

Poster 1595

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

Abstract

Background: The increasing threat of antibiotic resistance is of worldwide public health concern. Empirical therapy for treatment of infectious diseases has helped create bacterial strains with multiple antibiotic resistance mechanisms. Rapid molecular testing can offer a change in paradigm for susceptibility testing and can have a significant impact on patient care. In this study, a molecular diagnostic panel was designed to screen for twenty-six multidrug-resistance genes (MDRGs) encoding the most clinically prevalent mechanisms of resistance to three major classes of antibiotics: β -lactams, fluoroquinolones, and macrolides.

Methods: TaqMan® assays were designed for use on a high-throughput real-time platform (QuantStudio™ 12K Flex). DNA was isolated from samples using a KingFisher™ Flex instrument, followed by twelve-step cycling with a panel-specific PreAmp mix. Pre-amplification products were then processed on OpenArray® Custom Real-Time PCR Plates.

Results: The target specificity of TaqMan® assays was validated using clinical isolates (n=145) with known, sequence-validated resistance profiles and patient samples (n=457) for which phenotypic data was available. Clinical isolates yielded 100% correlation when compared to resistance profiles. One isolate, characterized as blaNDM-1 positive, was not detected by this panel, but repeated sequencing indicated loss of the NDM-1 plasmid. Screening of patient samples revealed a complex pattern of up to fourteen genes encoding resistance to three major classes of antibiotics, with TEM and *ermA-C* being the most prevalent (48% and 91% in respiratory and gastrointestinal samples, respectively). CTX-M Groups 1, 2, and 9; SHV; ACT/MIR; and *qnrA* and *qnrS* were also detected.

Conclusions: Molecular testing is a practical approach for the rapid detection of drug resistance. While phenotypic susceptibility methods are widely accepted in clinical practice, these tests are laborious, time-consuming, and results may be subjective. The direct testing on multidrug resistant organisms with a high-throughput TaqMan® assay panel provides a powerful screening tool that can be utilized in clinical settings to lower healthcare costs, reduce unnecessary antibiotic use, and improve patient care.

Introduction

Multidrug resistant organisms present a major healthcare threat worldwide. Effective antibiotic stewardship programs within healthcare facilities should include rapid and sensitive diagnostic methods. Quick implementation of infection control measures can prevent possible outbreaks, decrease patient's length of stay, and reduce healthcare costs. The objectives of this study were 1) to develop a molecular screening system for detection of genes associated with resistance to three major classes of antibiotics: β -lactams, macrolides and fluoroquinolones and 2) to assess the performance of the system using phenotypically characterized clinical isolates.

Materials & Methods

Samples

- Clinical isolates (n=145) from various sources (ATCC, BEI, CDC, IHMA, and Microbiologics) with characterized antibiotic resistance genotypes and phenotypic antibiotic susceptibility profiles were used for assay development.
- Nasopharyngeal (n=302) and rectal swabs (n=155) were tested from patients admitted to Alabama Infectious Disease Center.
- DNA from samples was extracted on the KingFisher™ Flex System.

Test System

- TaqMan® assays were designed to detect the most clinically relevant gene variants within individual gene classes.
- Assay specificity was verified using clinical isolates with established antibiotic resistance profiles.
- TaqMan® assays were printed on OpenArray® plates for testing.
- The PCR reaction included a pre-amplification step prior to real-time PCR amplification on OpenArray® plates.

Materials & Methods (continued)

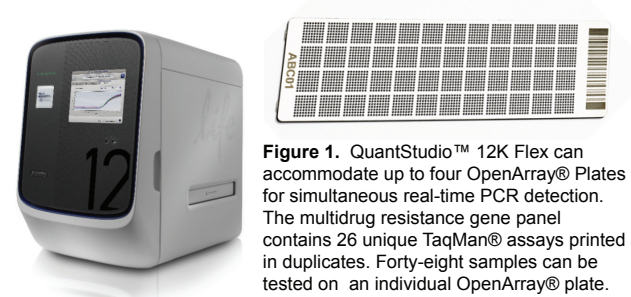


Figure 1. QuantStudio™ 12K Flex can accommodate up to four OpenArray® Plates for simultaneous real-time PCR detection. The multidrug resistance gene panel contains 26 unique TaqMan® assays printed in duplicates. Forty-eight samples can be tested on an individual OpenArray® plate.

