Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*

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ABSTRACT: 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 methicillin agar screen test. A total of 862 *S. aureus* isolates were tested. Six hundred and thirty eight of the isolates were methicillin – susceptible isolates from culture of various clinical samples. Also, 224 methicillin–resistant isolates that compared 205 different phage types were tested. Methicillin resistance was defined as the presence of the mec A gene product, PBP 2a. of the 224 methicillin – resistant isolates, 224 were positive by the MRSA screen test (Sensitivity, 100%), and all the methicillin – susceptible isolates were negative by the MRSA screen test (sensitivity, 98.6%). The sensitivity of the MRSA screen test was statistically significantly higher than the sensitivity of the oxacillin agar screen tests (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*

Key Words: Latex agglutination, MRSA, PBP 2a

Introduction

Over the last three decades methicillin – resistant *Staphylococcus aureus* (MRSA) has caused major problems in hospitals throughout the world (26). 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 tempered to hospital in the Netherlands. Because of the multitude of sources, these isolate show a wide variety of phage types (4, 26). All isolates of MRSA are sent to the National Institution of Public Health and Environmental Protection (Rivm; Bithoven, 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 carrier (26). Accurate and rapid detection of methicillin resistance in *S. aureus* is for the success of this policy. Moreover, it is of great importance for the institution of appropriate antimicrobial therapy for patents 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 mec A 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 oxacillin agar screen test, desk diffusion, or both micro dilutions, 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 of these methods a prolonged incubation period of 24 hours instead of 16 to 18 hours is required. Rapid methods with acceptable (>96%) sensitivity for detection of methicillin resistance include automated micro dilution systems such as the vitek GPS – SA card (bio merieux viték, Inc., Hazelwood, Mo.), the Rapid ATB staph system (bio merieux, La Balme – les Grottes. France), and the Rapid micro scan Panel system (Baxter Microscan, West sacramento, Calif.), which provide results after 35 to 15, 5, and 5 to 11 hours, respectively (12, 24, 30), the crystals 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 fluorescent sensor. Report sensitivities range from 91 – 100% after 4 hours of incubation (13, 20, 32). The limitation of all the methods mention above is that there 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 Mec A 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 mec A gene product, PBP 2a, could also be used to determine resistance and might be more clinically reliable than standard test method (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 Sugiyama (16) described 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 methicillin agar screen test 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 various clinical samples collected between January, 2008 and October, 2008 from consecutive patients at Murtala Mohammad Specialist Hospital, Kano, Nigeria. Only one isolates per patient was included. Isolates were identified by a latex agglutination test (Staphaurex Plus: murex diagnostics Ltd. Derefood, England) by the detection of free coagulase by the tube coagulase test which 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 perform according to the manufacturers instructions (14). The AccuProbe test was considered the gold standard. Isolates were classified as methicillin susceptible (MIC, ≤ 2g 1ml) by broth micro dilution susceptibility testing. Furthermore, no growth was observed by the oxacillin agar screen test (as described below).

Isolates were confirmed for susceptibility testing and phage typing results. Bacteriophage typing was performed as described before by using (i) the international set of phages ix and 100x 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 224 MRSA isolates included in the evaluation comprised 205 different page types. More than one isolate of the following phage types was included seven isolates of phage type Z - 115, five isolates of phage types Z – 151, three isolates of phage type III – 29, two isolates of phage type III – 7a, 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

Oxacillin agar screen test. All MRSA isolates were spot inoculated onto a maltur – Hinton agar plate (Difco laboratories, Detroit, Michi). Supplemented with 6µg/ml of oxacillin per ml and 4% NaCl by using
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a cotton swab dipped into a 0.5 Mac Farland standard suspension of each test isolate. The plates were incubated at 35°C for 24h. If any growth was detected, the isolate was considered methicillin resistant.

Methicillin agar screen test: All MRSA isolates were spot incubated onto a Muiller- Hinton agar plate (Difco laboratories Detroit, Michi) supplemented with 10µg/ml of methicillin per ml and 4% NaCl by using a cotton swab dipped into a 0.5 Mac Farland standard suspension of each test isolate. The plates were incubated at 35°C for 24h. If any growth was detected, the isolate was considered methicillin resistant.

MRSA screen test. The MRSA screen test is a latex agglutination test based on the reaction of latex particles sensitized with monoclonal antibodies against PBP 2a of *S. aureus* and PBP 2a extracted from tested colonies. The test was performed according to the manufacturers’ instructions. Briefly, isolates were subculture onto Columbia agar supplemented with 5% sleep blood (Oxoid Unipath Ltd) at 37°C for 18h to obtain fresh growth – Co extract PBP 2a from the tested colonies, a loopful of cells was suspended in 4 drops of extraction reagents 1. this suspension was placed in a heating block (>95°C) for 3 min. After allowing the suspension to cool to room temperature (± 10min), 1 drop of extraction reagent 2 was added and the mixture was vortexed thoroughly. The suspension as then centrifuge at 1,500 xg for 5min. The actual latex agglutination test was performed with the supernatant; 50µl of the supernatant was mixed with one drop of sensitized latex. For the negative control, 50µl of the supernatant was mixed with 1 drop of negative control latex. Mixing for 3min was performed with a shaker. The investigators that perform the test were blinded to the results of the susceptibility tests.

