxacin (5µg), Levofloxacin (5µg), Ciprofloxacin (5µg), and Azithromycin (15µg).

Sensititre™ Microbroth Dilution

- Minimum inhibitory concentrations (MIC) of antimicrobial agents were determined by the microbroth dilution method using the Sensititre™ Gram Negative Plate Format (GNF4, Thermo Fisher Scientific, Oakwood Village, OH).

The zones of growth inhibition and MICs were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI).[2]

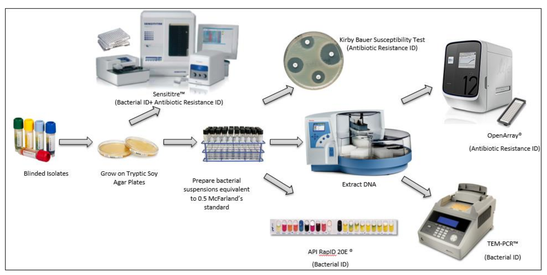


Figure 2. Testing strategy to compare phenotypic and genotypic susceptibility methods. Blinded isolates were grown on TSA plates and colonies were used to prepare 0.5 McFarland suspensions. Suspensions were used for performing Kirby-Bauer, API® strip testing, and DNA extraction used for testing on the ABRx™ Panel and for Target Enriched Multiplex PCR (TEM-PCR™). Colonies from overnight cultures were also streaked onto TSA slants and sent to Thermo Fisher Scientific for Sensititre™ microbroth dilution testing. Bacterial ID was verified with TEM-PCR™ and Biomérieux API® strips (Results not shown; See Poster #376).

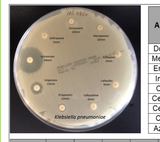
Results

Table 2. Comparison of ABRx™ detections to Kirby-Bauer disk diffusion and microbroth dilution method results. For this study, data is limited to the β-lactam classes of antibiotics.

