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Detection of Methicillin Resistance in Primary Blood Culture Isolates of Coagulase-Negative Staphylococci by PCR, Slide Agglutination, Disk Diffusion, and a Commercial Method

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The methicillin resistance of 363 coagulase-negative staphylococci isolated from blood cultures was determined by a slide latex agglutination (LA) test for penicillin-binding protein 2a (PBP 2a), the presence of the *mecA* gene by PCR, disk diffusion, and Vitek. LA was performed on primary cultures, and PBP 2a expression was induced by placing an oxacillin disk in the primary inoculum. Compared to the PCR results, LA was the most sensitive and specific in the detection of methicillin resistance. Without induction, LA failed to detect 50% of *mecA*-positive strains grown on two different media.

During the 1980s, the National Nosocomial Infection Surveillance surveys demonstrated the increasing significance of gram-positive organisms in hospital-acquired infections (3). Recent reports have confirmed that coagulase-negative staphylococci (CoNS) remain an important cause of bacteremia in hospitalized patients (1, 12). In North America over the last three decades, this increase in infections by staphylococci has been paralleled by an increase in resistance to methicillin among these organisms. In 1975, only 2% of *Staphylococcus aureus* strains were resistant to methicillin, while in 1991, 29% were resistant (4). Methicillin resistance is even more prevalent among CoNS, particularly with hospital-acquired infections (12).

The increase in CoNS as a cause of bacteremia and the prevalence of methicillin resistance in these organisms have led to increased use of vancomycin as empirical therapy. Concern about the spread of vancomycin-resistant enterococci has prompted the Hospital Infection Control Advisory Committee to recommend that vancomycin be used judiciously (7). In addition, beta-lactam agents are considered a better choice for their therapeutic profile than vancomycin against oxacillin-susceptible staphylococci (12). Early identification of methicillin resistance in staphylococci, especially from blood isolates, could, therefore, curtail unnecessary use of vancomycin and allow earlier optimal therapy of infections in some cases.

The MRSA Screen latex agglutination (LA) test (Denka Seiken, Niigata, Japan) is a simple slide agglutination test designed to detect the presence of penicillin-binding protein 2a (PBP 2a). It has been shown to be reliable for the detection of oxacillin resistance in CoNS (10). In this study, we evaluated the usefulness of this test for detecting the antibiotic susceptibilities of CoNS isolated from blood cultures by performing the test on the primary isolates with a rapid turnaround time.

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Blood cultures submitted to the microbiology laboratory were processed with the BacT/Alert system (Organon Teknika Corporation, Durham, N.C.). If gram-positive cocci in clusters were seen on a Gram stain, the blood culture was subcultured onto Columbia agar (BA; Oxoid, Napean, Ontario, Canada) with 5% sheep blood. A 1-μg oxacillin disk was placed in the primary inoculum. After overnight incubation, if the isolate was identified as *Staphylococcus*, growth around the disks was used to perform the LA test.

The LA test was carried out according to the instructions of the manufacturer, using a loopful of organisms, and results were recorded within 3 min. Identification and susceptibilities of isolates were determined using the GPI and GPS 105 cards of the Vitek system (bioMerieux Vitek, Inc., Hazelwood, Mo.). In addition, susceptibilities to oxacillin were determined by disk diffusion, as described by the National Committee for Clinical Laboratory Standards (NCCLS) (13). The presence of the *mecA* and *nuc* genes was determined by PCR as described previously (9, 10). The influence of induction of the *mecA* gene and the use of different agar media on the performance of the LA test were also tested. For this purpose, selected positive blood cultures were subcultured onto BA and Trypticase soy agar with 5% sheep blood (TSA; Oxoid, Napean, Ontario, Canada) plates with and without oxacillin disks. The manufacturer of the MRSA-Screen test recommends using BA, TSA, Mueller-Hinton agar, or brucella agar for the growth of organisms. The LA test was performed after overnight incubation. PCR results were compared with the results of LA, Vitek, and disk diffusion. The presence of the *mecA* gene was considered the gold standard for oxacillin resistance.

Three hundred sixty-three CoNS isolates from blood cultures were tested. Of these, 201 were positive and 162 were negative for the *mecA* gene. There was a complete concordance between the PCR and LA results except for one strain of *Staphylococcus epidermidis* and two strains of *Staphylococcus warneri*, which produced false-positive results with LA. Two strains of *S. epidermidis* and a CoNS strain that was not identified to species level gave weak reactions.

