Strategy for Rapid Identification and Antibiotic Susceptibility Testing of Gram-Negative Bacteria Directly Recovered from Positive Blood Cultures Using the Bruker MALDI Biotyper and the BD Phoenix System

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Decreasing the time to species identification and antibiotic susceptibility determination of strains recovered from patients with bacteremia significantly decreases morbidity and mortality. Herein, we validated a method to identify Gram-negative bacteria directly from positive blood culture medium using the Bruker MALDI Biotyper and to rapidly perform susceptibility testing using the BD Phoenix.

Bloodstream infections caused by Gram-negative bacteria are a leading cause of morbidity and mortality among hospitalized patients (12, 14). Inasmuch as decreasing the time to identify the causative organism and determine its antibiotic susceptibility can significantly improve outcomes in patients with bacteremia or sepsis (1, 7, 16), we validated a method to rapidly identify and perform susceptibility testing for Gram-negative bacteria directly recovered from positive blood culture medium. Our strategy used matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper; Bruker Daltonics, Billerica, MA) and automated antibiotic susceptibility testing (Phoenix, BD, Sparks, MD) instruments.

The study was performed in two parts. First, we created a training set of 50 simulated bacteremia specimens. The 10 Gram-negative species included in the training set were distributed in a ratio that is representative of the bacteremia specimens processed in our clinical microbiology laboratory. Organisms were selected from cryopreserved stocks of strains purchased from the American Type Culture Collection (ATCC, Manassas, VA), strains recovered from College of American Pathologists (CAP, Northfield, IL) proficiency testing surveys, and patient isolates that were collected simultaneously. For rapid organism identification, an aliquot of the pelleted bacteria was collected by gently touching it with a cotton swab (Pur-Wraps; Puritan Medical Products Company, Guilford, ME) and spotted onto the stainless steel target. Importantly, in evaluating several different swabs, we determined that a loosely wound, coarsely textured cotton tip provided the most consistent transfer of bacteria to the target. The bacteria were then allowed to dry at room temperature, overlaid with 1 μl of α-cyano-4-hydroxycinnamic acid (HCCA) matrix, and analyzed using the MALDI-TOF mass spectrometer (MALDI Biotyper with FlexControl software; Bruker Daltonics) according to the manufacturer’s instructions. Since MALDI-TOF mass spectrometry cannot reliably distinguish between Escherichia coli and Shigella spp., a spot indole test was performed to confirm the identification. Each unknown organism was also identified using conventional biochemical phenotyping methods (Phoenix; BD) on colonies isolated from subcultures of the positive blood culture medium.

Received 11 February 2012 Returned for modification 12 March 2012 Accepted 5 April 2012

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doi:10.1128/JCM.00409-12
Recent studies have identified and rapidly assessed for antibiotic susceptibility directly from positive blood cultures can be accurately determined for each strain. Antibiotic susceptibility results using the direct pellet method were compared to those generated by the conventional method (Phoenix; BD) that tests colonies isolated from subcultures of the positive blood culture medium. Any strain-antibiotic combination demonstrating a >1-fold dilution difference in the MIC or a change in the categorical drug-susceptible/resistant interpretation between the two methods was resolved by performing an epsilometer test (bioMérieux, Durham, NC). Categorical disagreements were scored as very major errors (susceptible by the direct method and resistant by the reference method), major errors (resistant by the direct method and susceptible by the reference method), or minor errors (intermediate by one method and susceptible or resistant by the other method) as previously described (6).

