COMPARISON OF PHENOTYPE AND GENOTYPE METHODS FOR DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS FROM CLINICAL ISOLATES IN A TERTIARY CARE TEACHING HOSPITAL, SOUTH INDIA

*Ms. Jayaraman Latha¹, Dr. Subburaya Umamaheswari², Dr. Paramasivam Rajendran³

¹Research Scholar, Department of Microbiology, Sri Ramachandra University and Research Institute (SRMC&RI), Porur, Chennai – 600 116, India.
²Professor, Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, and Research Institute (SRMC&RI), Porur, Chennai – 600 116, India.
³Professor, Department of Microbiology, Sri Ramachandra University and Research Institute, (SRMC&RI), Porur, Chennai – 600 116, India.

ABSTRACT
Objective: To compare the different phenotype methods of detection of methicillin resistance in Staphylococcus aureus from clinical isolates in a tertiary care centre South India. Methodology: 48 clinical isolates of S. aureus from different specimens such as blood and exudates collected from January through May 2010 were included in the study. Antibiotic susceptibility tests for various antibiotics were carried out. All the isolates were identified as MRSA by PCR for meca gene. Conventional methods like cefoxitin disc diffusion assay, MIC by E-test for oxacillin; Phenotype rapid methods for MRSA detection like MRSA-Screen slide latex agglutination test (Denka-Seiken, Japan) and ORSAB (Oxoid Ltd., UK) were carried out to compare their sensitivity, specificity, accuracy and cost-effectiveness. Results: Out of the n=48 clinical isolates included in the study, cefoxitin disc diffusion assay recorded n=47 (98%) as resistant to methicillin and n=1 (2.1%) strain as sensitive which was meca-positive. This strain was heterogeneously resistant to oxacillin by E-test recording a MIC of 0.38µg/ml (double drug strip), showed slight (1+) agglutination by MRSA-Screen and ORSAB showed mild growth. MIC by E-test for oxacillin was resistant

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for all the n= 48 strains with a optimal sensitivity of 100%. PBP2 detection by MRSA-Screen, showed n=45 (93.75%) strains as positive with n=3 (6.25%) false negative results. These n=3 (6.25%) strains were positive for mecA, resistant by cefoxitin disc diffusion test but heterogeneously resistant to oxacillin with MICs <32μg/ml. n=1 (2.1%) strain had a borderline MIC of 4μg/ml. In ORSAB n=2 (4.17%) out of the three strains showed mild growth on repeat and n=1 (2.1%) showed growth after 48 hrs. ORSAB recorded n=46 strains as resistant with a sensitivity of 95.8%. Conclusion: In the present study we found that though rapid methods like MRSA-Screen, ORSAB take less time, conventional methods like cefoxitin disc diffusion and MIC by E-test for oxacillin are more sensitive and accurate. Therefore in the absence of molecular techniques, conventional methods continue to be the gold-standard for the detection of methicillin resistance in Staphylococcus aureus.

Key words: Staphylococcus aureus, PCR, Cefoxitin disc diffusion, E-test, MRSA-Screen, ORSAB.

INTRODUCTION

MRSA is a worldwide concern because of their increasing frequency in health care settings and community causing superficial skin and soft tissue infections to serious systemic infections leading to illness and death of the person1. In India also there have been reports of increasing rates of MRSA2-4. Therefore, to reduce these rates it is important to accurately identify the organism from clinical specimens and decide in time on isolations procedures and antimicrobial treatment5-9. Moreover, early diagnosis of MRSA prevents their cross-transmission in the wards, decreases morbidity, helps to determine appropriate antimicrobial therapy, shortens patient’s hospital stay and lowers hospital costs10,11.

Rapid methods for the identification of MRSA from clinical specimens are a current need as the conventional methods of their detection take around 48 to 96hrs12. Further, conventional methods for identification of MRSA, such as disc susceptibility tests take more time and are influenced by environmental conditions like temperature, pH, salt concentration and incubation. Additionally, phenotypic expression of methicillin resistance is known to be heterogeneous leading to borderline MICs which are difficult to identify by conventional tests13,14.

