Comparison of four diagnostic methods for detection of methicillin resistant \textit{Staphylococcus aureus}

Mohammad Reza Pourmand\textsuperscript{1*}, Sepideh Hassanzadeh\textsuperscript{2}, Rahil Mashhadi\textsuperscript{3}, Emran Askari\textsuperscript{4}

\textsuperscript{1}Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. \textsuperscript{2}Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran. \textsuperscript{3}Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran. \textsuperscript{4}Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

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ABSTRACT

Background and Objectives: Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is a well-known pathogen with a worldwide distribution. Given the increasing rate of MRSA infections, implementing of reliable, accurate and rapid testing for diagnosis of MRSA is necessary. The aim of this study was to compare four diagnostic methods for detection of MRSA isolates.

Materials and Methods: From December 2012 to April 2014, 120 \textit{S. aureus} isolates were collected from three hospitals affiliated with Tehran University of Medical Sciences. MRSA isolates were detected by four different methods including cefoxitin disc diffusion test, oxacillin disc diffusion test, minimum inhibitory concentration (MIC) of oxacillin as determined by MIC test strip, and \textit{mecA} detection by PCR.

Results: Out of 120 \textit{S. aureus} isolates, cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC test strip identified 60 (50%), 48 (40%), 55 (45.83%) isolates as MRSA, respectively. The sensitivity and specificity for oxacillin disc diffusion, cefoxitin disc diffusion and MIC of oxacillin were 80% and 100%, 100% and 100%, and 91.6% and 100%, respectively.

Conclusion: Cefoxitin disc diffusion test is reliable substitute for detection of MRSA in clinical laboratory where MIC detection and molecular methods are not accessible.

Keywords: \textit{Staphylococcus aureus}, Methicillin resistance, Microbial sensitivity tests

INTRODUCTION

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is considered as a major pathogen both in hospital and community settings (1, 2). MRSA is endemic in many hospitals causing excess mortality and economic burden compared to methicillin-susceptible isolates (3). MRSA strains are resistant to nearly all of the beta-lactam antibiotics by producing an alternative penicillin-binding protein known as PBP\textsubscript{2a}. This protein is encoded by the \textit{mecA} gene and has a low affinity to many beta-lactam antibiotics (4). MRSA strains are not only resistant to beta-lactams and cephalosporins, but also often show resistance to a wide range of antibiotics (5).

Due to high prevalence of MRSA infections among hospitalized patients, rapid and accurate identification of MRSA is needed to initiate appropriate antimicrobial therapy and prevent the spread of MRSA infections. Usually, molecular methods such as detection of the \textit{mecA} gene are preferred for this task because of high sensitivity and specificity. The results of molecular methods are also usually available faster than that of phenotypic
Different phenotypic methods are available in clinical laboratories such as oxacillin and cefoxitin disc diffusion test, oxacillin agar screening test, and determination of minimum inhibitory concentration (MIC) for oxacillin and cefoxitin. However, the expression of resistance is affected in variant conditions such as difference in temperature, medium, inoculum size and NaCl concentration in the medium (8). In this study, we aimed to compare PCR of the \textit{mecA} gene with three phenotypic methods including cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC of oxacillin for detection of MRSA.

**MATERIALS AND METHODS**

**Isolation and identification of \textit{S. aureus}.** From December 2012 to April 2014, we collected 120 \textit{S. aureus} isolates from three hospitals affiliated with Tehran University of Medical Sciences. Samples were identified and confirmed by conventional biochemical tests (9). Control strains for methicillin-resistant and –susceptible \textit{S. aureus} were COL and ATCC 8325-4, respectively.

**Phenotype identification of MRSA.** We performed phenotypic methods for detection of MRSA strains according to clinical and laboratory standards Institute (CLSI) guideline as follows:

Cefoxitin and oxacillin disc diffusion tests were done using cefoxitin (30µg) and oxacillin (1µg) discs purchased from MAST Company (UK). Müeller-Hinton agar (MHA) plates containing 2% NaCl were inoculated with broth suspension equivalent to 0.5McFarland. Discs were applied on to the plates and incubated at 35°C for 24h. The zone inhibitions were measured and interpreted according to CLSI guideline (10).

For oxacillin MIC strip test, isolates were cultured on MHA containing 2% NaCl and oxacillin strip was placed on the medium and incubated for 24h at 35°C. After incubation, inhibitory concentration studied and interpreted according to the CLSI criteria (10).

