Rapid Slide Latex Agglutination Test for Detection of Methicillin Resistance in Staphylococcus aureus

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The MRSA screen test (Denka Seiken Co., Ltd.), a commercially available, rapid (20-min) slide latex agglutination test for the determination of methicillin resistance by detection of PBP 2a in Staphylococcus aureus, was compared with the oxacillin agar screen test and PCR detection of the mecA gene. A total of 563 S. aureus isolates were tested. Two hundred ninety-six of the isolates were methicillin-susceptible isolates from cultures of blood from consecutive patients. Also, 267 methicillin-resistant isolates that comprised 248 different phage types were tested. Methicillin resistance was defined as the presence of the mecA gene. Of the 267 mecA gene-positive isolates, 263 were positive by the MRSA screen test (sensitivity, 98.5%), and all the mecA-gene negative strains were negative by the MRSA screen test (specificity, 100%). The oxacillin agar screen test detected methicillin resistance in 250 of the mecA gene-positive isolates (sensitivity, 93.6%). The sensitivity of the MRSA screen test was statistically significantly higher than the sensitivity of the oxacillin agar screen test (P < 0.05). The MRSA screen test is a highly sensitive and specific test for the detection of methicillin resistance. Also, it offers results within half an hour and is easy to perform, which makes this test a valuable tool in the ongoing battle against methicillin-resistant S. aureus.

Over the last three decades methicillin-resistant Staphylococcus aureus (MRSA) has caused major problems in hospitals throughout the world (29). In The Netherlands the prevalence of MRSA is low (≤1.5%) (2, 28). MRSA isolates are usually found in patients who have been treated in foreign hospitals and who are transferred to hospitals in The Netherlands. Because of the multitude of sources, these isolates show a wide variety of phage types (4, 26). All isolates of MRSA are sent to the National Institute of Public Health and Environmental Protection (RIVM; Bilthoven, The Netherlands) for phage typing and confirmation of susceptibility test results. The low prevalence of MRSA in The Netherlands can be attributed to a stringent national policy. The mainstays of this policy are strict isolation of patients who carry MRSA, active search for carriers by screening, and treatment of those who are carriers (26). Accurate and rapid detection of methicillin resistance in S. aureus is essential for the success of this policy. Moreover, it is of great importance for the institution of appropriate antimicrobial therapy for patients with infections caused by these organisms.

The mechanism of methicillin resistance in S. aureus is based on the production of an additional low-affinity penicillin-binding protein (PBP; PBP 2a), which is encoded by the mecA gene (1, 9, 21). Many strains are heterogeneous in their phenotypic expression of methicillin resistance, despite their genetic homogeneity. Typically, only a few cells within the total population of cells express resistance, which makes detection of MRSA by conventional susceptibility testing methods difficult. Several factors are known to influence phenotypic expression of methicillin resistance (1, 9, 21). Commonly used methods for the detection of methicillin resistance, such as the oxacillin agar screen test, disk diffusion, or broth microdilution, rely on modified culture conditions to enhance the expression of resistance. Modifications include the use of oxacillin, incubation at 30 or 35°C instead of 37°C, and the addition of NaCl to the growth medium. Furthermore, for accurate detection by these methods, a prolonged incubation period of 24 h instead of 16 to 18 h is required. Rapid methods with acceptable (>96%) sensitivity for detection of methicillin resistance include automated microdilution systems such as the Vitek GPS-SS card (bioMérieux Vetik, Inc., Hazelwood, Mo.), the Rapid ATB Staph system (bioMérieux, La Balme-Les Grottes, France), and the Rapid Microscan Panel system (Baxter Microscan, West Sacramento, Calif.), which provide results after 3.5 to 15, 5, and 5 to 11 h, respectively (12, 24, 30). The Crystal MRSA ID system (Becton Dickinson, Cockeysville, Md.) is a rapid method based on detection of growth of S. aureus in the presence of 4 mg of oxacillin per liter and 2% NaCl with an oxygen-sensitive fluorescence sensor. Reported sensitivities range from 91 to 100% after 4 h of incubation (13, 20, 32). The limitation of all the methods mentioned above is that they are phenotypic methods, and their accuracies can be influenced by the prevalence of strains that express heterogeneous resistance. Therefore, the “gold standard” for the detection of methicillin resistance is PCR or DNA hybridization of the mecA gene (1). At present, these methods are becoming more feasible for some laboratories, but most clinical laboratories do not have the resources to efficiently perform these techniques on a routine basis. Furthermore, they take several hours to perform. Methods for the detection of the mecA gene product, PBP 2a, could be used to determine resistance and might be more clinically reliable than standard test methods (7). Until now the techniques described for the detection of PBP 2a were not feasible outside a research laboratory (7, 23). In a recent publication Nakatomi and Sugiya (16) describe the successful development of a slide latex agglutination assay...
for the direct detection of PBP 2a from isolates of *S. aureus* after a rapid extraction procedure.