The conventional methods for the detection of MRSA in the clinical laboratory are oxacillin agar screen test, oxacillin disk diffusion and MIC by agar, broth dilution or E-test method for determination of different degrees of oxacillin resistance13,15,16. Cefoxitin disc diffusion is
recommended by CLSI for the reliable detection of methicillin resistance\textsuperscript{17}. Cefoxitin is considered as a better inducer of \textit{mecA} gene expression than is oxacillin or methicillin, and can be used to screen heterogenous MRSA populations. The advantage of using cefoxitin is that the test conditions are similar to those used for other antibiotics\textsuperscript{16-18}. Many methods have been described to improve the turnaround time for the detection of MRSA.

Selective media are generally used to get a pure culture of MRSA. These media contain an indicator to distinguish \textit{S. aureus} from other organisms and also some antibiotics which prevent non-staphylococcal growth. Oxacillin resistance screening agar base (ORSAB) is a selective media which can detect MRSA directly from swabs within 24hrs. It contains mannitol and aniline blue for detection of mannitol fermentation, antibiotics like oxacillin which prevents the growth of methicillin sensitive \textit{Staphylococcus aureus} (MSSA) and polymixin B and high salt concentration prevents non-staphylococcal growth.

In recent times it is suggested that, in the identification of MRSA, it is more accurate to detect the gene that encodes methicillin resistance than the product, penicillin binding protein PBP2\textsuperscript{*} which is a modified form of penicillin binding protein expressed on the cell surface of all MRSA strains. As molecular techniques are expensive, technically demanding and all the laboratories may not be able to afford this facility, it is important to look for a simple, rapid and inexpensive method that is highly sensitive, accurate and can be used in routine clinical diagnostic laboratories.\textsuperscript{19-21}

For the detection of the PBP2\textsuperscript{*} protein, a rapid and commercially available latex agglutination test kit is used. In this method, PBP2\textsuperscript{*} or PBP2a protein is extracted from the bacterial colonies in a suspension and later detected by agglutination method. The sensitized latex particles are coated with monoclonal antibodies to PBP2a which agglutinate with the protein\textsuperscript{21}.

In the present study we have compared the different methods for MRSA detection for rapidity, sensitivity, specificity and cost-effectiveness.

\textbf{METHOD}

The institutional Ethical Clearance was received to use the patient samples for the study (REF: IEC-NI/09/DEC/13/43). 48 clinical isolate from specimens like blood and exudates collected from January through March 2010 and stocked in nutrient butts. All the isolates
were identified as *S. aureus* by conventional tests like catalyse, coagulase, Mannitol Salt Agar and urease. The isolates were tested by disc diffusion tests for the following antimicrobial agents— ampicillin cefotaxime, cefotaxime, oxacillin, erythromycin, clindamycin, gentamycin, co-trimoxazole, linezolid, and vancomycin to compare their susceptibility pattern. Methicillin sensitive *S. aureus* ATCC 25923 (MSSA) and methicillin resistant *S. aureus* ATCC 43300 (MRSA) were used as negative and positive controls respectively.

**Cefoxitin disc diffusion**

Cefoxitin disc diffusion test was carried out using a 30 μg disc of cefoxitin. Muller Hinton Agar was boiled and autoclaved, poured on petriplates in a sterile manner and cooled. Lawn culture of the bacterial suspension standardised to 0.5 Mc Farland standards was done on the agar plates. The plates were incubated at 37°C for 18 to 24 hrs and zone diameters were measured. Zone diameters <19mm was reported as methicillin sensitive and zone diameters >22mm was considered as methicillin resistant. Colonies that grew within the zones were tested again and the zone of inhibition reported.

**MIC by E-test:** The E-test (Himedia Laboratories Pvt. Ltd.) for determining oxacillin MICs were performed on Muller-Hinton agar supplemented with 2% NaCl as per the manufacturer’s instructions. Addition of 2% NaCl enhances the detection of heteroresistant MRSA. The plates were incubated at 37°C for full 24 hrs.