Results

A total of 638 MSSA and 224 MRSA isolates were included in the evaluation. All 638 MSSA isolates tested negative by MRSA screen test. The 224 MRSA strains were all MRSA screen test positive, 4 did not grow by the methicillin agar screen test and 17 did not grow by the oxacillin agar screen test (table 1). This is resulted in a sensitivity of 100% and specificity of 100% for the MRSA screen test. The sensitivity and specificity of the excelling agar test and methicillin agar screen test were 98.6 and 100%, 99.2 and 100% respectively. Upon testing, the results for all samples with discordant results were confirmed.

Table 1 evaluation of MRSA screen test, methicillin agar screen test for detection of methicillin resistance in *S. aureus* isolates (n= 862).

<table>
<thead>
<tr>
<th>MRSA Screen Test</th>
<th>Methicillin Agar Screen Test</th>
<th>Oxacillin Agar Screen Test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>224</td>
<td>0</td>
<td>220</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>638</td>
<td>0</td>
<td>224</td>
</tr>
</tbody>
</table>

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 résistance in *S. aureus*. In this evaluation MRSA isolates comprising 205 different phage type identified among the MRSA strains isolated in Kano, Nigeria. Between January and October, 2008 was included in the study. Since MRSA stains in Kano 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 N0. phage typing was performed with the methicillin- susceptible isolates these isolates were collected from various clinical samples in Murtala Mohammed Specialist
Hospital, Kano, Nigeria, for evaluation of tests for the detection of S. aureus it is. Essential to define the collection of isolates tested. S. aureus is a prime example of a microorganism which spreads colony in the environment (11). Consequently, many collections will contain many isolates of the same strains. This leads to over – or under estimation of the through value of the test under evolution. This evaluation is the first which include such a large poly colonel collection of MRSA strains for detection purposes. Therefore, it provides a valid estimation of the potential value of the MRSA screen test the detection of MRSA. The high sensitivity of the screen test makes this test suitable for detection purposes.

S. aureus strains that produce PBP 2a and do not grow on methicillin or oxacillin agar have been reported previously (15, 23, 25). These strains were all methicillin susceptible phenotipically Zt has been suggested that testing of those kind of strains by PCR or DNA probe techniques can lead to false positive results for resistance and that detection of PBP 2a may be more appropriate for the detection of MRSA (16) others have stated that these strains should be classified as MRSA, despite their phenotypic susceptibility to β – lactam antibiotics this is because of the possibility that methicillin resistance appears during therapy with β - lactam antibiotics (15, 18) for borderline MRSA strains, MICs are at or just above the susceptibility breakpoint (eg. Oxacillin MICs 4 to 8μg/ml) strains with borderline resistance do not contain the mec A gene and resistance is not based on the production of PBP 2a but has been hypothesized to result from modification of normal PBP genes over expiration of normal PBP, or over production of staphylococcus β- lactamases (!) differentiation of border line resistant mec A negative strains from heterogeneous mec A – positive, PBP 2a – producing strains is important in choosing the current antimicrobial treatment. Invitro susceptibility data, experimental data from studies with animal and some clinical data mediate that treatment with β – lactam antibiotics is effective for infections caused by these mec A gene negative, non PBP 2a producing strains (1, 7). Furthermore non PBP 2a producing strains of S. aureus may not require expensive and inconvenient patient isolation procedure (8). The MRSA screen test could provable be useful for the identification of these strains in this study, however, no borderline – resistance strains were included.

Methicillin resistance in coagulase – negative staphylococci (CONS) is also based on the mec A 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 resistance agar screen test is reported to be very reliable but 48h of incubation for CONS (31). It is possible that that 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.

The oxacillin/methicillin screen agar test is recommended by the national community for clinical laboratory standards (17) as one of the most reliable phenotypic tests for the detection of methicillin resistance. In this evaluation the sensitivity was 99.2% and 98.6% for the methicillin and an oxacillin agar respectively, which was statistically significantly (p < 0.01), lower than the sensitivity of the MRSA screen test. The risk of misclassification of an MRSA isolate a methicillin susceptible was 2.3 times higher by the oxacillin/methicillin agar screen test (95% confidence interval 1.5 to 12. 5)

In conclusion. The MRSA screen test is rapid, easy to – perform and highly reliable test for the detection of methicillin resistance in S. aureus. Results are available in approximately 20 min, where PCR detection of the mec A gene takes several Hours therefore the MRSA screen test offers a new valuable tool in the ongoing battle against MRSA.

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