

Identification of Multidrug Resistance Genes (MDRGs) in the Respiratory Tract Of Hospitalized Patients Using Integrated Molecular Techniques

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Introduction

- Early initiation of appropriate antibiotic therapy is essential in the management of any patient with respiratory tract infections, especially pneumonia. Delays in treatment can have a significant impact on morbidity and mortality. Empirical therapy using broad spectrum antibiotics is the accepted standard of treatment.
- Infections caused by multidrug resistant organisms (MDROs) are steadily increasing.
- Existing diagnostic methods employ identification of bacterial isolates followed by antimicrobial susceptibility profiling, delaying focused therapy by 2-3 days.
- Rapid identification of organisms causing respiratory tract infections, together with multidrug resistance gene profiles, may improve patient outcomes and prevent transmission of drug-resistant pathogens within the hospital setting.
- IDSA guidelines recommends a shift from single-analyte testing to multiplex testing in the molecular diagnostics for infectious disease¹.

Methods

Sample Collection

Sputum samples, patient demographics, discharge diagnoses, and sputum culture results were collected at Huntsville Hospital (Huntsville, AL) from 302 hospitalized patients (52% female) with a mean age of 55 years.

TEM-PCR™

Sputum samples were sent to Diatherix Laboratories, LLC (Huntsville, AL) for simultaneous processing on the Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™) Respiratory Panel, a molecular method (Figure 1) used to identify commonly encountered respiratory pathogens and four resistance genes associated with *Staphylococcus spp.*, including MRSA. TEM-PCR™ is protected by US 7,851,148 B2 properties of Diatherix Laboratories, LLC.



Figure 1. TEM-PCR™ Scheme. Low concentration nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal Superprimers (Fs and Rs) is used to amplify all targets. The Rs primer is labeled with biotin for subsequent detection. PCR products are detected by a complimentary detection probe covalently coupled to a glass microarray substrate.

MDRG Screening

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

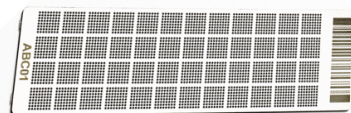
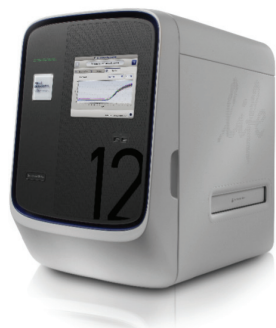


Figure 2. The QuantStudio® 12K Flex can accommodate up to four OpenArray® plates for simultaneous real-time PCR detection. The multidrug resistance gene panel contains 26 unique TaqMan® assays printed in duplicates. Forty-eight samples can be tested on an individual OpenArray® plate.

Results

Table 1. The Multidrug Resistance Gene Panel has been 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

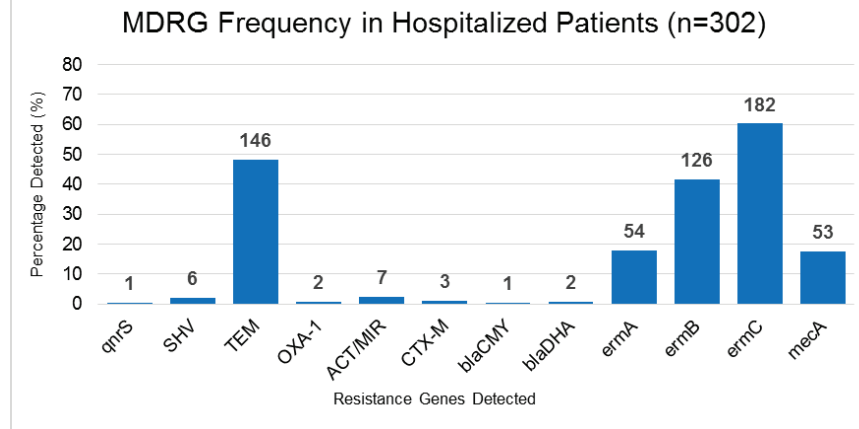


Figure 3. Multidrug Resistance Gene Detection Frequency. Among 302 patients, the following resistance genes were detected: *qnrS* (1), SHV (6), TEM (146), OXA-1 (2), ACT/MIR (7), CTX-M (3), blaCMY (1), blaDHA (2), *ermA* (54), *ermB* (126), *ermC* (182), and *mecA* (53).