**PBP2' detection**

MRSA-Screen (Denka Seiken Co., Ltd.) a slide latex agglutination kit was used for the detection of PBP2′ from the clinical isolates. This rapid assay uses a monoclonal antibody coated on latex particles which binds to the PBP2′ protein, expressed by the meca gene and determines phenotypic expression of methicillin resistance. The test was performed as follows:

4 drops viz., 200μl of extraction reagent 1 was taken in a microcentrifuge tube. Using sterile bacterial loop (1mm internal diameter) sufficient bacterial colonies were taken and thoroughly suspended in the tube. Note: excess number or fewer colonies give false positive or false negative results. The centrifuge tube was placed in a heating block at 100°C and heated for 3mins. After 3mins the tube was removed and cooled to room temperature. 1 drop viz., 50μl of extraction reagent 2 was added into the tube and mixed well and centrifuged at
4500rpm in microcentrifuge. After centrifugation the supernatant was removed and used as the test specimen.

3+ level of agglutination is reported as Strong agglutination, Agglutination against slightly turbid background is considered as 2+ level of agglutination and Slight agglutination against turbid background is reported as 1+ level of agglutination.

Oxacillin Resistant Screen Agar Base

(ORSAB: Oxoid Ltd. Basingstoke, England): ORSAB is a selective media developed to detect MRSA in clinical specimens. The medium uses aniline blue to detect mannitol fermentation in S. aureus. The antibiotic supplements (oxacillin, 2.0 µl; polymyxin B, 50, 000 IU/l) and of 5.5% NaCl reduce the growth of non-staphylococcal organisms and helps in the selection of MRSA. The test was carried out as per the manufacturer’s instructions.

51.75g of ORSAB was suspended in 500ml of distilled water and boiled to dissolve the contents. The medium was sterilised by autoclaving at 121°C for 15minutes, cooled to 50°C and aseptically added the contents of one vial of the antibiotic supplement after reconstituting it in 2ml of sterile water. 20ml of the medium was poured in sterile petriplates and cooled to solidify. Sufficient amount of each culture was taken in a loop and mixed in saline to bring it to 0.5 McFarland standards. The plates were subsequently inoculated and incubated at 37°C for 48 hrs.

Detection of mecA gene by PCR

The detection of mecA gene in MRSA isolates was considered as the reference method for establishing the rapidity, sensitivity, specificity of each technique studied. The DNA extraction was performed using bacterial genomic DNA kit (Medox Biotech Services Ltd.). We designed the following oligonucleotides using bioinformatic tools and standardized them to PCR conditions. mecA1, 5’-AGAGTAGCAGCTCGAATTAGGCAGT-3’; and mecA2, 5’TCTGCAATTACGGATATTGGCAA-3’, were designed which amplifies an internal region of the mecA gene and can be detected at 400bp. A 20µl PCR reaction consisted of 16µl of master mix [PCR buffer (2X), dNTP mix (400mM), HotStarTaq DNA polymerase (250 U) and MgCl2 (3mM)], 2µl primer, 2µl of template DNA and 1µl of distilled water. Cycling conditions were —initial TAQ activation according to the manufacturer’s instructions followed by 35cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 60 seconds and extension at 72°C for 60 seconds. Last elongation was at 72°C for 5 minutes.
The amplicon detection was carried out in 2% agarose gel electrophoresis with ethidium bromide and viewed under UV transilluminator.

RESULTS
A total of 48 clinical isolates of *S. aureus* were evaluated. The overall results obtained with the different techniques are tabulated (Table 1).

The disc diffusion test for Cefoxitin recorded n= 47 (97.9%) strains as resistant. n=1 strain (2.1%) was sensitive yielding a zone of inhibition measuring 22mm. Some strains were heterogeneously resistant to cefoxitin (Fig. 1)

MIC by E-test recorded all n=48 (100%) strains as resistant to oxacillin in which n=33 (68.75%) recording MICs >256µg/ml. One of the resistant strains was sensitive by disc diffusion assay. The different MIC values of the strains tested are shown in Table 2.

Among the resistant strains few strains showed lower MICs ranging between 0.38-4µg/ml with growth within the zone of inhibition (Fig. 2). When these colonies were scraped and MIC was carried out they showed resistance to oxacillin with MICs above 256µg/ml. This shows that among the sensitive colonies there are resistant colonies which induces mecA gene.

