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*Original Article*

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

**Farzaneh Khoei, *Haedeh Mobaiyen, Mohamamad Reza Nahaei, Sanam Sadeghi Mohammadi**

Department of Microbiology, College of Medicine, Tabriz branch, Islamic Azad University, Tabriz, Iran

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**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.

## INTRODUCTION

*Staphylococcus aureus* strains had become pandemic in 1950s after becoming resistant to penicillin [1]. Methicillin-resistance in *S. aureus was* first reported in 1961, 2 years after introduction of methicillin for the treatment of penicillin-resistant-Staphylococcal infections [2, 3], but the specific gene responsible for methicillin-resistance (*mecA*) has not been identified over the next 20 years. This gene encodes a low-affinity penicillin binding protein (PBP2a) [4]. The *mecA* gene that is located in a mobile cassette element improves our understanding of methicillin-resistance biology and provides an additional tool for clarifying the evolutionary relationships among methicillin-resistant *Staphylococcus aureus* (MRSA) strains [5].

At present, antibiotic resistance is a global problem and distributed widely in pathogenic bacteria. Emergence of community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA) isolates has become increasingly blurred [6]. One of the causes of the success of *S. aureus* strains is their high variability, occurring at different periods and places with different clonal types and antibiotic resistance pattern. Infections with these resistant bacteria lead to serious problems in the general population, especially young children, the elderly, and immunocompromised patients [7]. Furthermore, the MRSA strains carrying *mecA* LGA251 (a novel *mecA* homologue) are present in different host species other than human and cattle; therefore MRSA surveillance and control measures seem to be necessary [8]. Two recommendations are emerging for the control of MRSA. The first is to screen and treat carriers, and the second is universally treat everyone and run the theoretical risk of exacerbating bacterial resistance and changing the microbiome of both patient and facility [9]. This study was conducted first to evaluate the presence of *mecA* gene and MRSA isolates collected from inpatients with *S. aureus* infection in the orthopedic ward of Shohada Hospital in Tabriz by phenotypic and genotypic methods, and second to compare the frequency of MRSA in a period of three years.

## 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*.

***Correspondence:** Haedeh Mobaiyen

Department of Microbiology, College of Medicine, Tabriz branch, Islamic Azad University, Manzariyeh, SoleimanKhater St., Tabriz, Iran, 5157745155.

**Email:** drhmobaiyen@iaut.ac.ir

**Tel:** +98 (914) 3005489 **Fax:** +98 (41) 34781587

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'-GCG ATT GAT GGT GAT ACG GTT-3') and reverse primer sequence (5'-AGC 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 GGT AAA GGT GGC-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), 1µl of template DNA, and 3 µl of $ddH_2O$.

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 cefazolin. 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 cefazolin. Out of 85 methicillin sensitive *S. aureus* (MSSA) isolates, 85 (100%) isolates was susceptible to cefazolin, 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].

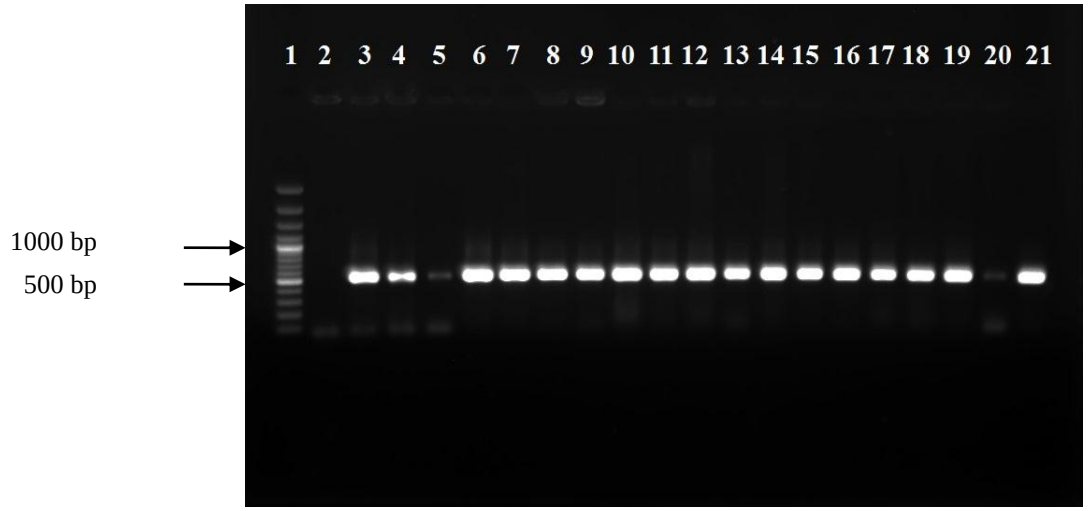


**Fig. 1.** Results of PCR for *mecA* gene in *S. aurues* isolates. Lane 1, Size marker 1 Kb; lane 2, *S. aurues* ATCC 25923 (negative control); lane3, *S. aurues* ATCC 33591 (positive control); lanes 4-21, Clinical isolates positive for *mecA* gene.

**Table 1.** Frequency of antibiotic resistance, presence of *mecA* gene, and gender of patients in clinical isolates of *S. aureus*

| Finding | Percent of isolates per year | | | *P*-value |
|---|---|---|---|---|
| | 2010 | 2011 | 2012 | |
| **Isolates from males** | 76.7 | 68.9 | 62.3 | 0.23 |
| **Isolates from females** | 23.3 | 31.1 | 37.7 | 0.23 |
| **Resistance to methicillin** | 96.7 | 98.4 | 95.1 | 0.59 |
| **Resistance to oxacillin** | 11.7 | 24.5 | 8.2 | 0.28 |
| **Resistance to cefazolin** | 10 | 21.3 | 6.6 | 0.21 |
| **Resistance to cefoxitin** | 43.3 | 39.3 | 50.8 | 0.44 |
| **Resistance to tetracycline** | 30 | 39.3 | 39.3 | 0.29 |
| **Presence of *mecA* gene** | 51.6 | 62.3 | 42.6 | 0.62 |

The main mechanism of methicillin-resistance in *S. 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. Johson'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 from different sites of body [21], isolation and barrier nursing by contact precautions, hand hygiene, and frequent cleaning and disinfection of environmental surfaces in this teaching hospital.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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