Susceptibility results were available for 292 strains only. Of

TABLE 1. Sensitivity and specificity of LA, disk diffusion, and Vitek for 158 *mecA*-positive and 134 *mecA*-negative CoNS strains

Test	% (no. correct/no. tested)	
	Sensitivity	Specificity
LA	100 (158/158)	98.5 (132/134)
Disk diffusion	99.4 (157/158)	91.8 (123/134)
Vitek	99.4 (157/158)	92.5 (124/134)

these, 158 were *mecA* positive. Disk diffusion and Vitek were equally reliable in their detection of *mecA*-positive strains, and only 1 of 158 strains (0.6%) was not recognized as such. However, both these methods proved to be less accurate in discerning strains lacking the *mecA* gene. By disk diffusion and Vitek, 10 of 134 (7.5%) and 11 of 134 (8.2%) *mecA*-negative strains appeared to be resistant. Sensitivity and specificity values for LA, disk diffusion, and Vitek are shown in Table 1. Not surprisingly, *mecA*-positive CoNS were more resistant to erythromycin (79.9% versus 32%), clindamycin (58.2% versus 25.3%), and co-trimoxazole (84.4% versus 25.3%) than were *mecA*-negative CoNS. Resistance to vancomycin was not observed.

Of 363 specimens, 110 were tested by LA from BA and TSA plates, with and without oxacillin disks. Eighty-two were *mecA* positive and 22 were *mecA* negative. All *mecA*-positive strains gave a positive result with LA after oxacillin induction. Without induction, only 41 of 82 (50%) strains yielded a positive agglutination test. Two *mecA*-negative strains of *S. warneri* produced a false-positive result with LA with induction, irrespective of the medium used. No false-positive LA results were seen if PBP 2a was not induced. Agglutination was on the whole stronger if the strains were induced for PBP 2a expression.

Methicillin resistance in staphylococci is due to the production of an additional nonnative penicillin-binding protein, PBP 2a (5). PBP 2a is encoded by the *mecA* gene and has low affinity for beta-lactam antibiotics. Many strains of staphylococci express the *mecA* gene heterogeneously, and only a few cells in a population of bacteria may be PBP 2a positive. This heterogeneity is more common in CoNS than in *Staphylococcus aureus* (2). This makes the phenotypic detection of methicillin resistance problematic, especially in CoNS (8). MICs for methicillin-resistant CoNS are usually lower than those for methicillin-resistant *S. aureus* (MRSA) (5, 12). To overcome this problem, the NCCLS has modified oxacillin breakpoints for susceptible and resistant CoNS (13). The new oxacillin MIC breakpoints, however, are less accurate when applied to species of CoNS other than *S. epidermidis*, *S. haemolyticus*, and *S. hominis* (9, 10, 15).

Using previously frozen isolates cultured on BA with oxacillin induction, we have shown that the LA test is more sensitive and specific than the NCCLS breakpoints for the detection of methicillin resistance in all species of CoNS (10). In the present study, we validated our past observation with fresh isolates and two kinds of media. LA failed to detect 50% of *mecA*-positive strains of CoNS if not induced.

Several investigators have challenged the need for induction (6, 11, 14, 15). All these investigators used a heavier inoculum than recommended by the manufacturer or extended the agglutination time. Yamazumi et al. found that 30 of 95 *mecA*-positive isolates were LA negative at 3 min, but the investigators could increase the sensitivity from 68% to 95.7% by extending the agglutination reaction to 10 min (14). The specificity of the LA test in the hands of Horstkotte and coworkers was initially 89.3%, but on retesting, they could achieve a specificity of 97.3% (6).

We believe that a 10-min reaction time and retesting are not practical in a busy diagnostic laboratory. Louie and coworkers achieved results similar to ours and their methodology was also similar, except they used a much heavier inoculum and the expression of PBP 2a was not induced (11). The use of oxacillin disks for the induction of PBP 2a as used in our laboratory is simple and does not cause a delay in the turnaround time. Other advantages of induction are a shorter reaction time, the requirement for a smaller number of organisms, and stronger agglutination reactions.

CoNS with the *mecA* gene were more resistant to erythromycin, clindamycin, and co-trimoxazole. However, resistance is not absolute, and a significant proportion of *mecA*-positive CoNS, 15 to 20%, remain susceptible to erythromycin, clindamycin, and co-trimoxazole. Tetracycline was equally active against *mecA*-positive and *mecA*-negative strains. No resistance against vancomycin was observed.

In conclusion, we would recommend that all staphylococci isolated from blood or sterile body sites be tested for the presence of PBP 2a with the LA test after overnight induction with oxacillin. This should enable clinicians to decide whether to continue with empirical vancomycin therapy or change to alternate therapies within 24 h of the Gram stain report. For LA-positive isolates, testing for susceptibility to any agent in most cases need not be performed, as vancomycin will be the drug of choice. Only when an agent other than vancomycin is being considered may susceptibility testing be required. In most cases, we regard susceptibility testing of LA-negative CoNS unnecessary, as the LA test is more sensitive and specific than MIC breakpoints for the detection of methicillin resistance (9, 10, 13). Further, it has been shown that the MIC of *mecA*-negative CoNS is rarely $\geq$4 μg/ml (9, 10, 11).

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