The MRSA-Screen latex agglutination test showed n=45 (93.75%) strains as positive for PBP2' (Fig. 3) and three as negative. Out the strains positive for the protein, n=32 (66.7%) strains showed 3+ n=8 (16.7%) strains 2+ and n=5 (10.42%) strains showed 1+ agglutination pattern. However, n=1 (2.1%) of these strains showed agglutination in control latex and n=1 (2.1%) strain showed susceptibility to methicillin by cefoxitin disc diffusion assay.

The results for ORSAB were recorded after 24 hrs and after 48 hrs. Among the n=48 strains, n=26 (54.16%) strains showed growth with fermentation after 24hrs (Fig. 4). n=7 (14.59%) strains showed growth with fermentation after 48 h. n=3 (6.25%) strains showed growth but no fermentation even after 48 hrs. n=9 (18.75%) strains showed moderate growth after 24 hrs on repeat. n=2 (4.17%) strains showed no growth even after 48 h but positive for PBP2'. n=1 (2.1%) strain which was sensitive by cefoxitin disc diffusion assay showed moderate growth in ORSAB. The specificity of ORSAB was found to be medium as there was good growth of *Proteus spp.* when tested but methicillin sensitive strains of *S. aureus* did not grow in the plate even after 48 hrs of incubation.

The mecA PCR assay showed all the strains as mecA-positive for *S. aureus* (Figure not included).
Table: 1 Comparison of different methods for detection of MRSA from clinical isolates

<table>
<thead>
<tr>
<th>Test method</th>
<th>Dedected as MRSA*</th>
<th>False negatives</th>
<th>False positives</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin disc diffusion (30µg)</td>
<td>47</td>
<td>1</td>
<td>0</td>
<td>97.9</td>
<td>100</td>
</tr>
<tr>
<td>E-test</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PBP2'</td>
<td>44</td>
<td>4</td>
<td>0</td>
<td>93.75</td>
<td>100</td>
</tr>
<tr>
<td>ORSAB</td>
<td>46</td>
<td>2</td>
<td>0</td>
<td>95.8</td>
<td>medium</td>
</tr>
</tbody>
</table>

*Cefoxitin resistant
*Resistant to oxacillin

Table: 2 Oxacillin MICs (µg/ml)

<table>
<thead>
<tr>
<th>E-test [MICs (µg/ml)]</th>
<th>Number of strains (n=48)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;256</td>
<td>33</td>
<td>68.75</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>4.16</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>2.08</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>6.25</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2.08</td>
</tr>
<tr>
<td>&lt;1 (Double Drug strips)</td>
<td>5</td>
<td>10.41</td>
</tr>
</tbody>
</table>

Fig: 1. Cefoxitin disc diffusion plate -
MRSA strain heterogeneously resistant to cefoxitin
Fig 2: Minimum Inhibitory Concentration (MIC) plate: MRSA strain heterogeneously resistant to Oxacillin

Fig 3: MRSA-Screen, Slide latex agglutination test: Strong (3+) agglutination in the test circle

Fig 4: Oxacillin Resistant Screen Agar Base (ORSAB): Growth with fermentation after 24hrs incubation
DISCUSSION

*S. aureus* causes serious infections in health-care settings and in the community with high rates of morbidity and mortality. Therefore, it is important to detect *S. aureus* infections early and accurately to determine appropriate therapy\(^ {22}\). Many methods have been developed for the detection of methicillin resistance, but they are slow and their sensitivity and specificity is also low. Phenotype expression of methicillin resistance is depended on many environmental factors as explained earlier. Identification of the gene coding for methicillin resistance is the most reliable method for the detection of MRSA isolates. However, molecular methods are expensive and not all laboratories can afford the necessary infrastructure and technical expertise. Therefore, it is essential to develop a rapid, accurate and sensitive method for detection of methicillin resistance in *S. aureus* which is independent of the environmental conditions.\(^ {23, 24}\)

Expression of Methicillin resistance in *S. aureus* isolates possessing the *mecA* gene could be heterogeneous or homogenous in nature. In homogenous expression, almost all cells express resistance. However, in hetero-resistant isolates the cells express varying degree of resistance to methicillin. In these strains methicillin MICs are at or just above the susceptibility breakpoint (e.g., oxacillin MICs of 4 to 8μg/ml). In in-vivo condition, when treatment with beta-lactams the PBP2a production may be induced and the cells which were susceptible to oxacillin in vitro become oxacillin resistant. Therefore, it is essential to detect the presence of *mecA* gene to precisely identify the strains to be MRSA and thus PCR is a reference method in most clinical laboratories\(^ {12, 25}\).