Organism	ABRx™ Detections	Info from Vendor (ABRx™ detections in bold font)	Carbapenems			Cephalosporins			Monobactam		
			KB	STT	OA	KB	STT	OA	KB	STT	OA
Acinetobacter baumannii	PER-1	**PER-1**	*	*		*	*	*	*		*
Escherichia coli	CTX-M Group 1	**CTX-M-32**				*	*	*	*	*	*
Escherichia coli	OXA-1,CTX-M Group 2	TEM-OSBL; **CTX-M-2**				*	*	*			*
Escherichia coli	CTX-M Group 8/25	**CTX-M-8**				*	*	*	*		*
Escherichia coli	CTX-M Group 9	**CTX-M-9**				*	*	*			*
Klebsiella oxytoca	KPC	TEM-OSBL; **KPC**-3	*	*	*	*	*	*	*	*	*
Klebsiella pneumoniae	FOX	SHV-83; TEM-OSBL; **FOX**-TYPE				*	*	*			
Klebsiella pneumoniae	OXA-1, QNR- A, FOX CTX-M Group 1, ERM-B	SHV-OSBL; TEM-OSBL; CTX-M-15; **FOX**-TYPE				*	*	*	*	*	*
Klebsiella pneumoniae	CTX-M Group 1	SHV-OSBL; TEM-OSBL; **CTX-M-12**				*	*	*			*
Klebsiella pneumoniae	OXA-1, NDM-1	SHV-OSBL; **NDM-1**	*	*	*	*	*	*			
Klebsiella pneumoniae	OXA-48	SHV-OSBL; **OXA-48**	*	*	*	*	*	*			
Klebsiella pneumoniae	VIM	SHV-OSBL; **VIM**-1	*	*	*	*	*	*			
Klebsiella pneumoniae	OXA-1, KPC	**KPC-2**	*	*	*	*	*	*	*	*	*
Klebsiella pneumoniae	CTX-M Group 8/25	SHV-OSBL; TEM-OSBL; **CTX-M**-8				*	*	*	*	*	*
Klebsiella pneumoniae	OXA-48	SHV-OSBL; **OXA-48**	*		*	*		*			
Klebsiella pneumoniae	CTX-M Group 9	SHV-OSBL; **CTX-M-9**				*	*	*			*
Proteus mirabilis	OXA-1, CTX-M Group 2	**CTX-M-2**				*	*	*			*
Providencia rettgeri	NDM-1	TEM-OSBL; **NDM-1**	*	*	*	*	*	*			
Pseudomonas aeruginosa	GES	**GES-5**	*	*	*	*	*	*			*
Pseudomonas aeruginosa	IMP-1	**IMP-1**	*	*	*	*	*	*	*	*	
Pseudomonas aeruginosa	GES	**GES-6**	*	*	*	*	*	*	*	*	*
Pseudomonas aeruginosa	OXA-1, VIM	**VIM-2**	*	*	*	*	*	*			
Pseudomonas aeruginosa	VEB	**VEB-1a**	*	*		*	*	*	*	*	*
Pseudomonas aeruginosa	VEB	**VEB-1**	*	*		*	*	*	*	*	*
Pseudomonas aeruginosa	PER-1	**PER-1**	*	*		*	*	*	*	*	*
Pseudomonas aeruginosa	IMP-1	**IMP-1**	*	*	*	*	*	*	*	*	
Klebsiella pneumoniae	VEB, KPC	SHV-OSBL; TEM-OSBL; **VEB**-1; **KPC**-2		*	*	*	*	*	*	*	*
Klebsiella pneumoniae	NO DETECTION	SHV-55; TEM-OSBL	*			*	*		*	*	*
Pseudomonas aeruginosa	GES	**GES-**13	*		*	*	*	*	*	*	*
Klebsiella pneumoniae	OXA-1, OXA-48	SHV-OSBL; OXA-48	*	*	*			*			
Pseudomonas aeruginosa	IMP-1	**IMP-1**	*	*	*	*	*	*			
Klebsiella pneumoniae	OXA-1, OXA-48	SHV-OSBL; TEM-OSBL; **OXA-48**	*		*			*			
Pseudomonas aeruginosa	GES	**GES-19**; **GES-20**	*	*	*	*	*	*	*	*	*
Escherichia coli	CTX-M Group 8/25	TEM-OSBL; **CTX-M-8**				*	*	*			*
Escherichia coli	CTX-M Group 2	TEM-OSBL; **CTX-M**-2				*	*	*	*		*
Escherichia coli	CTX-M Group 8/25	**CTX-M-8**				*	*	*			*
Klebsiella pneumoniae	NO DETECTION	SHV-OSBL; TEM-4				*	*				
Klebsiella pneumoniae	VEB, KPC	SHV-OSBL; TEM-OSBL; **VEB**-1; **KPC**-2	*	*	*	*	*	*	*	*	*
Klebsiella pneumoniae	FOX, KPC	SHV-OSBL; TEM-OSBL; **FOX**-5; **KPC**-3	*	*	*	*	*	*	*	*	*
Escherichia coli	CTX-M Group 9	**CTX-M-14**				*	*	*			*
Serratia marcescens	OXA-1, QNR-A, FOX	FOX-5		*	*						
Escherichia coli	CTX-M Group 2	TEM-OSBL; **CTX-M-2**				*	*	*			*
Staphylococcus aureus, MRSA	NO DETECTION	MUP-A					*				
Klebsiella pneumoniae	OXA-1, NDM-1, CTX-M Group 1	SHV-OSBL; TEM-OSBL; **CTX-M-15**; **NDM-1**	*	*	*	*	*	*	*	*	*
Klebsiella pneumoniae	CTX-M Group 9, QNR-S	SHV-OSBL; TEM-OSBL; CTX-M-14				*	*	*			*
Klebsiella pneumoniae	OXA-1, NDM-1, CTX-M Group 1	SHV-OSBL; **CTX-M-15**; **NDM**-1	*	*	*	*	*	*	*	*	*
Klebsiella pneumoniae	CTX-M Group 9, OXA-48	SHV-OSBL; TEM-OSBL; **CTX-M-14**; **OXA-48**; AAC(3)-IIa; AAC(6')-Ib; APH(3')-Via	*	*	*	*	*	*	*	*	*
Pseudomonas aeruginosa	PER-1	**PER-1**	*			*	*	*	*	*	*
Proteus mirabilis	NO DETECTION	TEM-129									
Klebsiella pneumoniae	VIM	SHV-OSBL; VIM-1	*	*	*	*	*	*			
Klebsiella pneumoniae	NDM-1	SHV-OSBL; NDM-1	*	*	*	*	*	*			
Klebsiella pneumoniae	OXA-1, CTX-M Group 1	SHV-31; TEM-OSBL; **CTX-M-15**				*	*	*	*	*	*
Proteus mirabilis	NO DETECTION	TEM-52									

