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Evaluation of MRSA-Screen, a Simple Anti-PBP 2a Slide Latex Agglutination Kit, for Rapid Detection of Methicillin Resistance in *Staphylococcus aureus*

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The MRSA-Screen test (Denka Seiken Co., Ltd., Tokyo, Japan), consisting of a slide latex agglutination kit that detects PBP 2a with a monoclonal antibody, was blindly compared to the oxacillin disk diffusion test, the oxacillin-salt agar screen, and PCR of the *mecA* gene for the detection of methicillin resistance in *Staphylococcus aureus*. A total of 120 methicillin-susceptible *S. aureus* (MSSA) and 80 methicillin-resistant *S. aureus* (MRSA) isolates, defined by the absence or presence of the *mecA* gene, respectively, were tested. The MRSA-Screen test, the oxacillin disk diffusion test, and the oxacillin-salt agar screening test showed sensitivities of 100, 61.3, and 82.5% and specificities of 99.2, 96.7, and 98.3%, respectively. We conclude that the MRSA-Screen is a very accurate, reliable, and fast test (15 min) for differentiation of MRSA from MSSA colonies on agar plates.

Resistance to methicillin (and to all β-lactam antibiotics) in *Staphylococcus aureus* is primarily associated with the acquisition of the *mecA* gene coding for the penicillin-binding protein 2a (PBP 2a), involved in bacterial cell wall synthesis (1, 13, 31). The detection of methicillin resistance, however, is complicated by the fact that its phenotypic expression in many strains is heterogeneous (9, 14). This has resulted in the development of various laboratory techniques to enhance the expression of this resistance in vitro (2, 6, 17). *mecA* gene detection tests based on PCR or DNA hybridization performed only by specialized laboratories has proved to be more specific and sensitive than conventional tests, particularly in very heterogeneous strains (16, 22). So far, no simple and rapid method aimed at the direct detection of PBP 2a has been commercialized (11). The purpose of the present study is to test such a candidate.

Bacterial isolates. A total of 200 *S. aureus* clinical isolates collected between 1987 and 1998 from our hospital and 15 neighboring hospitals were used in this study. All isolates were identified by conventional tests. Isolates from the neighboring hospitals (*n* = 33) were sent to our laboratory because of the difficulty of assessing susceptibility to oxacillin by phenotypic methods. The definitions of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) were based on the presence or absence, respectively, of the *mecA* gene by PCR. Among 120 MSSA isolates (19 penicillin susceptible and 101 penicillin resistant), 40 were susceptible to all non-β-lactam antibiotics tested, 51 showed resistance to one, and 29 showed resistance to more than one non-β-lactam antibiotic. Eighty MRSA isolates were carefully selected on the basis of molecular typing (60 different pulsed-field gel electrophoresis [PFGE] patterns) and on the basis of various levels of heterogeneous resistance to oxacillin (35 heterogeneously and 45 homogeneously resistant isolates).

Before being tested, the isolates were removed from storage

(−80°C), streaked onto Columbia blood agar plates, and incubated under aerobic conditions at 35°C for 24 h. An isolated colony was picked from each plate, streaked onto two new Columbia blood agar plates, and incubated for 24 h. All inocula were prepared from these subcultures. All isolates were blindly tested. Two control strains, one MRSA (ATCC 33591) and one MSSA (ATCC 29213) strain, were included in each batch.

MRSA-Screen test. The MRSA-Screen test (Denka Seiken Co., Ltd., Tokyo, Japan) was performed according to the manufacturer's instructions by batches of 20 isolates, including the control strains. The sample preparation was made as follows. Ten to 20 *S. aureus* colonies from a fresh blood agar plate were suspended in a 1.5-ml microtube containing 4 drops (200 μl) of extraction reagent no. 1 (0.1 M NaOH). The suspension was boiled for 3 min, and then 1 drop (50 μl) of extraction reagent no. 2 (0.5 M KH_2PO_4) was added and mixed well. After a centrifugation step (at 1,500 × *g* for 5 min at room temperature), 50 μl of the supernatant was placed on the slide for testing and mixed with 1 drop (25 μl) of anti-PBP 2a monoclonal antibody-sensitized latex. For the negative control, 50 μl of the supernatant was placed on the slide for testing and mixed with 1 drop (25 μl) of negative-control latex. Mixing for 3 min was performed with a shaker. When agglutination occurred within 3 min, it was visually quantified as a score between 1+ and 3+. All the isolates were tested twice, and the results were interpreted blindly by two different persons.

Phenotypic methods. The oxacillin disk diffusion test and oxacillin-salt agar screening test were carried out on all the isolates according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (23–25). Both tests were read after 24 h of incubation. Susceptibility to 11 other antibiotics (penicillin, cephalothin, ceftriaxone, gentamicin, ciprofloxacin, clindamycin, fusidic acid, erythromycin, trimethoprim-sulfamethoxazole, rifampin, and vancomycin) was also tested by the disk diffusion method according to NCCLS recommendations.