Another category of borderline resistant strains are those which are *mecA*-negative. Methicillin resistance has been hypothesized in these strains to result from modification in the normal PBP genes or excessive production of staphylococcal beta-lactamases. Borderline strains of MRSA that do not contain *mecA* gene can be differentiated phenotypically from extremely heterogenous *mecA*-positive strains in that in them the clones showing high resistance to methicillin do not occur \(^ {26, 27}\).

In recent studies, disc diffusion test using cefoxitin disc is considered superior to other phenotypic methods and is accepted method now for the detection of MRSA by many reference groups such as CLSI\(^ {28}\). In the present study, the cefoxitin disk diffusion test showed \(n=47\) strains as resistant with only one false negative result. This strain positive for *mecA* gene by PCR was heterogeneously resistant to oxacillin recording an MIC of 0.38μg/ml and
showed 1+ agglutination by MRSA-Screen slide latex agglutination test for PBP2'. ORSAB showed slight growth with fermentation in the mother inoculum. In previous study conducted by Velasco et al., 2005, cefoxitin disc diffusion test showed 100% sensitivity. In the study conducted by Anand et al., 2009, the sensitivity and specificity were optimal (100%) in the 50 strains tested. The false negative result in the present study can be explained by the conditional expression of PBP2' protein which is the reason for ambiguity in susceptibility tests.

MIC by E-test is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. Of the penicillinase-stable penicillins, Oxacillin is preferred in in-vitro testing as it is more likely to detect heteroreistant strains of staphylococci. In the present study MIC by E-test for oxacillin showed a optimal sensitivity (100%). In the study conducted by Velasco et al., 2005, E-test for oxacillin showed a lower sensitivity in that three out of 51 clinical strains tested which were positive for meca gene, showed false negative results. In previous study conducted by Cavassini et al., 1999, out of the 80true MRSA isolates, 20 were resistant heterogeneously to oxacillin with MICs ranging from 0.25 to 3.0μg/ml by E-test. The false negative results in previous study may be explained as due to growth conditions.

Detection of product of meca gene (PBP2' protein) is a rapid and easier method of predicting resistance to methicillin. In the present study, among the 48 clinical isolates of MRSA tested 45 isolates showed agglutination by the slide latex agglutination test and three were negative. One showed positive in control latex also. These three meca positive isolates were resistant by disc diffusion assay. E-test for all the three was heterogeneously resistant to oxacillin with MIC <32μg/ml. One strain recorded a borderline MIC of 4μg/ml. In ORSAB two of them showed growth in mother inoculum on repeat and one showed growth after 48 h. In the study conducted by Mohanasundaram KM and Lalitha MK, 2008, all the 33 MRSA tested showed 4+ reaction in latex agglutination test but they have reported false positive reaction in one which has been discussed as may be due to incorrect inoculum or test card rotating the test card for more than 3mins. In the study conducted by Cavassini et al., 1999, MRSA-Screen, slide latest agglutination test detected all the 80 MRSA isolates and misidentified as MRSA only 1 of 120 MSSA isolate. In the study conducted by Velasco et al., 2005, PBP2' detection by the kit showed optimal sensitivity with only two false positive results. Griethysen et al., 1999, reported in their study that out of 267 strains which were meca
positive by PCR; 4 tested negative by the kit which resulted in a sensitivity and specificity of 98.5% and 100% respectively. Louie et al. 2000, in their study reported a sensitivity ranging in the range of 97 to 100%, and the specificity as optimal. Even in studies which included borderline isolates the sensitivity was 98.5%.

