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Rapid Detection of *mecA*-Positive and *mecA*-Negative Coagulase-Negative Staphylococci by an Anti-Penicillin Binding Protein 2a Slide Latex Agglutination Test

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A rapid slide latex agglutination (LA) test, MRSA-Screen (Denka Seiken Co., Niigata, Japan), which detects PBP 2a, was tested for its ability to differentiate between *mecA*-positive and -negative coagulase-negative staphylococci. A total of 463 isolates from 13 species were included in the study. The *mecA* gene was detected by PCR, and the oxacillin MIC was determined by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The LA test was performed with oxacillin-induced isolates. The true-positive and true-negative results were defined on the basis of the presence or the absence of the *mecA* gene. By PCR, 251 isolates were *mecA* positive and 212 were *mecA* negative. The sensitivities, specificities, and positive and negative predictive values for the LA test compared to the NCCLS breakpoint for oxacillin resistance (≥0.5 mg/liter) were as follows: for the LA test, 100, 99.5, 99.6, and 100%, respectively; for the NCCLS breakpoint, 100, 60.8, 75.1, and 100%, respectively. One hundred twenty-five *mecA*-positive isolates were also tested by the LA test without induction of PBP 2a; only 72 (57.6%) gave a positive result and required 3 to 15 min for reaction. With induction, all 251 isolates were positive within 3 min. The LA test was reliable in classifying *mecA*-negative isolates, but it classified isolates for which the oxacillin MIC was ≥0.5 mg/liter as oxacillin susceptible. For the reliable detection of oxacillin resistance by the MRSA-Screen in coagulase-negative staphylococci, induction of the *mecA* gene appears to be necessary.

Coagulase-negative staphylococci (CoNS) are a common cause of bloodstream infection, and the majority of isolates are resistant to beta-lactam antibiotics (2, 13). The mechanism of methicillin resistance in CoNS such as *Staphylococcus aureus* is due to the production of an additional nonnative penicillin-binding protein (PBP), PBP 2a (6, 8). PBP 2a has a low affinity for beta-lactam antibiotics and is encoded by the *mecA* gene. Phenotypic detection of methicillin resistance in staphylococci is problematic, requiring several modifications to standard procedures (6, 8). The difficulty in the detection of phenotypic methicillin resistance is due to the heterogeneous expression of the *mecA* gene by many strains of staphylococci (6, 8). In the heterogeneous type of resistance, despite a homogeneous genetic composition of a population of bacteria, only a few cells express the gene and are thus PBP 2a positive. Commonly, laboratories use various susceptibility testing methods to determine methicillin resistance among staphylococci. These methods include an oxacillin screening test, disc diffusion, a broth microdilution method, or an agar dilution method. Molecular methods for the detection of the *mecA* gene are the most sensitive; however, this methodology is not feasible outside of reference laboratories. The MRSA-Screen latex agglutination (LA) test (Denka Seiken, Niigata, Japan) is a simple LA test designed to detect the presence of PBP 2a. This study was undertaken to determine the reliability of this test in detecting methicillin resistance in a variety of species of CoNS.

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MATERIALS AND METHODS

Bacterial isolates and susceptibility testing. All strains were originally isolated from clinical specimens, and only one strain per patient was included in the study. Isolates were identified by using susceptibility to desferrioxamine (12), conventional biochemical tests, and cellular fatty acid profile analysis as described previously (3, 15). Isolates were kept frozen at −70°C and were subcultured twice before testing. The oxacillin MICs (0.125 to 4.0 mg/liter) were determined by the agar dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (14). Mueller-Hinton agar (Oxoid Inc., Nepean, Ontario, Canada) supplemented with 2% sodium chloride and containing appropriate concentrations of oxacillin was inoculated with a replicator that delivered approximately 10^4 CFU to each spot. The plates were incubated at 35°C in ambient air and were read after 24 h. *S. aureus* ATCC 43300 and *S. aureus* ATCC 33591 were included in each run as control organisms. The MIC was recorded as the lowest concentration of oxacillin that completely inhibited growth. Oxacillin powder was obtained from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada).

