



Original Article

## Colonization and Antibiotic Resistance of Nasal *Staphylococcus Aureus* among Healthcare Workers in Southwestern Iran: Occurrence of OS-MRSA

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### Abstract

**Background & Objectives:** *Staphylococcus* spp. is a resident flora of the skin and mucosa of humans that can colonize the anterior nares of individuals. This cross-sectional study was conducted to determine the rate and antibiotic resistance pattern of nasal *Staphylococcus aureus* (*S. aureus*) carriers among the staff of Fasa hospital, southern Iran.

**Materials & Methods:** Nasal swab samples were collected from 117 hospital staff working in 12 wards. Microbiological culture method was applied for *S. aureus* identification. The isolates were confirmed by *tuf* gene identification using PCR assay. Five isolates were randomly sequenced and phylogenetically analysed using MEGA software. The antimicrobial resistance pattern of the isolates was evaluated using the disc diffusion assay and the amplification of the methicillin resistance (*mecA*) gene.

**Results:** The prevalence of *S. aureus* nasal carriers included 10.26% (n=12). The nasal carriers were identified in the wards of surgery ICU, gynecologic surgery, NICU, pediatric, internal surgery, and emergency. Among them, gynecologic surgery staff had the highest rate of nasal colonization (33.33%). Phylogenetic analysis showed that of five isolates, four had high similarities with each other. Also, the highest rate of resistance was related to penicillin (83.3%), followed by cefazolin (75%), and cephalexin (75%). However, the highest level of susceptibility (100%) was found for vancomycin, ceftazidime, and oxacillin. Furthermore, the methicillin resistance gene (*mecA*) was highly detected (75%) from the isolates, elucidating oxacillin-susceptible or ceftazidime-susceptible *mecA*-positive *S. aureus* (OS-MRSA).

**Conclusions:** The high rates of OS-MRSA can lead to antibiotic resistance among health care workers tremendously. Moreover, the high similarity probability in phylogenetic analysis shows the possibility of cross-infection between these health care workers, warning to exert effective strategies to control infection spread, especially in the surgery ward.

**Keywords:** *Staphylococcus aureus*, OS-MRSA, *MecA* gene, Healthcare workers, Colonization

### Introduction

*Staphylococcus* spp. is known as a resident flora of the skin and mucosa of most animals and humans. *S. aureus* can colonize the anterior nares of individuals particularly adults (1). About 20-30% of the people are nasal carriage of *S. aureus* asymptotically (2).

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This bacterium is identified as the most common strain of hospital-acquired pathogens (3). Nasal colonization of the bacterium makes the health care workers a major source of infection in nosocomial disease (4, 5). Identification of the bacterium carriers in hospitals is crucial to prevent the spread of the infection. Disregarding the patients with *S. aureus*, complications resulting even up to 40% mortality may occur (3). Nasal carriers of *S. aureus* seem to be healthy without any symptoms, while they have a remarkable load of

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bacteria in other parts of the body leading to bacteremia in comparison with non-carriers. Besides, in about 30% of healthy persons, the bacterium may colonize intermittently (6).

Moreover, increasing *S. aureus* resistance to antibiotic has become a global concern and the treatment or eradication is the main challenge (7). This bacterium is found to be a predominant nosocomial pathogen whose antibiotic resistance is reported up to 90%, leading to complicated infections (8). Methicillin-resistance *S. aureus* (MRSA) is enhancing globally and can especially spread among hospital staff and patients (9). The term MRSA is defined as *S. aureus* carrying the *mecA* gene or having a minimum inhibitory concentration (MIC) of oxacillin more than 4 mg/L (10). Nevertheless, it has been shown that some clinical *S. aureus* isolates possessing the *mecA* gene are phenotypically oxacillin or ceftiofur susceptible, which is nominated oxacillin-susceptible MRSA (OS-MRSA). In the last decade, the clinical OS-MRSA has been reported all around the world (11). Since OS-MRSA has low-level resistance to  $\beta$ -lactam antibiotics, the infected patients treated with  $\beta$ -lactams, high-level  $\beta$ -lactam resistant MRSA may occur. Hence, OS-MRSA exploration is significant (10). This study aimed to determine the frequency of nasal *S. aureus* carriage among Fasa hospital staff, and to identify the pattern of antibiotic resistance of the isolates.