The reason for reduced sensitivity of the MRSA-Screen kit used in the present study is unknown. The reduced beta-lactam resistance relies on the down-regulation of mecA transcription and is influenced by auxiliary genes such as mecR, mecl, and the fem genes. However, these cryptic methicillin-resistant strains, also called preMRSA, are potentially highly resistant, since they can generate highly resistant subclones in vitro. Therefore, their detection appears to determine the choice of antibiotic therapy and relies mainly on the detection of the gene encoding for methicillin resistance (mecA). The reason for the false positive reaction in the control latex is unknown. It may be due to incorrect inoculum or the test card was rotated more than 3 mins.

In case of borderline MRSA strains, MICs are just above the susceptibility breakpoint; around 4 to 8 μg/ml. Borderline resistance strains do not contain the gene coding for methicillin resistance and resistance is considered not due to the production of PBP2a but as a result of modifications in the normal PBP genes, their overexpression or excessive production of staphylococcal β-lactamases. To differentiate the borderline-resistant mecA-negative strains from the heterogeneous mecA-positive strains of MRSA which are PBP2a-producing, is important in choosing the appropriate antimicrobial therapy. In vitro susceptibility studies, experimental data from animal studies and some clinical data show that treatment with beta-lactam antibiotics is very effective for the infections caused by these mecA gene negative, non-PBP2a-producing BORSA. Additionally, these non-PBP2a-producing strains of S. aureus may not require very expensive, not convenient patient isolation procedures. The MRSA screen, slide latex agglutination test could be useful for the identification of these strains.

The ORSAB medium showed a sensitivity of 95.8%. In the study conducted by Velasco et al., 2005, ORSAB medium showed high sensitivity but low specificity. Accurate determination of methicillin resistance in staphylococci in a clinical laboratory by conventional method is subject to many environmental conditions as mentioned earlier such as temperature, pH, salt concentration and incubation time. Difficulties in detection occur.
when the organisms have their MICs near break point. In such instances that molecular techniques are useful in accurately diagnosing the infection.

CONCLUSIONS
In summary, in the present study, MIC for oxacillin by E-test method was the most sensitive for the detection of MRSA from clinical isolates. We found that rapid method like MRSA-Screen slide latex agglutination test though take less time, conventional methods are more accurate, sensitive and cost-effective. Therefore, if the PCR is not available or accessible, conventional methods like cefoxitin disc diffusion and MIC for oxacillin still continue to be the standard methods for the accurate detection of MRSA from clinical isolates.

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detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-screen


Comparison of rapid methods PBP2’ detection, ORSAB and mecA for detection of methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care centre, Chennai, India

Latha Jayaraman1*, Umapaheswari Subburaya2

From 2nd International Science Symposium on HIV and Infectious Diseases (HIV SCIENCE 2014) Chennai, India. 30 January - 1 February 2014

Background
Early detection of MRSA in clinical specimens is imperative to prevent cross transmission, morbidity, mortality and overall cost of treatment. Additionally, rapid detection of MRSA helps in preventing superficial skin infection to become deep seated.

Method
Fifty isolates of MRSA from different specimens like blood, urine, exudates and respiratory were collected from 2010 to 2011. MRSA screen slide latex agglutination test kit (Denka Seiken, Japan) was used for PBP2’ detection and Oxacillin Resistant Screen Agar Base (ORSAB) with supplement (Oxoid Ltd., UK) was used for detection of MRSA from the isolates with ATCC controls. The detection of mecA gene in the MRSA isolates was considered as the reference method for determining the sensitivity of each phenotype rapid method studied.

Results
All the 50 isolates of MRSA were positive for mecA by PCR. MRSA screen for PBP2’ detection and ORSAB had a sensitivity of 93.75% and 95.8%, respectively. Chi square statistical analysis was carried out for the comparison of the rapid methods. Chi square value was found to be 3.0928 (p-value=0.07864) and 2.008 (p-value=0.153127) at 5% level of significance. OR (Odds ratio) = 7.4421 (95% CI=0.3744-147.933) and OR=5.2602 (95%CI=0.2436-111.244), respectively.