Multiplex PCR for *mecA* and *nuc* genes. Overnight growth from plates containing Columbia agar supplemented with 5% sheep blood was used to perform a PCR for the *nuc* and *mecA* genes. DNA extraction, PCR master mixture concentrations, and amplification conditions were described previously (11). The primers used to detect the *nuc* and *mecA* genes were published previously (4, 18). The amplified products were detected by electrophoresis through a 2% agarose gel containing 0.5 mg of ethidium bromide per liter, and the bands were observed under UV light. Each run included *S. aureus* ATCC 15923, a local epidemiologically important methicillin-resistant *S. aureus* (MRSA) strain known as the "Ontario strain," and *S. epidermidis* ATCC 12228 as controls.

LA test. Test organisms were grown on Columbia agar with 5% sheep blood. A disc with 1 μg of oxacillin was placed in the main inoculum. After overnight incubation, the growth around the disc was used to perform the LA test. One hundred twenty-five known *mecA*-positive isolates were also tested without induction in parallel. The LA test was performed according to the manufacturer's instructions. Briefly, a loopful of bacterial growth was suspended in 200 μl of extraction reagent 1, and the mixture was boiled for 3 min. After cooling to room temperature, 50 μl of extraction reagent 2 was added to the lysate. (Reagents 1 and 2 were supplied in the kit.) The tubes were centrifuged at 1,500 × *g* for 5 min; 1 drop of sensitized latex particles and 1 drop of control latex particles were added to 50 μl of the supernatant on a test slide. The supernatant and latex particles were mixed by rotating the test slides. Agglutination was assessed visually after 3, 6, and 15 min of rotation.

TABLE 1. Comparison of NCCLS breakpoints and LA test results with PCR results

Category	No. of isolates				
	Total	Oxacillin MIC		LA test	
		≤0.25 mg/liter	≥0.5 mg/liter	Positive	Negative
mecA positive	251	0	251	251	0
mecA negative	212	129	83	1	211

RESULTS

A total of 463 isolates that belonged to 13 species of CoNS were tested. All the test organisms were negative for the *nuc* gene, but 251 (54.2%) of these strains were *mecA* positive. For all *mecA*-positive strains the oxacillin MIC was ≥0.5 mg/liter, and all of them also produced a positive LA reaction. Of the 212 *mecA*-negative strains, 211 were negative by the LA test. The oxacillin MIC for 125 of 212 (60.8%) *mecA*-negative isolates was ≤0.25 mg/liter, whereas the oxacillin MIC for 83 of 212 isolates was ≥0.5 mg/liter (Table 1). The oxacillin MICs for 81 of the 83 strains ranged between 0.5 and 2.0 mg/liter. For one strain of *S. cohnii* and one strain of *S. simulans*, oxacillin MICs were 4 and >4 mg/liter, respectively. The strain of *S. simulans* was the only isolate that was negative by PCR, but it produced a positive LA reaction. The number of *mecA*-positive and *mecA*-negative isolates of each species, the oxacillin MICs, and the LA test results are shown in Table 2.

The LA test was positive within 3 min for all (251 of 251) *mecA*-positive strains after induction of the *mecA* gene. In three instances the agglutination reaction was considered weak and remained unchanged when the rotation time was extended up to 15 min. Concurrently, 125 known *mecA*-positive isolates were also tested by the LA test without induction of PBP 2a. Forty-one (32.8%) of these were positive within 3 min; another 17 (13.6%) and 14 (11.2%) became positive after 6 and 15 min of rotation, respectively. Without induction, 53 (42.4%) of 125 isolates yielded a false-negative result (Table 3).

Using the detection of the *mecA* gene by PCR as a "gold standard," the sensitivities, specificities, and positive and negative predictive values for the LA test and the NCCLS breakpoint for oxacillin resistance for CoNS (≥0.5 mg/L) were as follows: for the LA test, 100, 99.5, 99.6, and 100%, respectively; for the NCCLS breakpoint, 100, 60.8, 75.1, and 100%, respectively. The calculations of sensitivities and specificities were based on the numbers of isolates shown in Table 1.