## Materials & Methods

### Sampling and culture

This cross-sectional study was conducted on 117 personnel (25 women and 92 men) with the mean age of 30.52 years (min. 20 and Max. 64) of the various wards of the Fasa hospitals, Southern Iran. Samples were collected from the anterior parts of the nasal cavities of the persons using sterile cotton swabs which were then transferred to the bacteriological laboratory of the Fasa University of Medical Science in the vicinity of the ice pack. The swabs were directly cultured on Blood agar, and in Giolitti-Cantoni broth (Merck, Germany) and incubated

for 48 h at 37°C in an aerobic and anaerobic condition, respectively. All cultures, were subsequently streaked onto Baird-Parker agar (Merck, Germany) supplemented with egg yolk–tellurite emulsion (Merck, Germany), and incubated aerobically for 24 to 48 h at 37°C. Gram staining and biochemical tests including catalase, coagulase, and DNase were carried out to confirm the suspected colonies (12).

### Antibiotic susceptibility test

Kirby-Bauer disc diffusion assay was performed based on the Clinical and Laboratory Institute Standard (CLSI) guidelines (13). Accordingly, the isolated bacteria with the turbidity of 0.5 MacFarland were inoculated onto the Mueller-Hinton agar (Merck, Germany), and the antibiotic discs comprising vancomycin (30  $\mu$ g), norfloxacin (10  $\mu$ g), amoxicillin (25  $\mu$ g), erythromycin (15  $\mu$ g), co-trimoxazole (25  $\mu$ g), penicillin (10  $\mu$ g), ceftiofur (30  $\mu$ g), cefazolin (30  $\mu$ g), oxacillin (1  $\mu$ g), cephalexin (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), were then placed on the agar surfaces. For oxacillin, 2% NaCl was added to 2 to Mueller-Hinton agar. The plates were subsequently incubated for 24 h at 37 °C, aerobically.

### PCR confirmation, sequencing, and phylogenetic analysis

To confirm the *S. aureus* isolates, DNA extraction was initially done using DNA kit (Cinnagen, Iran) according to the manufacturer's instruction. PCR assay was then performed to identify *tuf* gene using a pair of species-specific *Staphylococcus* primers, TstaG422 (5'-GGCCGTGTTGAACGTGGTCAAATCA-3') and Tstag765 (5'-TIACCATTTCAGTACCTTCTGGTAA-3'), to confirm the isolates. The target gene was then subjected to the thermal recycle program in a reaction volume of 25  $\mu$ L with an annealing temperature of 55 °C. The amplification program was accomplished as previously published (14). Amplified products (370 bp) were identified in a 1.4% agarose gel containing 0.5  $\mu$ g/mL safe stain.

To confirm the PCR result and show the evolutionary relationship of the isolates, five fragments were randomly picked out and sequenced (Macrogen, South Korea) in both strands. Nucleotide sequences were subsequently compared to data available in the GenBank library using the Basic Local Alignment Search Tool (BLAST) program cited in the National Center for Biotechnology Information (NCBI) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequences were analysed by applying MEGA (version 10.0) software for sequence alignment, identity percentage, and drawing the phylogenetic tree.

### Genotypic characterization of *mecA* gene

The methicillin resistance gene was distinguished using *mecA* primers (forward: 5'-GGGATCATAGCGTCATTATTC-3', reverse: 5'-AACGATTGTGACACGATAGCC-3'), specified for the isolated bacteria. *Staphylococcus epidermis* ST17 was considered as a positive control (15). Each amplification reaction was prepared in 20 µL volume comprising 0.5 µM of each primer, 0.2 mM dNTPs, 2 mM MgCl<sub>2</sub>. Thermal cycler program

was firstly run using gradient thermocycler (Eppendorf, Germany) with denaturation phase of 94 °C for 4 min, followed by 30 Cycles of 94 °C for 30 s, annealing step of 58 °C for 30 s and elongation at 72 °C for 30 s. 1.2% agarose gel electrophoresis containing safe stain was prepared to analyse the PCR products.

### Statistical analysis

The data were analyzed applying SPSS v. 16 software. The One-Way ANOVA test was used to calculate significant differences. The significance level was considered as p-value <0.05.

### Results

Of a total of 117 experimented staff of the hospital, 12 (10.26%) were found to be colonized with *S. aureus* according to the culture method. The nasal carriers of the bacterium were identified in the wards including surgery ICU, gynecologic surgery, NICU, pediatric, internal surgery, and emergency, among which gynecologic surgery staff had the most nasal colonization (33.33%). Details of the prevalence of the bacterium among hospital staff are included in Table 1.