Conclusion
In the present study, we found that the difference in sensitivity between PCR for mecA and the phenotype rapid methods is statistically not significant. Therefore, we conclude that phenotype rapid methods can be used for the detection of MRSA from clinical isolates in low resource health care settings.

Authors’ details
1Department of Microbiology, Sri Ramachandra University, Chennai, India
2Department of Pharmacology, Sri Ramachandra University, Chennai, India

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Characterization of Methicillin-Resistant Staphylococcus aureus in a Tertiary Care Teaching Hospital, South India

Lathe, J; Rajendran, S; Uma maheswari, G, and Sumathi, G

1Department of Microbiology, Sri Ramachandra University, Chennai, India
2Department of Microbiology, M.G.R Medical College, Chennai, India
3Department of Microbiology, Sree Ramachandra University, Chennai, India
4Department of Microbiology, M.G.R Medical College and Research Institute, Chennai, India

Corresponding author: Lathe, J. Department of Microbiology, Sri Ramachandra University, Chennai, India, Tel. +919952501179, Email: lathe_j73@yahoo.co.in


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Abstract

*S. aureus* causes superficial to deep seated infections in human beings. Methicillin-resistant *Staphylococcus aureus* (MRSA) evolved in the 1960s and since then has become a worldwide concern owing to increasing morbidity and mortality in healthcare settings and even community. (MRSA) is a resistant variant of *S. aureus* which are resistant to beta-lactam antibiotics and other classes of antimicrobials. Early and accurate detection of MRSA and their antimicrobial susceptibility profile is therefore imperative for the selection of appropriate antimicrobial therapy. A total of 300 isolates of *S. aureus* collected from January 2010 to December 2012 were included in the study. *S. aureus* was characterized based on morphological and biochemical characters. To receive a pure culture, the isolates were grown on mannitol salt agar with supplement 5% v/v egg yolk emulsion. Antibiotic susceptibility testing was carried out on the strains by disc diffusion technique and the results interpreted according to Clinical Laboratory Standards International (CLSI) guidelines. A significant proportion of the *S. aureus* isolates were obtained from the exudates (22%) in all the three years followed by blood (48), urine (16) and respiratory (10). The average resistance seen in the 300 isolates tested was ampicillin (67.2%), cephalaxin (94%), cefotaxime (96.4%), clindamycin (100%), erythromycin (92.6%), Gentamicin (76.3%), ciprofloxacin (84.4%), clindamycin (40.4%) and linezolid and vancomycin were susceptible for all the strains. In conclusion, the prevalence of MRSA in our healthcare setting is 45% among the clinical isolates of *S. aureus*. Active screening and proper infection control procedures need to be adapted to control the MRSA infection.

Keywords: *S. aureus*; MRSA; Antibiotic susceptibility

Introduction

*S. aureus* causes a variety of infections ranging from skin and soft tissue infections to invasive diseases such as bacteremia, endocarditis, pneumonia, visceral abscesses, osteoarthritis and septicemia [1]. Penicillin was the drug of choice for severe staphylococcal infections till the discovery of penicillin resistant strains in the hospital and community settings [2]. The development of beta-lactamase resistant penicillins such as methicillin, oxacillin and cloxacillin in the early 1960s revolutionized the treatment of Staphylococcal infections. But within a year of the introduction of methicillin, methicillin-resistant *Staphylococcus aureus* (MRSA) strains were reported worldwide and in the next few decades MRSA reached an epidemic proportion [3,4].

MRSA, a resistant variant of *S. aureus* is resistant to various classes of antibiotics such as penicillin, methicillin, cephalosporins and the fluoroquinolones. It is often referred to as a super-bug. Vancomycin, a Glycopeptide is the treatment of choice for MRSA infections. In recent times, there is development of vancomycin resistant Staphylococcus aureus (VRSA) strains around the world with highest being reported in USA. In India also, a few cases of VRSA have been reported in health care settings in the northern and southern states. But most isolates are still susceptible to Oxazolidines which includes linezolid and polyyclic compounds such as tetracycline and tigecycline [5,6].

