Evaluation of a Latex Agglutination Test (MRSA-Screen) for Detection of Oxacillin Resistance in Coagulase-Negative Staphylococci

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The MRSA-Screen (Denka-Seiken, Tokyo, Japan) latex agglutination test was evaluated for its ability to detect PBP 2a from 200 clinical isolates of coagulase-negative staphylococci (CoNS; 84 *mecA*-positive strains and 116 *mecA*-negative strains) consisting of 108 *Staphylococcus epidermidis*, 37 *S. saprophyticus*, 15 *S. haemolyticus*, 11 *S. hominis*, 10 *S. capitis*, 10 *S. warneri*, and 3 *S. lugdunensis* species as well as 6 other species of CoNS. The assay was compared with susceptibility testing with an agar screen plate with oxacillin at 6 µg/ml (OXA6), by oxacillin disk diffusion (DD), by broth microdilution (BMDIL), by the E test, and with Vitek GPS-SV and Vitek GPS-107 susceptibility cards. PCR for the detection of the *mecA* gene was used as the “gold standard.” The sensitivities and specificities for the methods evaluated were as follows: MRSA-Screen, 100 and 100%, respectively; OXA6, 100 and 99%, respectively; DD, 98 and 62%, respectively; BMDIL, 100 and 60%, respectively; E test, 100 and 51%, respectively; Vitek GPS-SV susceptibility card, 98 and 87%, respectively; and Vitek GPS-107 susceptibility card, 100 and 61%, respectively. The MRSA-Screen test accurately and rapidly detected oxacillin resistance in CoNS.

Coagulase-negative staphylococci (CoNS) are recognized as important causes of nosocomial infection, especially in neonates, those who are immunocompromised, and patients with indwelling prosthetic devices (6). The rate of resistance to oxacillin among CoNS has been increasing (2), and the empirical treatment of choice for infections caused by these organisms is often vancomycin. The rapid and accurate identification of oxacillin resistance is essential in order to determine the most appropriate antimicrobial therapy. The major mechanism of oxacillin resistance in staphylococci is mediated by the production of a modified penicillin-binding protein, PBP 2a, specified by the *mecA* gene. In 1999, the National Committee for Clinical Laboratory Standards (NCCLS) lowered the breakpoint for determination of oxacillin resistance in CoNS from ≥ 4 to ≥ 0.5 µg/ml (12) in order to more accurately reflect *mecA*-mediated resistance. In addition, the use of the oxacillin agar screen plate (Mueller-Hinton agar with 6 µg of oxacillin per ml supplemented with 4% NaCl) was no longer recommended by NCCLS for the detection of oxacillin resistance in CoNS, as reports in the literature had indicated that this method may not be able to detect *mecA*-mediated oxacillin resistance in these strains (15, 17).

A rapid latex agglutination test, the MRSA-Screen (Denka-Seiken, Tokyo, Japan), has recently been developed in order to detect the presence of oxacillin resistance mediated by PBP 2a. Although several studies have evaluated the use of this test for the detection of oxacillin resistance in *Staphylococcus aureus* (8, 18, 19), its accuracy for the detection of oxacillin resistance

in CoNS has not been extensively assessed (5, 9). In the study described here, we evaluated the MRSA-Screen for its ability to detect PBP 2a in clinical strains of CoNS. In addition, an evaluation of other test methods, including the oxacillin agar screen test, disk diffusion, broth microdilution, E test (AB Biodisk, Solna, Sweden), and tests with Vitek susceptibility cards (bioMérieux Inc., Hazelwood, Mo.), was also performed; and the results obtained by those tests were compared to those obtained by PCR detection of the *mecA* gene.

