

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%, 98.5 and 100%, and 98.5 and 98%, 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, PBP 2a (5). The *mec* gene complex regulates the production of PBP 2a. Detection of the *mecA* gene or of PBP 2a 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, PBP 2a. 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 PBP 2a (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 lysis 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

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TABLE 1. Primers used for multiplex PCR for the identification of methicillin resistance in *S. aureus*

Primer	Primer sequence (5'-3')	Amplicon size (bp)	Reference
<i>mecA1</i>	AAA ATC GAT GGT AAA GGT TGG C	533	12
<i>mecA2</i>	AGT TCT GCA GTA CCG GAT TTG C		
<i>nucA1</i>	GCG ATT GAT GGT GAT ACG GTT	270	3
<i>nucA2</i>	AGC CAA GCC TTG ACG AAC TAA AGC		

added, and the suspension was incubated at 55°C for a further 25 min. Cycle stop reagent was added, and the suspension was transferred to streptavidin-coated microtiter wells incubated at room temperature for 10 min. After two washes, detection substrate reagent was added. After color development a detection stop reagent was added. The development of a blue color was indicative of a methicillin-susceptible isolate (*mecA* negative); a colorless reaction indicated the presence of a methicillin-resistant strain (*mecA* positive). The test results could be determined spectrophotometrically or by visual inspection. For this evaluation, we used visual inspection for determination of the assay's sensitivity and specificity.

MRSA-Screen. The MRSA-Screen test was performed according to the manufacturer's instructions. A 1-µl loopful of the test isolate was emulsified in 4 drops of an extraction reagent and boiled for 3 min. This suspension was then allowed to cool to room temperature, and 1 drop of a second extraction reagent was added and mixed. This suspension was centrifuged at 1,500 × g for 5 min. A 50-µl aliquot of the supernatant was added to each of two circles on a disposable test card and mixed with 1 drop of the anti-PBP 2a monoclonal antibody sensitized latex and 1 drop of the negative control latex, respectively. The samples were then mixed for 3 min on a shaker, and agglutination was observed visually.

BBL Crystal MRSA ID. Testing with the BBL Crystal MRSA ID system was performed according to the manufacturer's instructions. The inoculated tray was incubated at 35°C for 4 h, and fluorescence in wells was observed by illuminating the panel with long-wave UV light.

Oxacillin agar screen and oxacillin susceptibility testing. Antimicrobial susceptibility testing of isolates using an oxacillin agar screen plate (Mueller-Hinton agar supplemented with 4% NaCl and 6 µg of oxacillin per ml) and by microbroth dilution were performed in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines (14).

Multiplex PCR. PCR was performed for the simultaneous detection of *mecA* (2) and *nucA* (3). The *nucA* gene is responsible for the production of thermostable nuclease and was included in the multiplex PCR assay in order to confirm that the isolates were indeed *S. aureus* and not other staphylococcal species. Bacterial DNA was extracted using two to three colonies of a test organism grown on a 5% sheep blood agar plate and then boiled for 10 min in 100 µl of Triton X-100 lysis buffer (100 mM NaCl, 10 mM Tris-HCl [pH 8], 1 mM EDTA [pH 9], and 1% Triton X-100) (20). The suspension was cooled at room temperature for 5 min and centrifuged at 14,000 rpm for 1 min. Next, 1 µl of the supernatant was used as the template. PCR was performed in a 25-µl volume, with 1× PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM concentrations of each deoxynucleoside triphosphate, 2.5 U of *Taq* polymerase, and 0.2 µM concentrations of each primer. Thermocycling conditions in a GeneAmp 9600 thermocycler (PE Biosystems, Mississauga, Ontario, Canada) were as follows: 94°C for 2 min, followed by 30 cycles of 94°C for 1 s and 55°C for 15 s, with a final 10-min extension at 72°C. The primer sequences for *mecA* and *nucA* are shown in Table 1. The control organisms included *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, and *S. epidermidis* ATCC 12228. Electrophoresis at 100 V for 40 min was performed to separate the products on 1% 1× TBE (8.9 M Tris, 8.9 M boric acid, and 0.2 M EDTA) agarose gels. Gels were stained with ethidium bromide and photographed under UV illumination.

A subset of 60 isolates was selected and retested by all methods in order to determine the reproducibility of the assays evaluated. To determine whether an increased inoculum size would improve test sensitivity without adversely affecting the specificity, an additional subset of 60 isolates (including MSSA, MRSA, and BORSA strains) was retested with the Velogene and MRSA-Screen assays using an inoculum of approximately 50 colonies (of 1-mm diameter), equivalent to a large "heaping" loopful.

RESULTS

The results of testing with the Velogene Rapid MRSA Identification, 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

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^a

Organism (no. of strains)	No. of strains found to be positive (+) or negative (-) by:									
	Velogene Rapid MRSA		MRSA-Screen		BBL Crystal		Oxacillin agar screen plate ^b		<i>mecA</i> PCR	
	+	-	+	-	+	-	+	-	+	-
MRSA (197)	194	3	194	3	194	3	195	2	197	0
MSSA (163)	0	163	0	163	0	163	0	163	0	163
BORSA (37)	0	37	0	37	4	33	29	8	0	37

^a 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 100%, 99.0 and 85.5%, and 100 and 100%, respectively.

^b For this method, the number of strains showing growth (+) or no growth (-) are indicated.

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

Identification (oxacillin MIC [µg/ml])	No. of isolates	Discrepancies with:				
		<i>mecA</i> PCR	Velogene ^a	MRSA-Screen ^b	Crystal ^c	OXA6 screen ^d
BORSA (4-8)	25	-	-	-	-	Growth
BORSA (4-8)	4	-	-	-	+	Growth
MRSA (>128)	3	+	-	+	+	Growth
MRSA (4-8)	2	+	+	-	-	No growth
MRSA (8)	1	+	+	-	-	Growth

^a -, *mecA* probe negative; +, *mecA* probe positive.

^b -, no agglutination (PBP 2a absent); +, agglutination (PBP 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 certain MRSA strains with lower MICs may produce smaller amounts of PBP 2a. The control strain, *S. aureus* ATCC 43300 (oxacillin MIC of 8 µg/ml) (11), also gave a negative result with an inoculum of only 1 µl (a loopful), as recommended by the manufacturer at the time of this evaluation. Using a larger 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.

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