**Original Article**

**Antibiotic Resistance Pattern and Frequency of meca Gene in Staphylococcus aureus Isolated from Shohada Hospital, Tabriz**

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Received Jun 15, 2015; accepted Sep 28, 2015

**INTRODUCTION**

*Methicillin*-resistant *Staphylococcus aureus* (MRSA) can cause serious and life-threatening hospital- and community-acquired infections. Colonized and infected patients represent the most important reservoir of MRSA in health care facilities. Therefore, in this study, MRSA isolates collected from Shohada Hospital in Tabriz were evaluated for the frequency of meca gene and their antimicrobial susceptibility in a period of three years, from 2010 to 2012. **Methods:** A total of 182 *S. aureus* isolates were collected from clinical specimens and first genotypically identified by detection of nuc gene. Antimicrobial susceptibility test was performed by disc agar diffusion method using cefazolin, methicillin, tetracycline, and cefoxitin according to clinical and laboratory standards institute (CLSI) recommendation. Phenotypic (cefoxitin 30 μg/disc) and genotypic (meca gene detection by PCR) methods were used for detecting methicillin sensitivity. **Results:** All isolates expressed *S. aureus* specific sequence gene (nuc) in their PCR products. Eighty-one (44.5%) isolates were confirmed as MRSA by cefoxitin disc screening test and 97 (53.3%) isolates by showing the presence of meca gene. All the methicillin sensitive *S. aureus* (MSSA) isolates and 64 (66%) MRSA isolates were found to be susceptible to cefazolin, but 25 (25.8%) MRSA were resistant to tetracycline and cefazolin. **Conclusion:** The results of this study showed high frequency (53.3%) of MRSA with no significant differences in rates within the three years of study, indicating the inefficiency of control programs to care for patients with MRSA. *J Med Microbiol Infec Dis, 2014, 2 (3): 105-108.*

**Keywords:** meca, Methicillin-Resistant *Staphylococcus aureus*, Polymerase Chain Reaction, Iran.

**MATERIAL AND METHODS**

This study was carried out in the department of Medical Microbiology, Tabriz branch, Islamic Azad University and Shohada Teaching Hospital, Tabriz, Iran. One hundred and eighty two *S. aureus* isolates obtained from different clinical (blood and wound) specimens, were studied to determine antimicrobial sensitivity patterns and presence of meca gene. Identification of the organism was made by growth in blood agar, colonial morphology, Gram stain, and positive results for catalase, coagulase and DNase. Coagulase and DNase positive *staphylococci* were considered as *S. aureus*.

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Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) recommendation for screening of MRSA, the test was performed with the same discs for all the samples, because most of the isolates were from wound. The used antibiotics included: cefazolin (30 μg), methicillin (5 μg), tetracycline (30 μg), cefoxitin (30 μg) (HiMedia, India), and oxacillin (1 μg) (Padtan Teb, Iran) [10]. The S. aureus ATCC 25923 was used as control strain for susceptibility testing.

To extract bacterial genomic DNA, an overnight culture in LB broth was harvested by centrifugation and processed according to the procedure of Kalia et al. [11]. The extracted DNA was stored at -20°C in 50 μl TE buffer for further use.

Genotypic identification of the isolates was done by tracking the presence of nuc gene. Forward primer sequence (5'-TGC ATT GAT GGT GAT AGC GTC-3') and reverse primer sequence (5'-AGG CAA GCC TTG ACG AAC TAA AGC-3') were used for amplification of 279 bp region [12]. The condition of PCR for this gene was first described by Brakstad et al. [12] and modified as follows: an initial denaturation at 95°C for 3 min, followed by 30 cycles of initial denaturation at 94°C for 60 s, annealing at 55°C for 30 s, extension at 72°C for 1.5 min, and a final extension at 72°C for 3.5 min.

For amplification of mecA gene (533 bp), the following primers were used: forward primer (5'-AAA ATC GAT GTT AAA GGT GCC-3') and reverse primer (5'-AGT TCT GCA GGT ACC GGA TTT GC-3') [13]. Each reaction mixture contained 5 μl of master mix buffer (Cinnagen Inc.), 0.5 μl of forward primer (30 mM), 0.5 μl of reverse primer (20 mM), 1 μl of template DNA, and 3 μl of ddH2O.

The PCR condition was described by Al-Ruaily et al. [13], and modified as follows: an initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The S. aureus ATCC 29213 and S. aureus ATCC 33591 strains were used as negative and positive controls for mecA gene, respectively. The PCR products of nuc and mecA genes were then electrophoresed on 1.2% agarose gel, and amplified bands were analyzed in UV transilluminator system (Intas, German).