DISCUSSION

CoNS are important pathogens in hospitalized patients (7). The National Nosocomial Infection Surveillance System (NNISS) identified CoNS as the number one cause of bacteremia in large and teaching hospitals (2). More significantly, the incidence of bloodstream infections due to CoNS from 1980 to 1989 radically increased; the increases seen were dependent on the size and the teaching affiliation of hospitals (2). Smaller nonteaching hospitals reported a 161% increase in the incidence of bacteremia caused by CoNS, whereas in large teaching hospitals the incidence of bacteremia caused by CoNS increased by 754%. In a recent report, NNISS confirms the undiminished significance of CoNS as a cause of nosocomial bacteremias in recent years (1). From January 1990 to May 1999, 13.9 to 44.6% of bacteremias in a variety of intensive care settings were due to CoNS. CoNS tend to be more resistant to antibiotics than *S. aureus*, and resistance to oxacillin in these organisms is widespread. In a recent report of the Surveillance and Control of Pathogens of Epidemiologic Importance surveillance program of bloodstream infections, 26% of *S. aureus*

TABLE 2. Oxacillin MICs and LA test results after overnight induction with an oxacillin disc

Species[a]	No. of isolates			
	Total	Oxacillin MIC, ≤0.25 mg/liter	Oxacillin MIC, ≥0.5 mg/liter	Positive LA test result
S. epidermidis				
mecA +	153	0	153	153
mecA −	68	68	0	0
S. haemolyticus				
mecA +	25	0	25	25
mecA −	6	6	0	0
S. hominis				
mecA +	47	0	47	47
mecA −	33	33	0	0
S. capitis subsp. *capitis*				
mecA +	0	0	0	0
mecA −	8	8	0	0
S. capitis subsp. *urealyticus*				
mecA +	6	0	6	6
mecA −	4	0	4	0
S. caprae				
mecA +	2	0	2	2
mecA −	6	0	6	0
S. cohnii				
mecA +	5	0	5	5
mecA −	15	0	15[b]	0
S. lugdunensis				
mecA +	0	0	0	0
mecA −	19	0	19	0
S. saprophyticus				
mecA +	2	0	2	2
mecA −	19	0	19	0
S. schleiferi				
mecA +	0	0	0	0
mecA −	10	10	0	0
S. simulans				
mecA +	5	0	5	5
mecA −	5	3	2[c]	1
S. warneri				
mecA +	6	0	6	6
mecA −	8	1	7	0
S. xylosus				
mecA +	0	0	0	0
mecA −	11	0	11	0
Total	463			
mecA +	251	0	251	251
mecA −	212	129	83	1

[a] *mecA* +, mecA positive; *mecA* −, *mecA* negative.
[b] For one strain the oxacillin MIC was 4 mg/liter.
[c] The oxacillin MICs for one these strains were >4 mg/liter.

TABLE 3. MRSA-Screen results for 125 *mecA*-positive strains with and without oxacillin induction

Species	No. of isolates					
	Total	LA with induction	LA without induction			
			Observed at:			Not observed
			3 min	6 min	15 min	
S. epidermidis	70	70	18	10	4	38
S. capitis subsp. *urealyticus*	5	5	5	0	0	0
S. cohnii subsp. *cohnii*	2	2	2	0	0	0
S. haemolyticus	11	11	11	0	0	0
S. hominis	33	33	1	7	10	15
S. warneri	4	4	4	0	0	0
Total	125	125	41	17	10	53

isolates and 66.7% of isolates of CoNS were described to be oxacillin resistant (13). Also in a 1999 NNISS survey, the oxacillin resistance rate among CoNS isolates from intensive care units patients tested during January to May of that year demonstrated an 87% increase compared to the rate during the same period 5 years earlier (1). Due to the emergence of vancomycin-resistant enterococci (10, 19) and vancomycin-intermediately resistant *S. aureus* (9), it is prudent to curtail the use of vancomycin in hospitals. The detection of oxacillin resistance in staphylococci is complex and time-consuming because of the heterogeneous nature of *mecA* gene expression. Furthermore, in some species of CoNS the differentiation between *mecA*-negative and *mecA*-positive strains may not be possible by susceptibility methods (11). Alternative methods for rapid and accurate detection of oxacillin-resistant strains are desirable.