Early and accurate detection of MRSA and their antimicrobial susceptibility profile is therefore imperative for the selection of appropriate antimicrobial therapy [7]. The present study was carried out to identify and characterize the MRSA strains and determine their prevalence in our healthcare setting.

*S. aureus* strains were isolated from various specimens such as blood, exudates (pus, wound swabs, ear swabs and body fluids) respiratory and urine from January 2010 to December 2012. *Staphylococcus aureus* were characterized by their morphology on Gram staining, growth characteristics on blood agar and chocolate agar. Biochemical characterization was determined by catalase production, coagulase (slide and tube), mannitol fermentation and urease activity.

To receive a pure culture, the isolates were grown on mannitol salt agar with supplement 5% v/v egg yolk emulsion.

A total of 300 clinically significant isolates of *S. aureus* were included in the study. Antibiotic susceptibility testing was carried out on the strains by disc diffusion technique and the results interpreted according to Clinical Laboratory standards International (CLSI) guidelines with quality controls ATCC 25923 (MSSA) and ATCC 43300 (MRSA). The antimicrobials tested included ampicillin (10 μg), cephalaxin (30 μg), cefotaxime (30 μg), clindamycin (5 μg), gentamicin (10 μg), erythromycin (30 μg), clindamycin (30 μg), ciprofloxacin (5 μg), vancomycin (30 μg) and linezolid (30μg).
Table 1: Susceptibility to various antibiotics in the three years of sample collection (n=300)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2010 (n=100)</th>
<th>2011(n=100)</th>
<th>2012(n=100)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>87(95)</td>
<td>91.6(95)</td>
<td>100(100)</td>
<td>95(95.7)</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>8.4</td>
<td>0</td>
<td>4.3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>81(93)</td>
<td>97.1(123)</td>
<td>87(88)</td>
<td>87.7(83.3)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3.1</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>86(96)</td>
<td>91.7(95)</td>
<td>100(100)</td>
<td>93.3(94)</td>
</tr>
<tr>
<td></td>
<td>8.3</td>
<td>8.3</td>
<td>0</td>
<td>6.6</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>100(100)</td>
<td>100(100)</td>
<td>100(100)</td>
<td>100(100)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>76(93)</td>
<td>81.7(179)</td>
<td>82(88)</td>
<td>80.8(85)</td>
</tr>
<tr>
<td></td>
<td>18.3</td>
<td>18.3</td>
<td>15.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>63(90)</td>
<td>68.9(289)</td>
<td>71(100)</td>
<td>73.3(90)</td>
</tr>
<tr>
<td></td>
<td>31.1</td>
<td>31.1</td>
<td>29</td>
<td>26.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>53(91)</td>
<td>57.1(291)</td>
<td>40.2(97)</td>
<td>51.7(95)</td>
</tr>
<tr>
<td></td>
<td>42.8</td>
<td>42.8</td>
<td>59.8</td>
<td>48.3</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>-</td>
<td>-</td>
<td>24(97)</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>64.2</td>
<td>64.2</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0(100)</td>
<td>0</td>
<td>0(100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0(100)</td>
<td>0</td>
<td>0(100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

As S. aureus can be a coloniser, clinical significance of the isolates were emphasised by correlating the laboratory results with the patient history. A strain was identified as MRSA based on its resistance to clindamycin in the Muller-Hinton agar plates. Oxacillin (1 μg) is generally used for the detection of MRSA. As oxacillin potency reduces with time, clindamycin was used for the identification of MRSA. A significant proportion of the S. aureus isolates were obtained from the exudates (226) specimens in all the three years followed by blood (48), urine (16) and respiratory (10). The average resistance seen in the 300 isolates tested was ampicillin (97.3%), cephalosporin (94%), cefotaxime (96.4%), clindamycin (100%), erythromycin (82.6%), Gentamicin (76.9%), ciprofloxacin (54.4%), cefazolin (40.4%) and linezolid and vancomycin were susceptible for the entire strains (Table 1). In conclusion, the prevalence of MRSA in our health-care setting is 45% among the clinical isolates of S. aureus. Active screening and proper infection control procedures need to be adopted to control the MRSA infection.

References