Evaluation of Three Rapid Methods for Detection of Methicillin Resistance in Staphylococcus aureus

L. LOUIE,* S. O. MATSUMURA, E. CHOI, M. LOUIE, AND A. E. SIMOR

Department of Microbiology, SD Laboratory Services, Sunnybrook and Women’s College Health Sciences Centre, and the University of Toronto, Toronto, Ontario M4N 3M5, Canada

Received 24 November 1999/Returned for modification 10 February 2000/Accepted 6 March 2000

The probe-based Velogene Rapid MRSA Identification Assay (ID Biomedical Corp., Vancouver, British Columbia, Canada) and the latex agglutination MRSA-Screen (Denka Seiken Co., Tokyo, Japan) were evaluated for their ability to identify methicillin-resistant Staphylococcus aureus (MRSA) and to distinguish strains of MRSA from borderline oxacillin-resistant S. aureus (BORA; mecA-negative, oxacillin MICs of 2 to 8 μg/ml). The Velogene is a 90-min assay using a chimeric probe to detect the mecA gene. MRSA-Screen is a 15-min latex agglutination test with penicillin-binding protein 2a antibody-sensitized latex particles. We compared these assays with the BBL Crystal MRSA ID System (Becton Dickinson, Cockeysville, Md.) and with PCR for mecA gene detection. A total of 397 clinical isolates of S. aureus were tested, consisting of 164 methicillin-susceptible strains, 197 MRSA strains, and 37 BORA strains. All assays performed well for the identification of MRSA with sensitivities and specificities for Velogene, MRSA-Screen, and BBL Crystal MRSA ID of 98.5 and 100%, 98.5 and 100%, and 98.5 and 100%, respectively. Three MRSA strains were not correctly identified by each of the Velogene and MRSA-Screen assays, but repeat testing with a larger inoculum resolved the discrepancies. The BBL Crystal MRSA ID test misclassified four BORA strains as MRSA. Both the Velogene and the MRSA-Screen assays are easy to perform, can accurately differentiate BORA isolates from MRSA isolates, and provide a rapid alternative for the detection of methicillin resistance in S. aureus in clinical laboratories, especially when mecA PCR gene detection is unavailable.

Materials and Methods

Clinical specimens. A total of 397 well-characterized clinical isolates of S. aureus were selected for testing, consisting of 163 methicillin-susceptible S. aureus (MSSA) strains (oxacillin MIC, ≤1 μg/ml; mecA negative), 197 MRSA strains (oxacillin MIC, ≥4 μg/ml; mecA positive), and 37 BORA strains (oxacillin MIC, 2 to 8 μg/ml; mecA negative). The isolates had been typed by pulsed-field gel electrophoresis and were shown to have represented distinct genotypes. All isolates were stored frozen in buffered glycerol at −70°C and were subcultured twice onto Trypticase soy agar supplemented with 5% sheep blood prior to testing. All isolates were subjected to “blinded” testing with the Velogene assay, the MRSA-Screen test kit, the BBL Crystal MRSA ID test, the oxacillin agar screen plate test (14), and determination of oxacillin MICs by broth microdilution testing (14). Control strains used for all assays included the MRSA strains ATCC 33592 and ATCC 43300 and MSSA strain ATCC 29213.

Velogene Rapid MRSA Identification Assay. Testing of isolates using the Velogene assay was performed in accordance with the manufacturer’s instructions. Briefly, a 1-μl loopful of growth from a blood agar plate was suspended in 50 μl of lysate buffer and incubated at 55°C for 20 min. The suspension was then incubated in a dry bath at 95°C for 5 min. A 50-μl aliquot of cycling reagent was
The results of testing with the Velogene Rapid MRSA Identification Assay, the MRSA-Screen, the BBL Crystal MRSA ID system, and the oxacillin agar screen plate are summarized in Table 2. Retesting a subset of 60 isolates yielded identical results. Discrepant test results obtained with these assays are summarized in Table 3. The Velogene Rapid MRSA Identification Assay was able to accurately detect methicillin resistance in almost all strains (sensitivity, 98.5%), including those with low-level resistance (oxacillin MICs, 4 to 8 μg/ml). There were no false-positive reactions when testing MSSA or BORSA strains (specificity, 100%). However, with visual interpretation of test results, three strains of MRSA were initially identified as methicillin susceptible (Table 3). These three strains were very “sticky” in consistency and were difficult to scrape off the plate. This created problems when emulsifying the organisms in the lysis buffer provided. Two of these three strains were identified as MRSA strains when the test results were read by spectrophotometer. Repeat testing of these two strains with a larger inoculum gave correct results both visually and spectrophotometrically. Use of the larger inoculum did not decrease the specificity of the assay.

The MRSA-Screen latex agglutination assay also had excellent sensitivity (98.5%) and specificity (100%) for the detection of methicillin resistance in S. aureus. However, methicillin resistance was not detected in three isolates (Table 3). Upon retesting these isolates with a larger inoculum, all three were found to agglutinate with the anti-PBP 2a-sensitized latex. No false-positive reactions were observed with a larger inoculum, and no autoagglutination was observed in the control latex reagent.

