

Identification of Multidrug Resistance Genes (MDRGs) in Stool Samples of Hospitalized Patients

Ali Hassoun, MD, FIDSA, FACP¹, Khushdeep Chahal, MD², Esmeralda Asis, MD², Donald Stalons, PhD, D(ABMM), MPH³, Stefan Brzezinski, MBA³, Elena Grigorenko, PhD³

¹Alabama Infectious Disease Center, Huntsville, AL, ²Internal Medicine, University of Alabama at Birmingham Huntsville Regional Medical Campus, Huntsville, AL, ³Diatherix Laboratories, LLC, Huntsville, AL

Ali Hassoun, MD
ali_hassoun@yahoo.com
Ph. No: 256-348-6392
Stefan Brzezinski, MBA
Email: stefan.brzezinski@diatherix.com



Introduction

- The emergence of multidrug resistant organisms and their increasing spread remains a significant challenge due to limited options of antimicrobial therapy and the technical limitations associated with common bacteriologic approaches and their interpretation.
- Multidrug resistance genes (MDRGs) are increasingly being found in the gut flora, specifically in the *Enterobacteriaceae* family.¹
- Horizontal gene transfer between members of the *Enterobacteriaceae* family can spread MDRGs between different organisms.²
- Widespread use of oral antibiotics puts a selective pressure on the gut microbiome, and multidrug resistant organisms can be carried in the gut without causing any symptoms.¹
- An OpenArray® TaqMan® assay panel was developed for detection of twenty-six MDRGs associated with resistances to three major classes of antibiotics: β -lactams, fluoroquinolones, and macrolides.
- This panel was utilized for screening stool specimens of patients admitted to Huntsville Hospital (Huntsville, AL).

Methods

Sample collection

Stool specimens (n=155) were collected from patients with diarrhea and vomiting, or from patients whose stool was submitted for routine occult blood testing (Huntsville Hospital, Huntsville, AL)

DNA extraction

DNA was extracted using a modified Omega Bio-Tek, Inc. Mag-Bind® kit (Norcross, GA) with additional reagents from MO BIO Laboratories, Inc. (Carlsbad, CA) on a Thermo Fisher Scientific, Inc. KingFisher™ Flex (Waltham, MA) semi-automated platform.

PCR detection for MDRGs

Diatherix Laboratories, LLC. (Huntsville, AL) tested DNA from stool samples for the presence of MDRGs on an OpenArray® Taqman® panel using a high-throughput real-time platform QuantStudio® 12K Flex (Thermo Fisher Scientific).

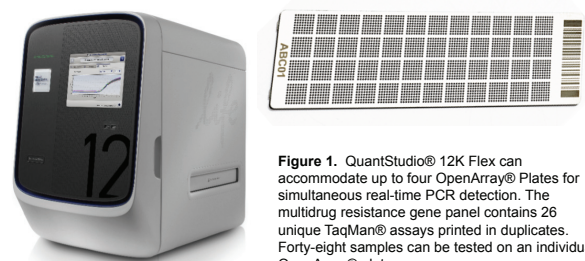


Figure 1. QuantStudio® 12K Flex can accommodate up to four OpenArray® Plates for simultaneous real-time PCR detection. The multidrug resistance gene panel contains 26 unique TaqMan® assays printed in duplicates. Forty-eight samples can be tested on an individual OpenArray® plate.

Results

Table 1. The Multidrug Resistance Gene Panel was developed at Diatherix Laboratories, LLC. The performance of TaqMan® assays were tested for specificity and sensitivity on the OpenArray® platform (see poster 1595).