Results

TaqMan® assay designs were based on an algorithm evaluating melting temperature, nucleotide composition of primer-pair combinations, and specificity of genomic sequences with closely related gene subtypes.

Table 1. TaqMan® Assay Inclusivity for Detection of Gene Resistance Targets. Assays in bold are targets detected *in silico* across different bacterial strains.

MDRG	Number of RefSeq Used	Target Inclusivity	Organisms Covered in Assay Design
TEM	791	TEM-1, -116, -121	<i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Enterobacter cloacae</i> , <i>Citrobacter spp.</i> , <i>Pseudomonas aeruginosa</i> , <i>Serratia marcescens</i> , <i>Proteus mirabilis</i> , <i>Acinetobacter spp.</i>
SHV	468	Broad coverage	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>Citrobacter freundii</i> , <i>P. aeruginosa</i> , <i>Salmonella enterica</i> , <i>Shigella sonnei</i>
KPC	73	KPC-2, -3, -4	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>C. freundii</i> , <i>P. aeruginosa</i> , <i>S. marcescens</i>
OXA-1	164	OXA-1, -30, -320	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>S. enterica</i> , <i>Shigella flexneri</i>
OXA-48	14	OXA-48, -162, -244	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i>
CTX-M Group 1	495	CTX-15, -3, -55	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>Enterobacter aerogenes</i> , <i>E. cloacae</i> , <i>Acinetobacter spp.</i> , <i>Aeromonas spp.</i> , <i>P. aeruginosa</i> , <i>C. freundii</i>
CTX-M Group 2	110	CTX-2, -39	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>P. mirabilis</i> , <i>S. enterica</i> , <i>P. aeruginosa</i>
CTX-M Group 9	222	CTX-14, -9, -27, -38, -65	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>P. mirabilis</i> , <i>E. cloacae</i>
CTX-M Group 8/25	12	CTX-8, -25, -26, -94, -100	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>Citrobacter amalonoticus</i> , <i>Kluyvera georgiana</i> , <i>S. enterica</i>
VIM	327	VIM-1, -2, -4, -6, 10, -11	<i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>C. freundii</i> , <i>P. aeruginosa</i> , <i>S. marcescens</i> , <i>Acinetobacter baumannii</i>
VEB	31	VEB-1, -2, 3-4, -5, -6	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>Aeromonas spp.</i> , <i>P. mirabilis</i> , <i>K. pneumoniae</i>
NDM	174	NDM-1, -2, -3, -6, -7, -8	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i> , <i>Acinetobacter baumannii</i> , <i>E. cloacae</i> , <i>Providencia stuartii</i>
PER	13	PER-1, -3, -5, -7	<i>P. aeruginosa</i> , <i>A. baumannii</i> , <i>Aeromonas spp.</i> , <i>Alcaligenes faecalis</i> , <i>Providencia rettgeri</i>
IMP	99	IMP-4, -6, -28, -29, 34	<i>Klebsiella oxytoca</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i> , <i>S. marcescens</i> , <i>Acinetobacter baumannii</i> , <i>E. coli</i> , <i>K. pneumoniae</i>
GES	47	GES-1, -5, -3, -4, -11, -12	<i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i>
FOX	10	FOX-4, -5, -6, -7, -10	<i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>E. coli</i> , <i>E. cloacae</i>
ACT/MIR	14	ACT-2, -6, MIR-1, -2, -6	<i>E. coli</i> , <i>Enterobacter asburiae</i> , <i>E. cloacae</i> , <i>K. pneumoniae</i>
<i>ermA</i>	185	<i>ermA</i>	<i>Staphylococcus aureus</i>
<i>ermB</i>	347	<i>ermB</i>	<i>Streptococcus pneumoniae</i> , <i>Enterococcus faecium</i> , <i>Enterococcus faecalis</i> , <i>Clostridium difficile</i> , <i>S. aureus</i> , <i>Pseudomonas aeruginosa</i>
<i>ermC</i>	25	<i>ermC</i>	<i>Staphylococcus spp.</i> , <i>E. faecium</i> , <i>E. faecalis</i> , <i>Neisseria meningitidis</i>
<i>qnrA</i>	72	<i>qnrA</i>	<i>E. coli</i> , <i>E. cloacae</i> , <i>A. baumannii</i> , <i>C. freundii</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i>
<i>qnrS</i>	59	<i>qnrS1</i> , <i>qnrS4</i>	<i>K. pneumoniae</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>S. enterica</i> , <i>S. flexneri</i>