The MRSA screen test (Denka Seiken Co., Ltd.) is a commercially available, rapid (20-min) slide latex agglutination test for the detection of PBP 2a. This study compared the MRSA screen test with the oxacillin agar screen test and PCR detection of the *mecA* gene for the detection of methicillin resistance in *S. aureus*.

**MATERIALS AND METHODS**

**Bacterial isolates.** The methicillin-susceptible *S. aureus* (MSSA) isolates used in the study were from cultures of blood collected between January 1995 and December 1998 from consecutive patients at St. Elisabeth Hospital and Twesteden Hospital, Tilburg, The Netherlands; Pasteur Hospital, Oosterhout, The Netherlands; Twesteden Hospital, Waalwijk, The Netherlands; and S. Ignatius Hospital and Hospital de Baronic, Breda, The Netherlands. Only one isolate per patient was included. Isolates were identified by a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd., Dartford, England), by the detection of free coagulase by the tube coagulase test with rabbit plasma (10), and by the detection of Dnase (DNase agar; Oxoid Unipath Ltd., Basingstoke, England). If the results of the tests were discordant, an AccuProbe culture identification test (Gen-Probe; San Diego, Calif.) was performed according to the manufacturer’s instructions (14). The AccuProbe test was considered the gold standard. Isolates were tested for susceptibility to the supernatant (MIC £2 µg/ml) by broth microculture susceptibility testing. Furthermore, no growth was observed by the oxacillin agar screen test (as described below).

MRSA isolates were selected from the strain collection of RIVM. This collection contains all MRSA strains isolated in The Netherlands since 1989. Isolates were sent to RIVM for confirmation of susceptibility testing and phage typing results. Bacteriophage typing was performed as described before by using (i) the international set of phages at 1× and 10× routine test dilution concentrations, (ii) an additional set of Dutch phages, and (iii) a set of experimental MRSA phages. Phage typing patterns were given a type designation (6, 19, 22, 27). Strains were selected on the basis of their different phage types. The 267 MRSA isolates included in the evaluation comprised 248 different phage types. Moreover, one isolate of each strain was chosen for the following purposes: seven isolates of phage type Z-115, five isolates of phage type Z-151, three isolates of phage type III-29, two isolates of phage type III-70, two isolates of phage type III-169, two isolates of phage type III-172, and two isolates of phage type XI-5. Three isolates were not typeable.

**Multiplex PCR for the *mecA* and coagulase genes.** A 298-bp fragment of the *mecA* gene was amplified with the primers 5'-GTT GTA GTC GTC TTT TTG GG-3' (upstream) and 5'-CTT CCA CAT ACC TTC TCT TAC AAC-3' (downstream) specific for the *mecA* gene (GenBank accession no. X52593). A set of primers was included in each reaction mixture to amplify a polymorphic region of the coagulase gene that varied between approximately 350 and 600 bp. The coagulase primers specific for the coagulase gene (GenBank accession no. X71132) were 5'-CAT CAG GTA TCT ATT AT-A-3 (downstream) and 5'-TGT TTA CGT TAT GTC TTT GGA-3' (downstream). The latter primers provided an internal control to check for the presence of *S. aureus* DNA and for the absence of PCR inhibitors. MSSA isolates yield only one PCR product (coa amplicon), whereas MRSA isolates yield two PCR products; one PCR product is specific for *mecA* in the tested colonies, a loopful of cells was suspended in 4 drops of extraction buffer (10 mM Tris, 89 mM boric acid, and 2 mM EDTA) at 27°C for 10 min, 55°C for 10 min, and 72°C for 5 min; and (iv) a final primer extension at 72°C for 5 min, followed by (ii) 10 cycles of touchdown PCR of 95°C for 60 s, 65°C for 60 s, and 72°C for 60 s; and (v) a final primer extension at 72°C for 6 min. After amplification, the PCR products were separated by electrophoresis through 0.8% agarose gels in 0.5× TBE buffer (1× TBE buffer is 89 mM Tris, 89 mM boric acid, and 2 mM EDTA) at 150 V for 45 min. The gels were then stained with ethidium bromide (0.5 µg/ml) and viewed under UV light.