***mecA* gene.** Detection of the *mecA* gene was performed blindly for all isolates by using the method described by Tokue et al. with some modifications (28). The extraction technique was simplified by directly suspending 2 to 5 colonies in 200 μl

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TABLE 1. Comparison of the MRSA-Screen test and two phenotypic tests to classify 200 *S. aureus* clinical isolates as MRSA or MSSA[a]

Test method	MRSA (*mecA* gene positive) (n = 80)			MSSA (*mecA* gene negative) (n = 120)		Sensitivity (%)	Specificity (%)
	True positive	False negative	Indeterminate	True negative	False positive		
MRSA-Screen	80	0	0	119	1[b]	100	99.2
Oxacillin disk diffusion	49	20	11	116	4	61.3	96.7
Oxacillin-salt agar screen	66	14	0	118	2	82.5	98.3

[a] Defined by the presence or absence, respectively, of the *mecA* gene.
[b] This isolate, when retested, gave no agglutination.

of water; the suspension was diluted 1:10 and 1:100 in water, and the DNA was released by boiling the suspensions for 5 min at 95°C. One positive MRSA control strain (ATCC 33591), one negative MSSA control strain (ATCC 25923), and water as an extraction control were included in each run.

Molecular typing. Molecular typing was performed by PFGE on all MRSA isolates (4).

Repeat testing. When *S. aureus* isolates yielded discrepant results among the MRSA-Screen test, oxacillin-salt agar screening test, and *mecA* gene detection, the tests were repeated blindly twice from the same Columbia blood agar plate.

β-Lactamase testing. Chromogenic nitrocefin disks (Cefinase; BBL Becton Dickinson, Cockeysville, Md.) were used according to the manufacturer's instructions to test MSSA isolates that appeared resistant to oxacillin by phenotypic methods.

E-tests. Oxacillin MICs were measured for all isolates showing discordant results between phenotypic methods and the MRSA-Screen. E-tests (AB Biodisk, Solna, Sweden) were performed according to the manufacturer's advice on Mueller-Hinton agar supplemented with 2% NaCl and read after 24 h of incubation. In addition, penicillin, amoxicillin, and amoxicillin-clavulanate MICs were measured for the four MSSA isolates that appeared resistant to oxacillin by phenotypic methods.

Results. Table 1 summarizes the results obtained with the MRSA-Screen test, oxacillin disk diffusion method, and oxacillin-salt agar screening test. Table 2 lists the isolates with discrepant results. The positive and negative control strains, included blindly in each of 11 batches of MRSA-screen tests, gave the expected results. No agglutination occurred when the 200 *S. aureus* isolates were tested with the negative-control latex. The MRSA-Screen test by slide latex agglutination showed complete concordance with the detection by PCR of the *mecA* gene, except for one false-positive result among the 120 *mecA* gene-negative *S. aureus* isolates tested. This false-positive agglutination was graded 1+ and gave no agglutination upon retesting. Results of duplicate testing were in agreement for the remaining 199 isolates, which proves the good reproducibility of the test (99.5%). Quantification of the agglutination test reaction (1+ to 3+) for the 80 MRSA isolates did not correlate with their level of heterogeneous resistance to methicillin. Among the 35 heterogeneously resistant MRSA isolates, 31 showed a 3+ agglutination test reaction and 4 showed a 2+ reaction. Of the remaining 45 isolates homogeneously resistant to methicillin, one showed a 1+, 6 showed a 2+, and 38 showed a 3+ agglutination test reaction. Upon retesting, the grading of the agglutination reaction was identical for 69 of 80 MRSA isolates; of the remaining 11 isolates (4 heterogeneously resistant and 7 homogeneously resistant), the grading changed from 3+ to 2+ or from 2+ to 3+ for 10 and from 1+ to 2+ for the last isolate.

Of 80 true MRSA isolates, the oxacillin disk diffusion method falsely read 20 as MSSA (false negative), with inhibition zone diameters ranging from 14 to 27 mm (Table 2). All of them

were heterogeneously resistant, with oxacillin MICs ranging from 0.25 to 3.0 μg/ml by the E-test. These 20 isolates were easily classified as MRSA by the MRSA-Screen test. Among them, only six were also detected as MRSA by the oxacillin-salt agar screening test read after 24 h of incubation.

Among 120 true MSSA isolates, the oxacillin disk diffusion method falsely classified 4 as MRSA (false positive), with inhibition zone diameters between 6 and 8 mm. Two of these isolates were correctly classified as MSSA by the oxacillin-salt agar screening test, whereas the two remaining isolates were also false positive by the latter method. Oxacillin MICs by the E-test ranged from 2.0 to 3.0 μg/ml for these four isolates. Production of β-lactamase, associated with a reduction in the MIC from 24 to 1 μg/ml when clavulanate was added to amoxicillin, was demonstrated in only one isolate. Overproduction of β-lactamase in this isolate may contribute to the oxacillin resistance observed with the oxacillin disk diffusion test (21). The three remaining strains were not β-lactamase producers and could thus be classified as MOD-SA (modified *S. aureus*) isolates (15, 29).