*Not all strains shown

Results (continued)

Genotypic, multidrug resistant gene profiles for all tested assays were 100% concordant with reported phenotypic antibiotic resistance profiles, independent PCR, and sequencing tests.

Table 2. TaqMan® Assay Specificity for Detection of Gene Resistance Targets. Characterized bacterial strains were used to determine assay specificity.

MDRO Class	Assay	Resistance Genes Verified	Number of Strains Tested	Species Tested
Class A β -lactamases	TEM	TEM-1, TEM-2, TEM-3, TEM-10, TEM-12, TEM-89	32	<i>C. freundii</i> , <i>E. asburiae</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i>
	SHV	SHV-1, SHV-11, SHV-12, SHV-18, SHV-28	24	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. pneumoniae</i>
	CTX-M Group 1	CTX-M-1, CTX-M-3, CTX-M-12, CTX-M-15, CTX-M-32, CTX-M-55, CTX-M-116	26	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>P. mirabilis</i>
	CTX-M Group 2	CTX-M-2, CTX-M-74	4	<i>E. coli</i> , <i>K. pneumoniae</i>
	CTX-M Group 8/25	CTX-M-8, CTX-M-40	3	<i>E. coli</i> , <i>K. pneumoniae</i>
	CTX-M Group 9	CTX-M-9, CTX-M-14, CTX-M-24, CTX-M-27, CTX-M-45, CTX-M-57, CTX-M-134	17	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. pneumoniae</i>
Class B metallo- β -lactamases	KPC	KPC-2, KPC-3, KPC-12	15	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>
	IMP-1	IMP-1, IMP-4, IMP-26	5	<i>E. cloacae</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>
	VIM	VIM-1, VIM-2, VIM-4, VIM-5, VIM-23, VIM-26, VIM-27, VIM-31, VIM-32	12	<i>C. freundii</i> , <i>E. cloacae</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i>
AmpC β -lactamases	NDM	NDM-1, NDM-5, NDM-7	14	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i>
	ACT/MIR	ACT-type, ACT-16, MIR-type, MIR-3, MIR-12	8	<i>E. cloacae</i> , <i>K. pneumoniae</i>
Class D oxacillinases	FOX	FOX-3, FOX-10	3	<i>K. pneumoniae</i> , <i>E. cloacae</i>
	OXA-1*	OXA-1	13	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> , <i>Morganella morganii</i>
	OXA-48	OXA-48	7	<i>E. cloacae</i> , <i>K. pneumoniae</i> , <i>M. morganii</i>
	PER	PER-1, PER-3	4	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i> , <i>P. aeruginosa</i>
Minor ESBLs	VEB	VEB-1, VEB-2, VEB-4	4	<i>K. pneumoniae</i> , <i>E. asburiae</i> , <i>P. mirabilis</i> , <i>P. aeruginosa</i>
	GES	GES-1, GES-5, GES-6, GES-11, GES-13, GES-19, GES-20	7	<i>A. baumannii</i> , <i>K. oxytoca</i> , <i>P. aeruginosa</i>
	<i>ermA</i>	<i>ermA</i>	3	<i>S. aureus</i>
Macrolides	<i>ermB</i>	<i>ermB</i>	5	<i>E. coli</i> , <i>E. faecalis</i> , <i>E. faecium</i>
	<i>ermC</i>	<i>ermC</i>	5	<i>E. faecalis</i> , <i>E. faecium</i>
	<i>qnrA</i>	<i>qnrA</i>	6	<i>C. freundii</i> , <i>E. cloacae</i> , <i>E. faecalis</i> , <i>K. pneumoniae</i>
Fluoroquinolones	<i>qnrS</i>	<i>qnrS</i>	9	<i>E. cloacae</i> , <i>E. coli</i> , <i>E. faecalis</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i>