MDRG Class	Assay
Class A β -lactamases	CTX-M Group 1; CTX-M Group 2; CTX-M Group 8/25; CTX-M Group 9, KPC, TEM, SHV
Class B β -lactamases	IMP-1; VIM, NDM
AmpC β -lactamases	FOX; ACC; ACT/MIR; blaDHA; blaCMY
Class D oxacillinases	OXA-1; OXA-48
Minor ESBL	PER; VEB; GES
Macrolide resistance	<i>ermA</i> , <i>ermB</i> , <i>ermC</i>
Fluoroquinolone resistance	<i>qnrA</i> , <i>qnrS</i>

* ESBL- extended spectrum β -lactamase

Detection of MDRGs in Patients (n=155)

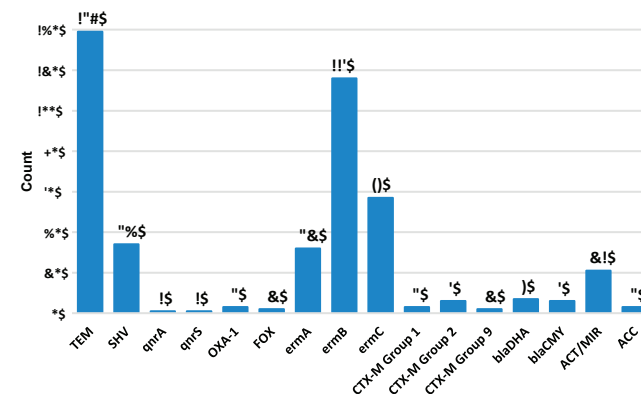


Figure 2. Sixteen MDRGs of the twenty-six gene targets present on the OpenArray® were detected in tested samples. The number above the columns represents the number of positive samples for each detected drug resistance gene.

Symptomatic vs Asymptomatic

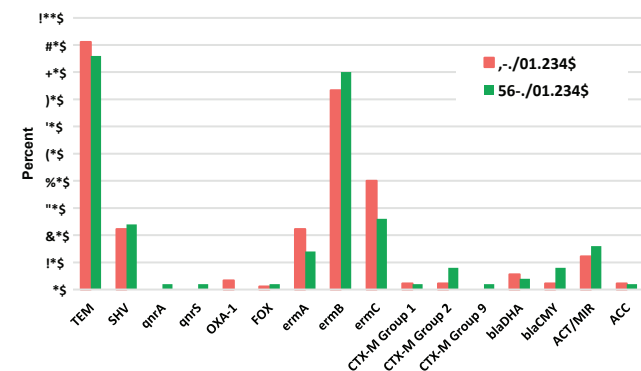


Figure 3. MDRGs detected in symptomatic gastroenteritis patients (n=99), and in asymptomatic (n=56) patients whose stool samples were submitted for occult blood testing.

MDRG Detection by Age Group

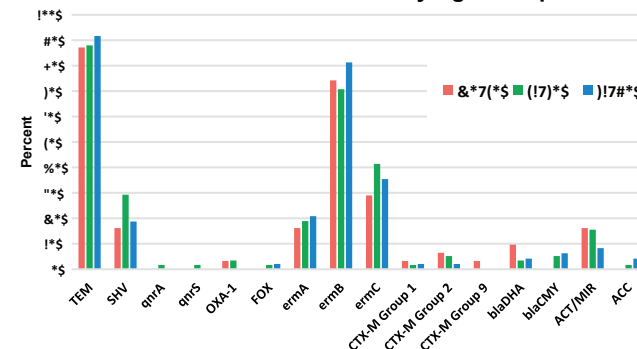


Figure 4. Age-related MDRG distribution (20-50, n=31; 51-70, n=58; 71-90, n=48). Mean age is 59.

Table 2. The number and percentage (parenthesis) of MDRGs co-detected in tested samples. Low prevalence (*qnrA*, *qnrS*, FOX) and high prevalence MDRGs (TEM and *ermB*) were excluded from analysis. blaDHA was excluded due to cross-reactivity.