KB: Kirby-Bauer method; STT: Sensititre™ microbroth dilution method; OA: OpenArray® (ABRx™ Panel)

Results *(continued)*



Antibiotic	Resistance Zone of Inhibition (CLSI)	Zone of Inhibition Observed	Kirby-Bauer Interpretation	MIC Criteria for Resistance (CLSI)	Sensititre™ Results	Sensititre Interpretation
Doripenem	≤19 mm	10 mm	Resistant	≥4 µg/mL	>4 µg/mL	Resistant
Meropenem	≤19 mm	10 mm	Resistant	≥4 µg/mL	>8 µg/mL	Resistant
Ertapenem	≤18 mm	10 mm	Resistant	≥2 µg/mL	>8 µg/mL	Resistant
Imipenem	≤19 mm	15 mm	Resistant	≥4 µg/mL	>8 µg/mL	Resistant
Cefazolin	≤19 mm	0 mm	Resistant	≥8 µg/mL	>16 µg/mL	Resistant
Ceftazidime	≤17 mm	0 mm	Resistant	≥16 µg/mL	>16 µg/mL	Resistant
Ceftriaxone	≤19 mm	0 mm	Resistant	≥16 µg/mL	>32 µg/mL	Resistant
Cefepime	≤18 mm	0 mm	Resistant	≥16 µg/mL	>32 µg/mL	Resistant
Aztreonam	≤17 mm	34 mm	Susceptible	≥16 µg/mL	1 µg/mL	Susceptible

	VEB		OXA-1		VIM		CTX-M Grp 2		CTX-M Grp 9		NDM-1		qnrA		CTX-M Grp 1		qnrS		CTX-M Grp 8/25		FOX		GES		OXA-48		IMP-1		KPC		PER-1		ermB		APX1 Internal Ctrl	
	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD
K. pneumoniae					14.00	0.71																													21.09	0.071

Figure 5. Phenotype/Genotype Correlation of a *Klebsiella pneumoniae* isolate. The ABRx™ Panel detected the VIM gene which is concordant with the molecular characterization provided by the vendor. VIM confers resistance to penicillins, cephalosporins, and carbapenems. Phenotypic testing confirmed these results.

Table 2. Accuracy Summary. Phenotypes corresponding to resistance genes were determined based on published studies.[1] The three methods were compared by correlating results from two methods with those from the third method as the reference.

Antibiotic Class	Resistance Genes Detected by ABRx™	Organisms Tested	OA Detection as Reference		KB Detection as Reference		STT Detection as Reference	
			KB	STT	OA	STT	KB	OA
Carbapenems	KPC, IMP, VIM, NDM, OXA-48, GES	*K. pneumoniae*, *P. aeruginosa*, *P. rettgeri*, *K. oxytoca*, *S. marcescens*	92	88	80	83	93	85
Cephalosporins	KPC, IMP, VIM, NDM, OXA-1, OXA-48, FOX, GES	*K. pneumoniae, E. coli*, *P. aeruginosa, P. rettgeri*, *P. mirabilis, A. baumannii*, *S. marcescens*	98	96	96	98	98	94
Monobactams	CTX-M Group 1, 2, 8/25, 9, KPC, PER, VEB, GES	*K. pneumoniae, E. coli*, *P. aeruginosa, K. oxytoca*, *P. mirabilis, A. baumannii*	68	59	92	88	100	91

Discussion

- Susceptibility profiles from the phenotypic methods were concordant with multidrug resistance genes detected on the ABRx™ Panel.
- Correlation of the phenotypic AST methods with ABRx™ as the reference was between 88-98% for the cephalosporins and carbapenems. However, correlation was lower (80-94%) when using phenotypic methods as the reference, indicating resistant phenotypes in the absence of corresponding ABRx™ gene detections. This could be due to antibiotic resistance genes present in the isolates that are not targeted by the panel.
- Kirby-Bauer and Sensititre™ did not detect as many monobactam resistant phenotypes as was reported by the ABRx™ Panel. This could be due to resistance genes that may not be expressing *in vitro* but may be expressed *in vivo*.[3,4]
- All targeted resistance genes were correctly identified by the ABRx™ Panel when compared to the genotypic profiles provided by the vendor. This indicates the accuracy of the panel in detecting these genes.
- The accuracy of the ABRx™ Panel is comparable to the conventional methods in predicting antibiotic class resistance phenotypes and is an innovative and rapid alternative to conventional AST methods.

References & Acknowledgements

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