RESULTS

CLSI (2013) [10] has recommended cefoxitin disc screening test to be used instead of methicillin disc diffusion test for detection of MRSA, because cefoxitin is a good inducer of mecA gene. In our study, in disc diffusion method 176 (96.7%) isolates, 27 (14.8%) isolates, and 81 (44.5%) isolates were identified as MRSA by methicillin, oxacillin, and cefoxitin discs, respectively. During the period of study (2010-2012), 43.3%, 39.3%, and 50.8% of the isolates were identified as MRSA by cefoxitin disc diffusion method.

Sixty-six (36.3%) isolates were resistant to tetracycline and 23 (12.6%) isolates were resistant to cefoxitin. In our isolates, the presence of mecA gene was confirmed in 97 (53.3%) cases (Figure 1). Table 1 shows antibiotic resistance and presence of mecA gene in our isolates. No significant differences were found among the isolates over the three-year period of the study. Twenty-five (25.8%) isolates of MRSA were resistant to either tetracycline or cefoxitin.

Out of 85 methicillin-sensitive S. aureus (MSSA) isolates, 85 (100%) isolates was susceptible to cefoxitin, but 25 (29.41%) isolates showed resistance to tetracycline.

DISCUSSION

S. aureus as an opportunistic pathogen plays an important role in community- and hospital-acquired infections [14]. Increasing frequency of MRSA poses a serious and growing global problem [15]. According to CLSI recommendation, cefoxitin disc screening test is better than methicillin or oxacillin disc screening test for detection of methicillin-resistance [10].

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**Fig. 1.** Results of PCR for mecA gene in S. aureus isolates. Lane 1, Size marker 1 Kb; lane 2, S. aureus ATCC 25923 (negative control); lane 3, S. aureus ATCC 33591 (positive control); lanes 4-21, Clinical isolates positive for mecA gene.
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Table 1. Frequency of antibiotic resistance, presence of mecA gene, and gender of patients in clinical isolates of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Finding</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates from males</td>
<td>76.7</td>
<td>68.9</td>
<td>62.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Isolates from females</td>
<td>23.3</td>
<td>31.1</td>
<td>37.7</td>
<td>0.23</td>
</tr>
<tr>
<td>Resistance to methicillin</td>
<td>96.7</td>
<td>98.4</td>
<td>95.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Resistance to oxacillin</td>
<td>11.7</td>
<td>24.5</td>
<td>8.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Resistance to cefazolin</td>
<td>10</td>
<td>21.3</td>
<td>6.6</td>
<td>0.21</td>
</tr>
<tr>
<td>Resistance to cefoxitin</td>
<td>43.3</td>
<td>39.3</td>
<td>50.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Resistance to tetracycline</td>
<td>30</td>
<td>39.3</td>
<td>39.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Presence of mecA gene</td>
<td>51.6</td>
<td>62.3</td>
<td>42.6</td>
<td>0.62</td>
</tr>
</tbody>
</table>

The main mechanism of methicillin-resistance in Staphylococcus aureus is production of low affinity penicillin-binding proteins (PBPs), which is identified by the presence of mecA gene [4-5].

In this study, most of the isolates were collected from male orthopedic inpatients (126 of 182 isolates). There was no significant difference in isolation of pathogenic bacteria over the three-year period of study, which indicates that programs have been unsuccessful in controlling or reducing MRSA frequency. The most effective drug was cefazolin, since 100% of MSSA isolates and 67% of MRSA isolates were sensitive to this antibiotic. The frequency of MRSA in Shohada Hospital of Tabriz was determined to be 96.7%, 14.8%, 44.8%, and 53.3% by methicillin disc diffusion method, oxacillin disc diffusion method, cefoxitin disc screening test, and PCR for mecA gene, respectively. There was no significant difference between cefoxitin disc screening test and PCR for mecA gene. There was a significance difference between methicillin-resistance (96.7%) and PCR (53.3%) for mecA gene confirming the invalidity of this disc in routine sensitivity tests.

Our findings were similar to those of Moghadami et al. [16] in Shiraz hospitals, which reported 52.7% MRSA, meta-analysis and systematic review by Askari et al. [17] with 52.7%±4.7% MRSA, Azimian et al. [18] in Tehran hospitals with 47% MRSA, and Jarvis et al. [19] in the USA with 50% MRSA in health care facilities. Johnson’s study [20] showed that more than one-third of European countries share >25% proportion of hospital-acquired infections caused by MRSA.

It is strongly believed that the dissemination of MRSA clones must be controlled via screening patients by culture and blood samples and for mecA homologue, mecALGA251, is present in methicillin-resistant Staphylococcus epidermidis and Staphylococcus haemolyticus strains among outpatients from four countries. Antimicrob Agents Chemother. 2009; 53 (2): 442-9.

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Phenotypic and Genotypic Detection of MRSA