**Genotype identification of MRSA by PCR.** We extracted genomic DNA using Viogene kit (UK) based on manufacturer’s instructions. We used the extracted genomic DNA as template for PCR of the \textit{mecA} gene. Forward (5’-TCC AGA TTA CAA CTT CAC CAG G-3’) and reverse (5’-CCA CTT CAT ATC TTG TAA CG-3’) primers were used to amplify the 162 bp \textit{mecA} gene of MRSA as described previously (Fig. 1) (11). Each PCR mixture was composed of 2 µl DNA template, 0.5 µl of each primer (10 µM), 12.5 µl master mix (SinaClon, Iran), and 9.5 µl sterile distilled water. PCR program began with an initial denaturation step at 97°C for 6 min followed by 30 cycles of 92°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 second, and ended with a final extension step at 72°C for 10 min. The \textit{mecA}-positive strain COL and the \textit{mecA}-negative ATCC8325-4 were included as positive and negative controls, respectively. The amplified PCR products were electrophoresed in 1% agarose gel at 120 V for 1h, stained with KBC (0.5 µg/ml) (Kawsar,Iran), and photographed under UV light.

**Table 1.** Comparison of various laboratory methods for detection of methicillin-resistant \textit{Staphylococcus aureus} isolates in this study

<table>
<thead>
<tr>
<th>Method</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{mecA} gene</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Cefoxitin disc</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Oxacillin disc</td>
<td>100%</td>
<td>80%</td>
<td>100%</td>
<td>83%</td>
</tr>
<tr>
<td>Oxacillin strip</td>
<td>100%</td>
<td>91.6%</td>
<td>100%</td>
<td>92%</td>
</tr>
</tbody>
</table>

PPV= Positive Predictive Value; NPV= Negative Predictive Value

![Fig. 1. 1%Agarose gel electrophoresis of the PCR-amplified \textit{mecA} methicillin resistance gene. Lanes: 1: 50-bp ladder; 2: Negative control (\textit{S. aureus} ATCC 8325-4); 3: Positive control (\textit{S. aureus} strain COL); 4-7: \textit{S. aureus} isolates showing 162 bp \textit{mecA} amplicon.](image)
RESULTS

All *S. aureus* isolates were subjected to MRSA detection by four phenotypic methods. The PCR assay targeting *mecA* gene and cefoxitin disc diffusion identified 60 (50%) isolates as MRSA. Forty-eight MRSA (40%) were identified by oxacillin disc diffusion method, including the intermediate zones (Table 1). MIC test strip found 55 (45.83%) MRSA phenotype with MIC between 8-16 μg/ml. The remaining five MRSA strains were only identified by cefoxitin disc diffusion and had an MIC of oxacillin between 0.125-0.5 μg/ml (Table 2).

DISCUSSION

In recent years, detection of *mecA* by PCR is considered as the gold standard for identification of MRSA. In this study, we evaluated other methods as alternatives to PCR (12). Cefoxitin disc diffusion test was perceived to be the most sensitive method for detection of *mecA*-mediated resistance. CLSI has also recently substituted the oxacillin disc with cefoxitin disc for detection of MRSA (13). Numerous studies including the current one have informed that the results of the cefoxitin disc diffusion test correlates better with the presence of *mecA* compared with those of the oxacillin disc diffusion test (14-16).

Our results about cefoxitin disc diffusion method are consistent with previous report (15). However, Broekeme *et al.*, reported the sensitivity and specificity of this method 97.3% and 100%, respectively among 1,611 *S. aureus* isolates (16).

In current study, MIC strip test showed the sensitivity and specificity about 91.6% and 100%, respectively. In the study of Rahbater *et al.*, sensitivity and specificity were both 100% (17). Five isolates in our study showed discordant results for MIC of oxacillin and PCR. This can be probably explained by the fact that not all *S. aureus* isolates express their *mecA* gene (18). In our study, the sensitivity and specificity of oxacillin disc diffusion test were 80% and 100%, respectively. In the study of Farahani *et al.*, the sensitivity and specificity of the oxacillin disc diffusion method was 100 and 73.6%, respectively (19). In previous study that performed by Pillaiet *et al.*, the sensitivity and specificity were reported 93.5% and 83.5%, respectively (20).

In conclusion, the present study showed that cefoxitin disc diffusion has both high sensitivity and specificity as compared with *mecA* PCR. Therefore, it can be a good alternative to molecular methods due to its low cost for clinical laboratories.

ACKNOWLEDGEMENT

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REFERENCES


Table 2. Discordant results of minimum inhibitory concentration of oxacillin and PCR of *mecA*

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>MIC (μg/dL) of Oxacillin</th>
<th>mecA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTH-3-25</td>
<td>0.5</td>
<td>+</td>
</tr>
<tr>
<td>TTH-1-17</td>
<td>0.25</td>
<td>+</td>
</tr>
<tr>
<td>TTH-3-11</td>
<td>0.25</td>
<td>+</td>
</tr>
<tr>
<td>TTH-3-8</td>
<td>0.25</td>
<td>+</td>
</tr>
<tr>
<td>TTH-3-3</td>
<td>0.125</td>
<td>+</td>
</tr>
</tbody>
</table>

TTH: Tehran Teaching Hospital; MIC: Minimum Inhibitory Concentration