A total of 200 clinical isolates of CoNS recovered from blood, cerebrospinal fluid, urine, and other normally sterile sites were selected for testing. These included *S. epidermidis* (108 strains), *S. saprophyticus* (37 strains), *S. haemolyticus* (15 strains), *S. hominis* (11 strains), *S. capitis* (10 strains), *S. warneri* (10 strains), *S. lugdunensis* (3 strains), *S. simulans* (2 strains), and one strain each of *S. cohnii*, *S. sciuri*, *S. schleiferi*, and *S. xylosum*. All isolates were identified with the API Staph ID 32 system (bioMérieux sa, Marcy-l'Étoile, France). The MRSA-Screen test was performed according to the manufacturer's instructions but with one modification: a large, “heaping” 1-µl loopful of test organism (approximately 30 to 50 colonies, depending on the colony size) was used instead of the standard 1-µl loopful. Antimicrobial susceptibility testing of isolates was performed with an oxacillin agar screen plate (11) prepared in-house (BBL Mueller-Hinton II agar; Becton Dickinson, Cockeysville, Md.) and by disk diffusion and broth microdilution in accordance with the recommendations of NCCLS (13). Oxacillin MIC determination by the E test and breakpoint MIC testing with Vitek GPS-SV and Vitek GPS-107 susceptibility cards were performed according to the manufacturers' instructions. Multiplex PCR assays were performed for the simultaneous detection of the *mecA* (10) and *nucA* (1) gene sequences, as described previously (8).

Of the 200 isolates tested, 84 were *mecA* positive and 116

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TABLE 1. Results of oxacillin broth microdilution and *mecA* PCR for 200 isolates of coagulase-negative staphylococci

Organism	Oxacillin MIC ($\mu\text{g/ml}$)	No. of isolates	
		<i>mecA</i> negative	<i>mecA</i> positive
<i>S. epidermidis</i> (n = 108)	≤ 0.06	17	
	0.12	28	
	0.25	6	
	1		7
	2		14
	≥ 4	1	35
<i>S. saprophyticus</i> (n = 37)	0.5	10	
	1	27	
<i>S. haemolyticus</i> (n = 15)	≤ 0.06	1	
	0.12	1	
	0.25	2	
	≥ 4		11
<i>S. hominis</i> (n = 11)	≤ 0.06	1	
	0.12	2	
	2		2
	≥ 4		6
<i>S. capitis</i> (n = 10)	0.12	1	
	0.25	5	
	0.5	1	
	2		1
	≥ 4	1	1
<i>S. warneri</i> (n = 10)	0.25	5	
	0.5	2	
	≥ 4		3
<i>S. lugdunensis</i> (n = 3)	0.5	3	
<i>S. simulans</i> (n = 2)	≥ 4		2
<i>S. cohnii</i> (n = 1)	≥ 4		1
<i>S. schleiferi</i> (n = 1)	0.12	1	
<i>S. sciuri</i> (n = 1)	1		1
<i>S. xylosum</i> (n = 1)	0.5	1	

were *mecA* negative by PCR (Table 1). All strains positive for the *mecA* gene were detected by the MRSA-Screen latex agglutination test, and all strains negative for *mecA* produced no agglutination. The sensitivities and specificities of the assays

evaluated are summarized in Table 2A. The MRSA-Screen latex agglutination assay rapidly and accurately detected oxacillin resistance that was mediated by the *mecA* gene in CoNS, regardless of the species. Induction of oxacillin resistance was not required in this study, as was previously reported by Hussain et al. (5). This may have been because we used a larger inoculum (a "heaping" loopful). This modification was made on the basis of previous experience with the MRSA-Screen test and testing of *S. aureus* isolates (8). This did not result in any loss of specificity.

Current NCCLS susceptibility test breakpoints (14) provide improved sensitivity for detection of oxacillin resistance in CoNS, especially *S. epidermidis*. For all of the isolates of CoNS that were tested in the present evaluation and that were found to have the *mecA* gene by PCR, oxacillin MICs were $\geq 1 \mu\text{g/ml}$. For a total of 18 isolates, including all 3 strains of *S. lugdunensis*, several strains of *S. saprophyticus*, *S. warneri*, and one strain each of *S. capitis*, *S. epidermidis*, and *S. xylosum*, oxacillin MICs were $0.5 \mu\text{g/ml}$, but the strains did not have the *mecA* gene. In addition, for 27 *S. saprophyticus* isolates, oxacillin MICs were $1 \mu\text{g/ml}$, but the isolates were negative for the *mecA* gene. Discrepancies between the results of the PCR assay for the detection of the *mecA* gene and the results of the oxacillin broth microdilution susceptibility test occurred primarily for species of CoNS other than *S. epidermidis* (most notably, *S. saprophyticus* and *S. lugdunensis*). Mechanisms of oxacillin resistance other than that mediated by the *mecA* gene may be responsible for this observation. Alternatively, these strains may be susceptible to oxacillin and the present NCCLS breakpoint guidelines may need to be reevaluated for certain staphylococcal species, such as *S. saprophyticus* and *S. lugdunensis* (4, 17). For this reason, the sensitivities and specificities of the assays in this evaluation were recalculated without the inclusion of these two staphylococcal species (Table 2). In fact, the present NCCLS guidelines no longer recommend routine susceptibility testing of *S. saprophyticus*, as urinary tract infections with this organism respond well to the concentrations of antimicrobial agents achieved in urine (14). In the present study, there were two isolates, one *S. epidermidis* isolate and one *S. capitis* isolate, for which oxacillin MICs were $\geq 4 \mu\text{g/ml}$ but that were both *mecA* negative. These strains may possess an alternate resistance mechanism, such as other altered penicillin-binding proteins, as described by Suzuki et al. (16), but this was not investigated further.