**Table 1.** The prevalence of nasal *S. aureus* isolated from hospital staff of different wards

Ward	Negative (%)	Positive (%)	Total (%)
Dialysis	3 (100)	0 (0)	3 (100)
Surgery ICU	8 (80)	2 (20)	10 (100)
Cardio-surgery ICU	8 (100)	0 (0)	8 (100)
Gynecologic surgery	4 (66.67)	2 (33.33)	6 (100)
Male surgery	3 (100)	0 (0)	3 (100)
NICU	4 (80)	1 (20)	5 (100)
Pediatric	6 (75)	2 (25)	8 (100)
GYN	9 (100)	0 (0)	9 (100)
Internal surgery	3 (75)	1 (25)	4 (100)
Internal	11 (100)	0 (0)	11 (100)
Emergency	16 (80)	4 (20)	20 (100)
Operatory room	30 (100)	0 (0)	30 (100)
Total	105 (89.74)	12 (10.26)	117 (100)

ICU: intensive care unit, NICU: neonatal intensive care unit, GYN: Gynaecology



**Disc diffusion antibiotic susceptibility of isolates**

The antibiotic susceptibility of the isolated bacteria to various antibiotic discs are summarized in Table 2. Respectively, the highest antimicrobial resistance of the isolates

belonged to penicillin (83.3%) followed by cefazolin (75%) and cephalixin (75%) based on the disc diffusion method. The highest sensitivity rates were towards vancomycin, ceftiofur, and oxacillin, and none of the isolates were found methicillin-resistant *S. aureus* (MRSA).

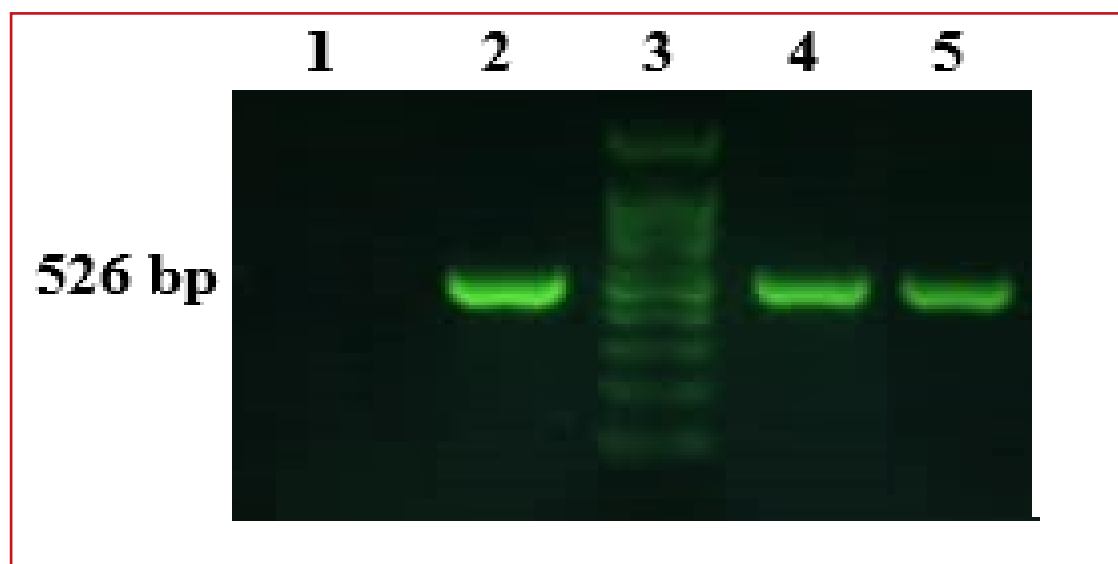
**Table 2.** Antibiotic susceptibility of *S. aureus* isolates using disc diffusion assay

Antibiotic	Susceptibility (%)		
	Resistant	Intermediate	Susceptible
Vancomycin	0 (0)	0 (0)	12 (100)
Norfloxacin	3 (25)	0 (0)	9 (75)
Amoxicillin	5 (41.7)	0 (0)	7 (58.3)
Erythromycin	7 (58.3)	0 (0)	5 (41.7)
Co-trimoxazole	2 (16.7)	2 (16.7)	8 (66.7)
Penicillin	10 (83.3)	0 (0)	2 (16.7)
Ceftiofur	0 (0)	0 (0)	12 (100)
Cefazolin	9 (75)	0 (0)	3 (25)
Oxacillin	0 (0)	0 (0)	12 (100)
Cephalixin	9 (75)	0 (0)	3 (25)
Ciprofloxacin	2 (16.7)	0 (0)	10 (83.3)

### Characterization of *mecA* gene

Out of 12 *S. aureus* isolates, 9 (75%) samples harbored the methicillin resistance

gene (*mecA*). The PCR results are demonstrated in Figure 1, representing the amplicon size of 526 bp for *mecA* gene.