Results of the rapid identification study using the MALDI-TOF mass spectrometer were highly concordant with those based on conventional biochemical phenotypes and 16S rRNA gene sequences (108/110 identifications were concordant [98%]) (Table 1). Both discordant identifications occurred in the training set. The MALDI-TOF mass spectrometer did not generate a reliable identification score for one Enterobacter cloacae complex strain, and it identified one Stenotrophomonas maltophilia strain to only the genus (Table 1). A similar level of diagnostic accuracy for identifying Gram-negative bacteria directly from positive blood culture bottles has been previously reported (4, 11, 15). Results of the rapid susceptibility study using pelleted bacteria directly recovered from the positive blood culture medium were also highly concordant with those of the conventional method using colonies isolated by subculture (Table 1). A similar level of accuracy for susceptibility testing directly from positive blood culture bottles has been previously reported (3, 5, 8–10). The training set and validation set included totals of 821 and 1,074 organism-antibiotic combinations, respectively. In total, 5 very major errors (0.26%), 6 major errors (0.32%), and 26 minor errors (1.37%) were recorded. These data are consistent with accepted error rates for clinical microbiology laboratories (2, 13). No significant trend in the errors was observed for any organism or antibiotic agent tested (chi-square test, Prism 4; GraphPad Software, La Jolla, CA). Importantly, by bypassing the time-consuming subculture steps included in the conventional method, results from the direct testing method were generated a full day earlier (mean, 24.06 h; standard error of the mean [SEM], 0.84 h; range, 9.57 to 35.07 h).

In summary, the data demonstrate that Gram-negative organisms recovered from positive blood cultures can be accurately identified and rapidly assessed for antibiotic susceptibility directly from the primary broth. Although other investigators have also recently reported the successful identification of bacteria directly from positive blood culture medium using MALDI-TOF mass spectrometry (4, 11, 15), we are the first to couple rapid identification with rapid antibiotic susceptibility testing. To have a maximum benefit to patient care, particularly in Gram-negative sepis, appropriate antibiotic therapy must be administered as soon as possible (12, 14). To this end, our strategy markedly reduced turnaround times for species identification and susceptibility testing. Due to operational constraints in our laboratory, specimens are processed in batches at predetermined time points. However, if the workflow were modified to immediately process positive blood cultures for Gram stain interpretation, MALDI-TOF analysis, and susceptibility testing when flagged by the instrument, clinically actionable results could be generated even more quickly.

### Table 1: Organism identification and antibiotic susceptibility testing results

<table>
<thead>
<tr>
<th>Gram-negative strain</th>
<th>No. of concordant identifications/total no. of identifications (%)</th>
<th>No. of organism-antibiotic susceptibility testing (% of total no. of tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training set</td>
<td>Validation set</td>
</tr>
<tr>
<td><em>Achromobacter</em> species</td>
<td>13a</td>
<td>0</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td><em>E. cloacae</em> complex</td>
<td>114</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em>b</td>
<td>854</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em>c</td>
<td>442</td>
<td>1d</td>
</tr>
<tr>
<td><em>Morganella morgani</em></td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>126</td>
<td>3e</td>
</tr>
<tr>
<td><em>Providencia stuartii</em></td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>80</td>
<td>1f</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td><em>S. maltophilia</em></td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,882</td>
<td>5 (0.26)</td>
</tr>
</tbody>
</table>

a For all organisms, analysis of the antibiotic susceptibility test data of the direct testing method compared to those of the conventional method was limited to the agents reported for each strain.
b Ten *E. coli* strains were extended-spectrum β-lactamase (ESBL)-producing organisms.
c Seven *K. pneumoniae* strains were ESBL-producing organisms.
d The discordant agent was tetracycline.
e The discordant agents were cefazolin in 1 strain and cefazolin and cefuroxime in 1 strain.
f The discordant agent was aztreonam.
g The discordant agents were ampicillin in 1 strain, tetracycline in 1 strain, and trimethoprim-sulfamethoxazole and cefepime in 1 strain.
h The discordant agents were imipenem in 1 strain and piperacillin-tazobactam in 1 strain.

JCM 50(7):2453-2463, 2012
The direct testing method described herein has been successfully implemented in our clinical microbiology laboratory that serves as the reference laboratory for The Methodist Hospital System. Validation studies are now under way to expand the strategy of rapid identification and antibiotic susceptibility testing to bacteria caused by Gram-positive bacteria and yeast.

ACKNOWLEDGMENTS

We thank the clinical microbiology laboratory staff for assistance with the study and K. Stockbauer and P. Randall for assistance with manuscript preparation.

REFERENCES