The LA test fulfills both these criteria. It can accurately detect methicillin-resistant CoNS, and the turnaround time is shorter than that of susceptibility testing methods. Of 463 CoNS isolates tested, 251 were *mecA* positive by PCR. The LA test and the NCCLS breakpoint for oxacillin resistance correctly identified all of them as oxacillin resistant. Several investigators have reported similar results for the detection of MRSA by the LA test. In those studies the presence or absence of the *mecA* gene was used to define MRSA and methicillin-susceptible *S. aureus* (MSSA) (5, 16, 17). Cavassini et al. (5) tested 200 clinical isolates of *S. aureus*: 120 MSSA and 80 MRSA isolates. In their hands, the LA test correctly identified all MRSA isolates and one strain of MSSA was falsely identified as methicillin resistant (5). In another study, the LA test correctly categorized 90 and 87 of 90 genetically diverse MRSA with and without induction, respectively, and gave a negative reaction with 106 MSSA isolates and a small number of isolates that were not staphylococci (17). Van Griethuysen and coworkers (16) demonstrated that the sensitivity of the LA test was 98.5% and that the specificity was 100% in a study of 267 MRSA isolates of 248 phage types and 296 MSSA isolates.

In this study, all strains were tested for oxacillin resistance after overnight induction of the *mecA* gene by placing an oxacillin disc in the main inoculum. Concurrently, 125 *mecA*-positive strains were also tested by the LA test without induction of the *mecA* gene. Only 72 (57.6%) of these strains produced agglutination. For 31 of 72 strains a positive reaction was discernible only after the rotation time was extended from 3 to 6 or 15 min. The LA test, similar to conventional susceptibility testing methods, depends upon the production of PBP 2a. Due to the heterogeneous expression of the *mecA* gene, without induction there are too few cells with PBP 2a to be detected. However, growth conditions can alter the number of cells that express the *mecA* gene. Addition of NaCl or sucrose to the culture medium, incubation at 30°C, and growth in the presence of beta-lactam antibiotics enhance the expression of resistance. Previous publications have shown that induction is usually not necessary to detect MRSA by the LA test (5, 16, 17). Overnight induction does delay the final results; however, at least for isolates from blood cultures and sterile body sites, the delay can easily be avoided. If gram-positive cocci are seen in positive blood culture bottles or sterile body fluids, an oxacillin disc can be placed in the main inoculum at the time of plating. If a staphylococcus is isolated the following day, the growth around the disc can be used to perform the slide LA test. In our laboratory, we have used this approach with success (data not shown). CoNS from other sites are usually less significant.

In 1999, NCCLS lowered the breakpoint of oxacillin resistance for CoNS to $\geq$0.5 mg/liter to enhance the accuracy of susceptibility testing (14). The present study confirms the finding of past reports that have demonstrated the accuracy of the new breakpoint in identifying *mecA*-positive strains as oxacillin resistant (11, 13). However, it was shown that for the majority of strains of CoNS without *mecA* the oxacillin MIC can be $\geq$0.5 mg/liter (11). By a conventional susceptibility test, such isolates were falsely designated as oxacillin resistant. In this study, for 83 of 212 *mecA*-negative strains the oxacillin MIC was 0.5 to 2 mg/liter; for 1 strain of *S. cohnii* the MIC was 4 mg/liter, and for a strain of *S. simulans* the MIC was >4 mg/liter. This strain of *S. simulans* was the only *mecA*-negative strain that yielded a false-positive result by the agglutination test. We are not aware of any mechanism of resistance for oxacillin other than the one mediated by the *mecA* gene. A false-negative result by PCR for this strain cannot be ruled out, and in view of the MIC for the strain and the positive LA test result, this seems likely. The slide LA test was more reliable in classifying *mecA*-negative strains of CoNS as oxacillin susceptible than the conventional susceptibility test. Such discrepancies between the conventional methods and the LA test were not observed for *S. epidermidis*, *S. haemolyticus*, and *S. hominis* species, which are responsible for over 90% of the bacteremias due to CoNS.

In conclusion, the LA test is a reliable and rapid test for the detection of *mecA*-positive strains of staphylococci and is better than the conventional susceptibility tests in classifying *mecA*-negative CoNS as oxacillin susceptible. The slide agglutination test may be useful in place of conventional susceptibility tests for the detection of oxacillin resistance in CoNS. MIC determination methods need to be performed only if the LA test is negative, and in these cases susceptibility or resistance to oxacillin can be determined by using the NCCLS oxacillin breakpoints for *S. aureus*. However, with CoNS, the LA test should be performed only after the induction of the oxacillin resistance gene.

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