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

Ali Hassoun, MD FIDSA FACP,1 Mounika Boyeinpally, MD,2 Ashokkumar Gaba, MD,2 Mickala Tompson, PharmD3, Jonathan Edwards, PharmD3, Leslie Malone, MS, MB(ASCP)CM4, Matthew Huff, BS5, Elena Grigorenko, PhD6 and Donald Stalons, PhD, D(ABMM), MPH7
1Alabama Infectious Diseases Center, Huntsville, AL, 2Internal Medicine, UAB Huntsville Campus, Huntsville, AL, 3Huntsville Hospital, Huntsville, AL, 4Diatherix Laboratories, LLC, Huntsville, AL, 5Huntsville Hospital, Huntsville, AL, 6Internal Medicine, UAB, Birmingham, AL, 7Diatherix Laboratories, LLC, Huntsville, AL

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 mortality and morbidity. Empirical therapy using broad-spectrum antibiotics is the accepted standard of treatment.

• Infections caused by multidrug resistant organisms (MDROs) are increasingly recognized.

• 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 profiling, may improve patient outcomes and prevent transmission of drug-resistant pathogens within the hospital setting.

• IDSA-guidelines recommends a shift from single-antigen testing to multiplex testing in the molecular diagnostics for infectious diseases.1

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 real-time PCR detection. The TEM-PCR™ Respiratory Panel, a molecular method (Figure 1) used to identify commonly encountered respiratory pathogens and four resistance genes associated with Staphylococcus spp, was developed by Diatherix Laboratories, LLC. TEM-PCR™ is protected by US 7,851,148 B2.

Figure 1. TEM-PCR™ Scheme. Low concentration threshold gene-specific primers are used to form a nested PCR reaction. A Superprimer is used to amplify all targets. PCR products are detected by a complementary detection probe covalently coupled to a glass microarray slide. 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).

Results

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

<table>
<thead>
<tr>
<th>MDRG Class</th>
<th>Class A β-lactamases</th>
<th>Class B β-lactamases</th>
<th>AmpC β-lactamases</th>
<th>Class D oxacillinases</th>
<th>Minor ESBL</th>
<th>Macrolide resistance</th>
<th>Fluoroquinolone resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M Group 1</td>
<td>CTX-M Group 2</td>
<td>CTX-M Group B2/5</td>
<td>CTX-M Group 9</td>
<td>TEM</td>
<td>SHV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP-1</td>
<td>VIM</td>
<td>NDM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOX</td>
<td>ACC</td>
<td>ACT/IMP</td>
<td>blaHDA</td>
<td>blaCMY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-1</td>
<td>OXA-48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qnrA</td>
<td>qnrB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TEM-PCR Positive* 142 (86%) 24 (14%)

Sputum Culture Positive 17 (85%) 3 (15%)

*Includes viral pathogens

Table 2. Sputum cultures were positive in 12% of 302 specimens tested at Huntsville Hospital. The most common organisms were Pseudomonas aeruginosa and MRSA. TEM-PCR™ was positive in 129 of 302 specimens. MDRG screening on sputum samples resulted in 82% of TEM-PCR™ positive samples.

Conclusions

• MDRGs associated with resistance to three major classes of antibiotics (-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 (75%).

• The class A β-lactamases, TEM and macrolide resistance genes were 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.

Acknowledgements

We would like to thank the Clinical Laboratory at Diatherix Laboratories for processing all TEM-PCR™ specimens for this study. Megan Don摔倒 in Diatherix Laboratories for processing all MDRG panel samples, and Thermo Fisher Scientific for providing OpenArray® supports used in this study.

Reference