Table 2. Sputum cultures were positive in 18% of 111 specimens tested at Huntsville Hospital. The most common organisms were *Pseudomonas* and MRSA. TEM-PCR™ was positive in 55% of 302 specimens. MRSA, pneumococci, and *Haemophilus influenzae* were the most commonly detected organisms. At least one MDRG was detected in 76% of patients with negative sputum cultures and 82% of TEM-PCR™ negative samples.

Laboratory Result	MDRG Detected	MDRG Not Detected
Sputum Culture Positive	17 (85%)	3 (15%)
Sputum Culture Negative	73 (80%)	18 (20%)
TEM-PCR Positive*	142 (86%)	24 (14%)
TEM-PCR Negative	112 (82%)	24 (18%)

*Includes viral pathogens

The most common discharge diagnoses were community-acquired pneumonia (CAP) 31%, upper respiratory infection (URI) 11%, sepsis 10%, chronic obstructive pulmonary disorder (COPD) 8%, and healthcare-associated pneumonia (HCAP) 8%.

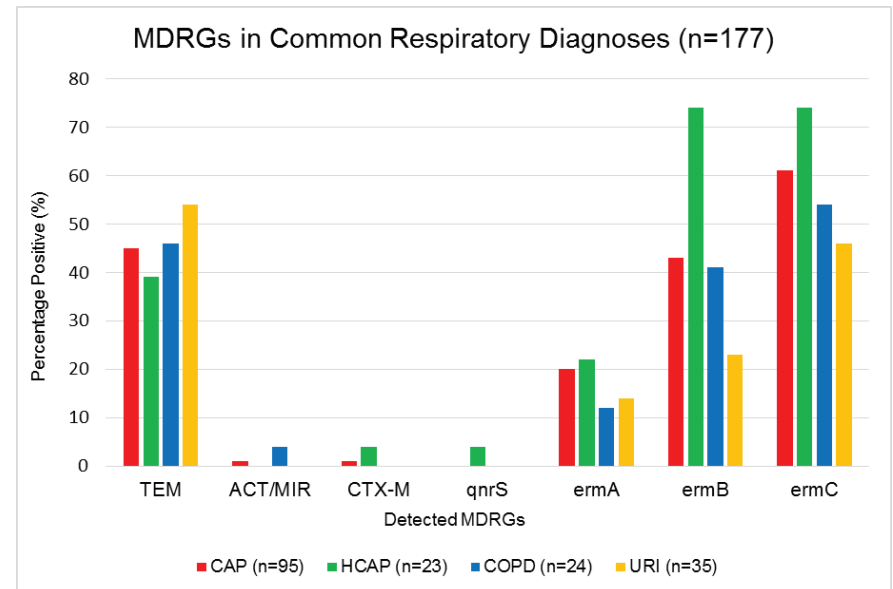


Figure 4. Multidrug Resistance Genes Present in the Most Common Respiratory Discharge Diagnoses. TEM, *ermA*, *ermB* and *ermC* genes were frequently detected in respiratory tract samples. Patients with HCAP were more likely to have the CTX-M, *qnrS*, *ermB* and *ermC* genes. Patients with COPD were more likely to have presence of ACT/MIR genes.

Conclusions

- MDRGs associated with resistance to three major classes of antibiotics (β -lactams, macrolides and fluoroquinolones) were found in samples from the respiratory tract in hospitalized patients.
- Patients with negative sputum cultures had a high rate of MDRG carriage (76%).
- The class A β -lactamase, TEM and macrolide resistance genes were the most frequently detected in sputum samples of hospitalized patients.
- The TEM-PCR™ Respiratory Panel detected more bacterial and viral pathogens than standard microbiological techniques. Viral pathogens would be missed by the hospital laboratory using conventional test methods.
- Further studies are needed to assess the benefit of molecular testing of MDRGs for screening and management of patients at high risk of infection with multidrug resistant organisms.

Reference

- Caliendo, AM, Gilbert, DN, Ginocchio et al. Better Tests, Better care: Improved Diagnostics for Infectious Diseases. CID 2013;57 (Suppl 3), S139-S170.

Acknowledgements

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