*OXA-1 sequence verification TBD

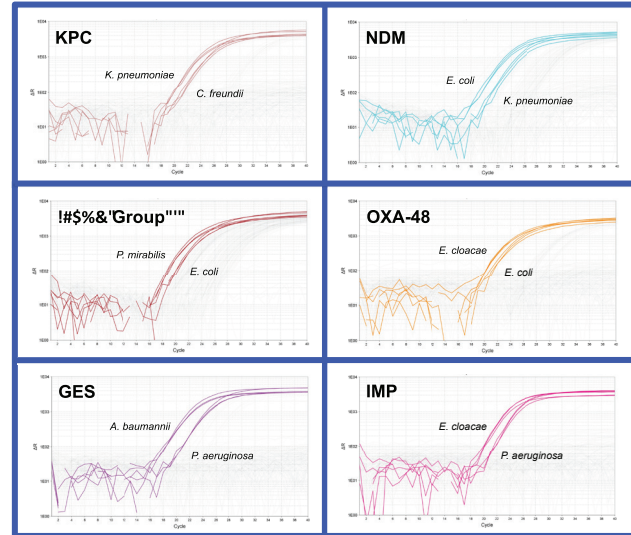


Figure 2. Representative amplification curves of target-specific TaqMan® assays using the OpenArray® platform. Samples were tested in triplicate.

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Results (continued)

The pattern and frequency of specific MDRG detection in stool and sputum samples is related to microbiota present in GI or respiratory tracts of tested samples.

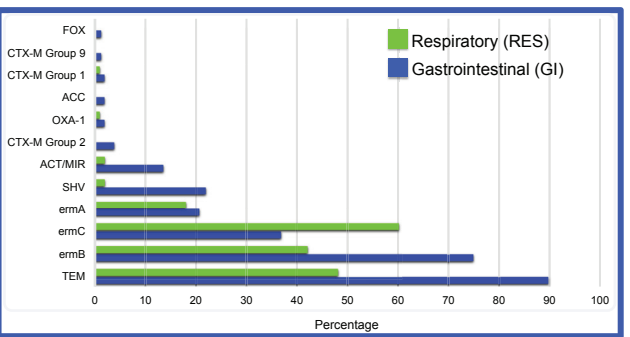
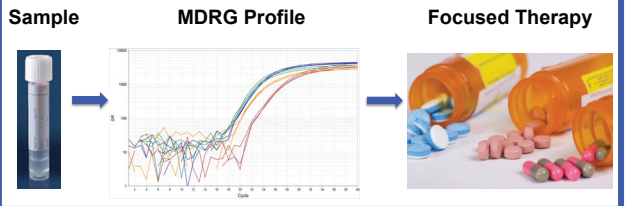


Figure 3. Multidrug resistance genes were detected in clinical samples collected from hospitalized patients: RES sputum samples (n=302, mean age=55 years) GI stool samples (n=155, mean age=59 years).

Utility of a Multidrug Resistance Diagnostic Panel: Sample-to-Answer in 5 hours



Conclusions

- An initial TaqMan® assay panel for detection of twenty-six MDRGs was developed using the spacial multiplexing OpenArray® platform.
- TaqMan® assays were designed to capture multiple clinically relevant subtypes within each major antibiotic resistance gene class found across representative bacterial species.
- Assay specificity was validated using 145 clinical isolates with characterized phenotypic profiles and was 100% concordant with reported phenotypic antibiotic resistance profiles.
- The MDRG Panel provides direct sample testing with no additional enrichment and can be used to evaluate antibiotic resistance patterns in variable patient populations.
- Accurate and rapid detection of antibiotic resistance genes provides a powerful screening tool in clinical settings to improve antibiotic stewardship by lowering healthcare costs, reducing unnecessary antibiotic use, and using focused patient therapy.