The BBL Crystal MRSA ID System performed well for the detection of MRSA strains, although some BORSA isolates and MRSA strains with oxacillin MICs of ≤8 μg/ml were

### Table 1. Primers used for multiplex PCR for the identification of methicillin resistance in S. aureus

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5′–3′)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>meca1</td>
<td>AAA ATC GAT GGT AAA GGT TGG C</td>
<td>533</td>
<td>12</td>
</tr>
<tr>
<td>meca2</td>
<td>AGT TCT GCA GTA CAG GAT TGC</td>
<td>270</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2. Results of testing 397 S. aureus strains with the Velogene Rapid MRSA Identification Assay, the MRSA-Screen, the BBL Crystal MRSA ID system, the oxacillin agar screen plate, and meca PCR

<table>
<thead>
<tr>
<th>Organism (no. of strains)</th>
<th>Velogene Rapid MRSA</th>
<th>MRSA-Screen</th>
<th>BBL Crystal</th>
<th>Oxacillin agar screen plate</th>
<th>meca PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (197)</td>
<td>194</td>
<td>194</td>
<td>194</td>
<td>195</td>
<td>2</td>
</tr>
<tr>
<td>MSSA (163)</td>
<td>0</td>
<td>163</td>
<td>0</td>
<td>163</td>
<td>0</td>
</tr>
<tr>
<td>BORSA (37)</td>
<td>37</td>
<td>0</td>
<td>37</td>
<td>34</td>
<td>29</td>
</tr>
</tbody>
</table>

* For the five tests, the sensitivity and specificity values for the detection of methicillin resistance were 98.5% and 100%, 98.5% and 100%, 98.5% and 98.0%, 99.0% and 85.5%, and 100% and 100%, respectively.

* For this method, the number of strains showing growth (+) or no growth (–) are indicated.

### Table 3. Discrepancies between meca PCR detection, Velogene Rapid MRSA Identification Assay, MRSA-Screen, BBL Crystal MRSA ID system, and oxacillin agar screen test results

<table>
<thead>
<tr>
<th>Identification (oxacillin MIC [μg/ml])</th>
<th>No. of isolates</th>
<th>Discrepancies with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>meca PCR</td>
<td>Velogene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MRSA-Screen&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BORSA (4–8)</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>BORSA (4–8)</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>MRSA (&gt;128)</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>MRSA (4–8)</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>MRSA (8)</td>
<td>1</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> meca probe negative; +, meca probe positive.

<sup>b</sup> no agglutination (PBP 2a absent); +, agglutination (PBP 2a present).

<sup>c</sup> no fluorescence (no growth in oxacillin at 4 μg/ml well); +, fluorescence (growth in oxacillin at 4 μg/ml well).

<sup>d</sup> OXA6, oxacillin agar screen plate; growth, growth after 24 h of incubation; no growth, no growth after 24 h of incubation.
misidentified (Table 3). The sensitivity of this assay was 98.5%, and the specificity was 98%.

The oxacillin agar screen plate performed well for the detection of MRSA strains, missing only two, both with oxacillin MICs of 4 μg/ml (sensitivity, 99%). Growth of all of the MSSA strains and of eight BORSA strains was suppressed on the screen plate; 29 BORSA strains did grow on the oxacillin screen plate (specificity, 85.5%).

DISCUSSION

It is known that many strains of MRSA demonstrate heterogeneous expression of oxacillin resistance (5, 7). As a result, laboratory methods have been developed to enhance the expression of resistance in staphylococci, including the supplementation of media with NaCl and prolonging the incubation period to 24 h (7). The use of the oxacillin agar screen plate containing 6 μg of oxacillin per ml, as recommended by the NCCLS (14), has been very useful for identifying MRSA, although many BORSA strains will also grow on this medium. Rapid commercially available methods of detecting methicillin resistance in staphylococci, such as the BBL Crystal MRSA ID test kit, have been developed and, as in the present study, these methods have been found to be useful (9, 17). Nevertheless, difficulties exist in accurately identifying MRSA and in differentiating these strains from BORSA strains (18, 19, 23). In this study, we wished to determine the accuracy of two new rapid diagnostic tests for the detection of methicillin resistance in S. aureus. A large number of BORSA isolates was included in this evaluation in order to challenge the assays.

The Velogene Rapid MRSA Identification Assay was rapid and easy to perform, providing results in approximately 90 min. This test compares favorably with conventional susceptibility test methods and provides more rapid results. Test results can be interpreted visually or by using a spectrophotometer. Problems were occasionally encountered with certain strains of S. aureus with a very “sticky” or “waxy” consistency. Since these strains were difficult to pick off an agar plate, a one-loopful inoculum of approximately 50 colonies (a large, “heaping” loopful) resulted in improved sensitivity of the assay without loss of specificity. The manufacturer has recently changed its recommendations, indicating the need for use of a higher inoculum or more time for agglutination. In another recent report (25), MRSA strains that initially failed to agglutinate with the MRSA-Screen assay were retested after incubation in the presence of a 5-μg methicillin disk in order to increase the level of PBP 2a expression. However, this would add to the total amount of time required for laboratory confirmation of MRSA. The MRSA-Screen assay is simple to perform, highly sensitive and specific, and can easily be incorporated into any clinical diagnostic laboratory.

Attempts to use genotypic methods for the identification of MRSA have generally been limited to specialized reference laboratories. With the introduction of newer assays, such as the Velogene Rapid MRSA Identification Assay and the MRSA-Screen, diagnostic laboratories will have better tools at their disposal for rapid and accurate detection of methicillin resistance in S. aureus. The time and cost savings that are realized with these newer, genotype-based assays will allow clinicians and infection control practitioners to more effectively manage patients and control the spread of MRSA. These newer tests show great promise in providing rapid, sensitive and specific alternatives for clinical laboratories where PCR or DNA hybridization for the mecA gene is not readily available.

REFERENCES