MDRG Detected	MDRG Co-detection									
	SHV	OXA-1	ACC	ACT/MIR	CTX-M*	qnrA	FOX	ermC	ermA	blaCMY
ACC (n=3)	2 (66%)	---	---	1 (33%)	---	---	---	2 (66%)	1 (33%)	---
ACT/MIR (n=21)	11 (52%)	---	1 (5%)	---	4 (19%)	1 (5%)	1 (5%)	7 (33%)	4 (19%)	1 (5%)
CTX-M* (n=11)	5 (45%)	2 (18%)	---	3 (27%)	---	1 (9%)	1 (9%)	4 (36%)	3 (27%)	---
ermA (n=32)	7 (22%)	1 (3%)	---	4 (13%)	3 (9%)	---	---	16 (50%)	---	---
ermC (n=57)	13 (23%)	1 (2%)	2 (4%)	7 (12%)	4 (7%)	---	---	---	16 (28%)	2 (4%)
OXA-1 (n=3)	1 (33%)	---	---	---	2 (66%)	---	---	1 (33%)	1 (33%)	---
SHV (n=34)	---	1 (3%)	2 (6%)	11 (22%)	5 (15%)	1 (3%)	1 (3%)	13 (38%)	7 (21%)	3 (9%)

* Includes CTX-M groups 1, 2, and 9.

Antibiotic Use

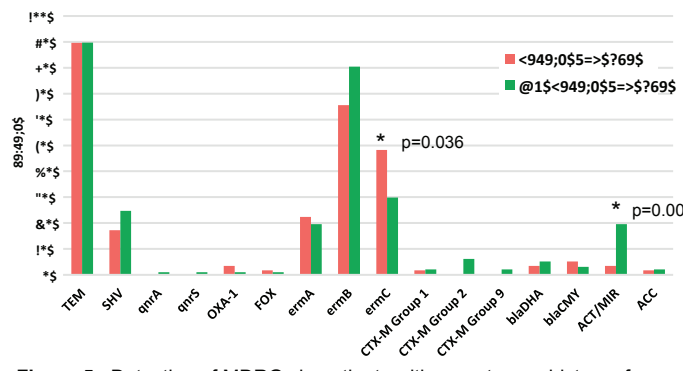


Figure 5. Detection of MDRGs in patients with recent or no history of antibiotic (Abx) use (recent use n=58; no recent use n=97). The difference in *ermC* and ACT/MIR detection between two groups is statistically significant based on Fisher's exact test.

Conclusions

- MDRGs associated with resistances to three major classes of antibiotics (β -lactams, macrolides, and fluoroquinolones) were found in stool samples of hospitalized patients.
- The Class A β -lactamases TEM and SHV and the macrolide resistances were the most common resistance genes found in this study being detected in 94% of tested samples.
- The TaqMan® assays for detection of TEM and SHV gene classes are inclusive for detection of TEM-1 and SHV-1, narrow spectrum β -lactamases predominantly found in *Escherichia coli* and *Klebsiella pneumoniae*.³
- MDRGs associated with macrolide resistance (*ermA*, *ermB*, and *ermC*) were found in 21%, 75%, and 37% samples, respectively.
- No Class B β -lactamases or minor extended spectrum β -lactamases were detected in tested samples.
- There was no difference in gender or age-specific MDRG detection rates.
- There was no difference in antibiotic resistance gene profiles between patients symptomatic for gastroenteritis or the asymptomatic patient population.
- Patients with recent use of antibiotics had a statistically significant increase and decrease of *ermB* and ACT/MIR detection rate, respectively, compared to patients with no recent history of antibiotic use.
- The most common co-detections were identified among the macrolide resistances gene groups.
- This study demonstrated widespread carriage of the older gene resistances that have been present in the environment the longest but did not show high prevalence of the extended spectrum β -lactamases or fluoroquinolones.
- Further studies are urgently needed to incorporate these evolving rapid molecular tests for early detection of multi-drug resistant organisms to improve infection control and prevention in hospitals.

References

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Acknowledgements

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