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

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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* (BORSA; *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 BORSA 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%, 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 BORSA strains as MRSA. Both the Velogene and the MRSA-Screen assays are easy to perform, can accurately differentiate BORSA 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.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become increasingly prevalent worldwide. In the United States and in some European countries, MRSA accounts for 10 to 40% of all *S. aureus* isolates (16, 26). Increased surveillance, including screening of high-risk patients, has been recognized as an important component of effective infection control programs to limit the spread of MRSA in hospitals. Therefore, rapid and accurate identification of MRSA is essential. Traditional antimicrobial susceptibility test methods such as disk diffusion or broth microdilution require at least 24 h to perform. In addition, problems in the laboratory identification of MRSA may occur due to low-level expression of oxacillin resistance in certain strains of *S. aureus*. Difficulties in the differentiation of MRSA from borderline oxacillin-resistant *S. aureus* (BORSA) strains may also occur (8, 10).

Methicillin resistance in *S. aureus* is mediated by the production of an altered penicillin-binding protein, PBP2a (5). The *mec* gene complex regulates the production of PBP2a. Detection of the *mecA* gene or of PBP2a appears to most accurately detect methicillin resistance in *S. aureus* (1, 5, 6, 15, 21, 22). However, the use of these assays is largely restricted to reference centers, and they are not currently utilized by most routine diagnostic laboratories.

Bekkaoui et al. (2) recently described the development of a 2-h assay utilizing cycling probe technology with a DNA-RNA-DNA chimeric probe designed to detect the *mecA* gene in *S. aureus*. The resulting Velogene Rapid MRSA Identification Assay (ID Biomedical Corp., Vancouver, British Columbia, Canada) is a colorimetric enzyme immunoassay (EIA) utilizing a fluorescein-labeled *mecA* probe. This subtractive assay uses a streptavidin-coated 96-well microtiter plate format, and the detection of uncut probe from *mecA* negative strains results in the development of a blue color, whereas *mecA*-positive strains result in a colorless reaction.

In 1998, Nakatomi and Sugiyama (13) reported on the development of a simple test for the detection of the *mecA* gene product, PBP2a. The resulting commercially available assay, the MRSA-Screen (Denka Seiken Co., Tokyo, Japan), is a 15-min slide latex agglutination test using latex particles sensitized with a monoclonal antibody against PBP2a (4).

In this study, we evaluated these two new tests for the detection of methicillin resistance in *S. aureus*. The assays were compared to standard methods of susceptibility testing and to another commercially available kit, the BBL Crystal MRSA ID System (Becton Dickinson, Cockeysville, Md.) (9). Detection of the *mecA* gene by PCR was used as the “gold standard” in this evaluation.

**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 BORSA 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, MRSA-Screen, and BBL Crystal MRSA ID assays are summarized in Table 2. Retesting a subset of 60 isolates with these assays 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 not detected by this method. The sensitivity and specificity of the MRSA-Screen test were 98.5 and 100%, respectively. However, there were a few false-negative reactions when testing MRSA strains (specificity, 98.5%).

### 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 TCT GAT GGT AAG GTG TGG C</td>
<td>533</td>
<td>12</td>
</tr>
<tr>
<td>mecA2</td>
<td>AGI TCA GGA GTA CGG TAT TGG C</td>
<td>270</td>
<td>3</td>
</tr>
<tr>
<td>nucA1</td>
<td>GGCG ATT GAT GAT ACG GGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nucA2</td>
<td>ACA GCC TTG ACG AAC TAA AGC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Results of testing 397 S. aureus strains with the Velogene Rapid MRSA Identification Assay, 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 3 194 3</td>
<td>194 3 195 2</td>
<td>197 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA (163)</td>
<td>0 163 0 163</td>
<td>0 163 0 163</td>
<td>0 163 0 163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BORSA (37)</td>
<td>37 0 37 0 37 0 37 0</td>
<td>0 33 29 0 37 0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For the five tests, the sensitivity and specificity values for the detection of methicillin resistance were 98.5 and 100%, and 98.5 and 98.0%, 98.5 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>mecA PCR</th>
<th>Velogene</th>
<th>MRSA-Screen</th>
<th>Crystal</th>
<th>OXA6 screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>BORSA (4–8)</td>
<td>25</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Growth</td>
</tr>
<tr>
<td>BORSA (4–8)</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Growth</td>
</tr>
<tr>
<td>MRSA (&gt;128)</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Growth</td>
</tr>
<tr>
<td>MRSA (4–8)</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>No growth</td>
</tr>
<tr>
<td>MRSA (8)</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Growth</td>
</tr>
</tbody>
</table>

a = mecA probe negative; +, mecA probe positive.
b = no agglutination (PPB 2a absent); +, agglutination (PPB 2a present).
c = no fluorescence (no growth in oxacillin at 4 μg/ml well); +, fluorescence (growth in oxacillin at 4 μg/ml well).
d = 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 (approximately two to five colonies), as recommended by the manufacturer, may not provide sufficient target for the test assay. As a result, a small number of MRSA strains were not correctly identified. However, improvements to the assay's sensitivity could be achieved by using a heavier inoculum, without affecting the excellent specificity of the assay. Because of its microwell-EIA detection format, the test can easily be adopted for testing of multiple isolates simultaneously, and 20 isolates can easily be tested in less than 2 h.

The MRSA-Screen accurately detected the *mecA* gene product, PBP 2a, in almost all of the test strains of MRSA, as previously reported by other investigators (4, 24, 25). False-negative results occurred with three of the MRSA isolates with oxacillin MICs of either 4 or 8 μg/ml. Similar results had been reported by van Griethuysen et al. (24), who speculated that oxacillin MICs of either 4 or 8 μg/ml can easily be adopted for testing of multiple isolates simultaneously, and 20 isolates can easily be tested in less than 2 h.

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**REFERENCES**