The oxacillin agar screen plate prepared in-house performed

TABLE 2. Sensitivities, specificities, and positive and negative predictive values for tests evaluated in comparison with PCR for detection of the *mecA* gene for oxacillin resistance and for tests evaluated in comparison with PCR without inclusion of 37 *S. saprophyticus* and 3 *S. lugdunensis* isolates^a

Test	PCR for <i>mecA</i> gene as comparison test				PCR with <i>S. saprophyticus</i> and <i>S. lugdunensis</i> as comparison test			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
M-S	100	100	100	100	100	100	100	100
OX6	100	99	99	100	100	99	99	100
DD	98	62	65	97	98	95	95	97
BMDIL	100	60	65	100	100	92	93	100
ET	100	51	60	98	100	78	83	100
GPS-SV	98	87	85	98	98	97	98	97
GPS-107	100	61	65	100	100	93	94	100

^a Abbreviations: M-S, MRSA-Screen; OX6, oxacillin agar screen; DD, disk diffusion; BMDIL, broth microdilution; ET, E test; GPS-SV, Vitek GPS-SV susceptibility card; GPS-107, Vitek GPS-107 susceptibility card; PPV, positive predictive value; NPV, negative predictive value.

well for the detection of methicillin resistance in CoNS when the results were examined after 48 h of incubation. These results are similar to those reported in some previous investigations (7, 20) but not all previous investigations (15). Optimal performance of the oxacillin agar screen plate may depend in part on the source of the Mueller-Hinton agar base (3, 17), as well as on how the medium is prepared, on the inoculum size used, and on the length of incubation (15, 17).

Both the Vitek GPS-SV and Vitek GPS-107 cards were able to accurately detect methicillin resistance in CoNS when *S. saprophyticus* and *S. lugdunensis* strains were excluded from the evaluation. The E test had a relatively lower level of specificity (78%), even when these two species were excluded. This may have occurred because the oxacillin MIC for several of the CoNS strains by the E test was 0.38 µg/ml. As this MIC falls between the NCCLS breakpoints for susceptibility and resistance (0.25 and 0.5 µg/ml, respectively), the E test MIC was interpreted as 0.5 µg/ml to allow comparison with NCCLS breakpoint values. As a result, these strains were interpreted by the E test to be resistant, although they lacked the *mecA* gene and may, in fact, have been susceptible.

In summary, compared to PCR as the "gold standard," the MRSA-Screen latex agglutination test was able to rapidly and accurately determine the presence of oxacillin resistance mediated by the *mecA* gene in *S. epidermidis* and most other species of CoNS. Our findings suggest that induction of oxacillin resistance may be unnecessary if a large initial inoculum is used. All of the phenotypic methods evaluated in the present study appeared to perform very well for the detection of oxacillin resistance. The methods evaluated were very sensitive, although the E test had a lower level of specificity than the other phenotypic methods when the results were compared with those of PCR for the detection of *mecA*-mediated resistance. Future studies of oxacillin susceptibility testing for certain species of CoNS (such as *S. lugdunensis*) may indicate the need for reevaluation of the NCCLS breakpoints. Until such breakpoints are reassessed, the MRSA-Screen latex agglutination test may be considered an accurate method for confirmation of an oxacillin MIC of ≥ 0.5 µg/ml, regardless of the phenotypic method used for susceptibility testing, for the detection of resistance which is mediated by the *mecA* gene.

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