Discussion. The MRSA-Screen test detected all the 80 MRSA isolates and misclassified as MRSA only 1 of 120 MSSA isolates. Compared to the standard agar disk diffusion test and to the oxacillin-salt agar screening test, the MRSA-Screen test showed higher sensitivity and specificity. Fourteen heterogeneously resistant isolates, while they were *mecA* gene positive by PCR, did not express any resistance to oxacillin by the conventional phenotypic methods. However, these *mecA* gene-positive isolates were categorized as MRSA by the MRSA-Screen test, which detects PBP 2a, confirming that expression of the resistance is under the control of other determinants, such as the *BlaI*, *mecR1-mecI*, and *fem* genes, whose mechanisms and interactions are still not fully understood (3, 8, 12, 18).

TABLE 2. Discrepancies between the MRSA-Screen test, *mecA* gene detection by PCR, and phenotypic tests for the detection of methicillin resistance among 200 *S. aureus* clinical isolates

No. of isolates	Result[a] for:			
	MRSA-Screen[b]	*mecA* gene detection	Oxacillin disk diffusion[c]	Oxacillin-salt agar screen[d]
1	+[e]	−	−	−
4	−	−	+	− (2), + (2)
20	+	+	−	− (14), + (6)

[a] +, resistant; −, susceptible.
[b] Agglutination.
[c] Inhibition zone diameters were ≤10 mm for resistant isolates and ≥13 mm for susceptible isolates.
[d] Growth indicated resistance; no growth indicated susceptibility. The number of isolates with each result is given in parentheses after the symbol.
[e] This isolate, when retested, gave no agglutination.

Technically the test was easy to perform, requiring only microtubes, boiling water, a table centrifuge, and a manual pipetter. The test gave results in 15 min, making it the fastest test to our knowledge to reliably detect oxacillin resistance in *S. aureus* isolates. Even if most MRSA isolates agglutinated after 30 s of mixing time, some needed almost the 3 min recommended by the manufacturer to produce visible agglutination. The correct inoculum is also important, as at the beginning of the study we experienced two false-positive agglutination reactions when the inoculum used was about 10 times heavier than that recommended. The influences of various solid growth media on the sensitivity and specificity of the MRSA-Screen test were not investigated in the present study.

As already reported, the oxacillin disk diffusion test was the least reliable test for detection of resistance to oxacillin in *S. aureus* (5, 20, 30). Of the 80 MRSA isolates, 20 had inhibitory-zone diameters in the susceptible range ($\geq$13 mm), 11 in the intermediate range (11 to 12 mm), and 49 in the resistant range ($\leq$10 mm). As recommended by the NCCLS, we looked for associated resistance and cross-resistance to other antibiotics as clues to suspect methicillin resistance. Of the 11 antibiotics tested in addition to oxacillin, ceftriaxone gave the best indication for oxacillin resistance when both intermediate and resistant zone diameters were considered as clues for oxacillin resistance. Among 120 MSSA isolates, the ceftriaxone inhibitory-zone diameters would have suggested resistance to oxacillin in only 4 cases. Of these four, two were also falsely resistant to oxacillin by the oxacillin disk diffusion test. Among 80 MRSA isolates, only 4 were ceftriaxone susceptible, 3 of which were also susceptible to oxacillin by disk diffusion. Although associated resistance to non-β-lactam antibiotics is often found among MRSA isolates, there were 13 MRSA isolates without any associated resistance in this study.

The oxacillin-salt agar screening test is recognized as a sensitive and specific test (7, 10, 27). In our study the sensitivity was low (82.5%). This is due to a bias of selection of the MRSA isolates, as we included strains that were difficult to detect as being resistant to methicillin by the oxacillin-salt agar screening test and oxacillin disk diffusion. However, 13 of the 14 MRSA isolates not detected by the oxacillin-salt agar screen test after 24 h of incubation showed clear growth after reincubation for an additional 24 h. Therefore, in order to further increase the sensitivity of the test, we suggest looking for growth after 24 and 48 h of incubation. In our study, reading all the oxacillin-salt agar screening plates after 48 h of incubation would not have decreased the specificity of the test compared to reading after 24 h of incubation.

Many methods have been evaluated for more accurate detection of methicillin resistance in *S. aureus* (10, 19, 26). The cost and workload of DNA probes or PCR to detect the *mecA* gene have prevented their broad use in a clinical microbiology laboratory. Therefore, detection of PBP 2a with this simple agglutination kit offers an interesting new approach to the rapid characterization of *S. aureus* as MRSA or MSSA. The test has the major advantage over the phenotypic methods of not being influenced by the various levels of expression of the resistance, a parameter which in highly heterogeneously resistant isolates tends to render classical and automated methods less accurate (11). When applied to overnight primary culture agar media, the MRSA-Screen test will shorten the delay for the detection of MRSA to 1 day, versus 2 or 3 days for conventional methods, a potentially significant improvement for both directed antibiotherapy and epidemiological measures.

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