**Oxacillin agar screen test.** All MRSA isolates were spot inoculated onto a Mueller-Hinton agar plate (Difco Laboratories, Detroit, Mich.) supplemented with 6 µg of oxacillin per ml and 4% NaCl by using a cotton swab dipped into a 0.5 McFarland standard suspension of each test isolate. The plates were incubated at 35°C for 24 h. If any growth was detected, the isolate was considered oxacillin resistant (17).

**RESULTS**

A total of 296 MSSA and 267 MRSA isolates were included in the evaluation. All 296 MSSA isolates tested negative by the *mecA* gene PCR, MRSA screen, and oxacillin agar screen tests. The 267 MRSA strains were all *mecA* gene PCR positive; 4 tested negative by the MRSA screen test and 17 did not grow by the oxacillin agar screen test (Table 1). This resulted in a sensitivity of 98.5% and a specificity of 100% for the MRSA screen test. The sensitivity and specificity of the oxacillin agar screen test were 93.6 and 100%, respectively. Upon retesting, the results for all samples with discordant results were confirmed. The MICs determined by the E-test for the 19 discordant strains are presented in Table 2. According to the National Committee for Clinical Laboratory Standards breakpoint (±2 µg/ml) (17), the E test identified 11 *mecA* gene-positive isolates as oxacillin susceptible.

**DISCUSSION**

This study shows that detection of PBP 2a by the MRSA screen test is a highly sensitive and specific means for the detection of methicillin resistance in *S. aureus*. In this evaluation MRSA isolates comprising 248 different phage types were included. In fact, at least one isolate of each phage type identified among the MRSA strains isolated in The Netherlands between 1989 and 1998 was included in the study. Since MRSA strains in The Netherlands are usually recovered from patients who have been hospitalized in other countries, this collection can be considered a reflection of MRSA strains from throughout the world. Most isolates are of European origin (4, 26). No phage typing was performed with the methicillin-susceptible
Methicillin resistance in coagulase-negative staphylococci (CoNS) is also based on the mecA gene product PBP 2a; therefore, thorough identification of the tested strain is necessary. Detection of methicillin resistance in CoNS by conventional susceptibility tests is even more difficult than detection of methicillin resistance in S. aureus. The oxacillin agar screen test is reported to be very reliable but requires 48 h of incubation for CoNS (31). It is possible that the MRSA screen test could also successfully detect methicillin resistance in CoNS. The manufacturer does not recommend use of the MRSA screen test for the detection of methicillin resistance in CoNS, and this study did not include CoNS. Further testing for this purpose is warranted.

Five mecA-positive strains showed only weak agglutination after 3 min of rotation of the test card, as recommended by the manufacturer’s instructions. When rotated for another 3 min the agglutination pattern became strongly positive. It is important to check carefully for any sign of agglutination. If a weak agglutination pattern is seen, one can rotate the test card for another 3 min, which can clarify how one should interpret the test result. To evaluate the chance of false-positive results as a result of an increase in the duration of rotation, 100 MSSA isolates were rotated for 6 min. No agglutination was observed.

The oxacillin screen agar test is recommended by the National Committee for Clinical Laboratory Standards (17) as one of the most reliable phenotypic tests for the detection of oxacillin resistance. The MRSA screen test was tested by the E test. Of this subset, 11 strains were mecA-positive, PBP 2a-producing strains showed only weak agglutination after 3 min of rotation of the test card, as recommended by the manufacturer’s instructions. When rotated for another 3 min the agglutination pattern became strongly positive. It is important to check carefully for any sign of agglutination. If a weak agglutination pattern is seen, one can rotate the test card for another 3 min, which can clarify how one should interpret the test result. To evaluate the chance of false-positive results as a result of an increase in the duration of rotation, 100 MSSA isolates were rotated for 6 min. No agglutination was observed.

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hours. Therefore, the MRSA screen test offers a new, valuable tool in the ongoing battle against MRSA.

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