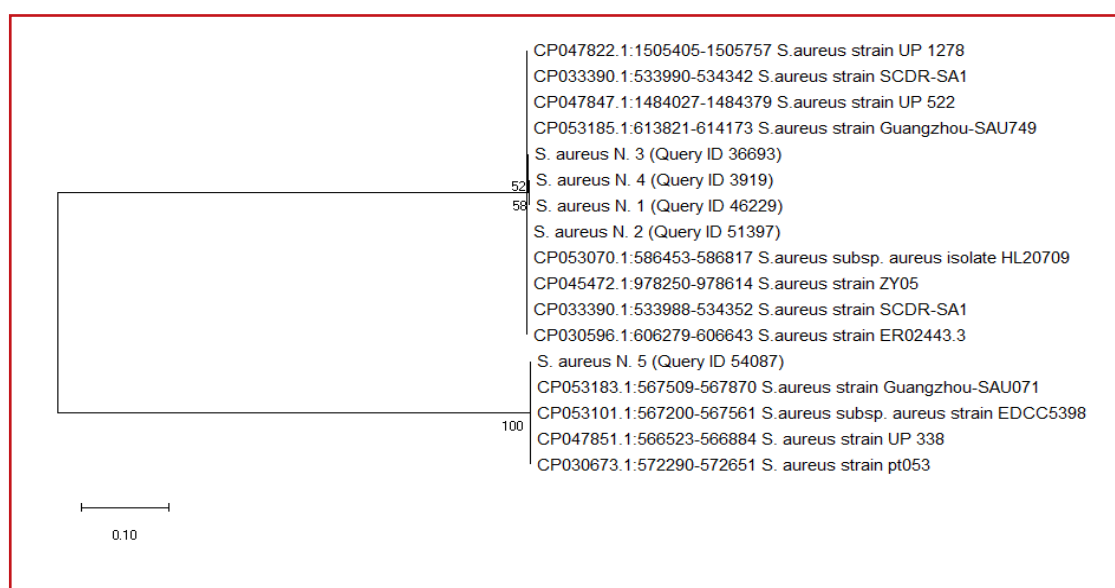


**Figure 1.** PCR gel electrophoresis based on *mecA* gene resistance. Lane 1: Negative control, Lane 2: Positive control (526 bp), Lane 3: Marker 100 bp, Lanes 4 and 5: Positive samples (526 bp)

### Phylogenetic analysis

Nucleotide BLAST compression of the five sequences against the GenBank database indicated 99.72%-100% identity to documented *S. aureus* sequences. Aligning the sequences exerting MEGA software, a phylogenetic tree

was deduced using the Neighbor-Joining method established 1000 replicates in the bootstrap test. The whole five sequences are phylogenetically defined (with their Query IDs) in Figure 2. Four of the isolated fragments showed more similarity, whilst sequence number 5 (Query ID 54087) is categorized in a different cluster.



**Figure 2.** The phylogenetic tree is inferred from DNA sequences using Neighbor Joining method





## Discussion

Health care providers play a critical role in promoting the health of patients at hospitals. Any possibility of nasal carriage of the pathogen is the main risk factor in infecting patients admitted in various wards of a hospital (16). A previous study has illuminated a well-appointed relationship in gene coding between *S. aureus* colonizing nasal mucus and those isolated from clinical samples (6). In the last decade, nasal carriage of *S. aureus* is tremendously focused, and plenty of studies are confined to patients, route of transmission, and treatment while numerous reports have focused on health care workers in different wards of hospitals. In this research, we studied hospital staff carrying the nasal bacterium and categorized them in various wards. The antibiotic susceptibility test was implicated. Accordingly, 10.26% of the contributors were infected as nasal carriers in agreement with the studies from Zabol province of Iran (10.8%), Ethiopia (12%), and Oman (20.5%) (17-19). This study is in contradiction with nasal colonization in healthcare workers of France (37.4%), Argentina (30%) and Tehran (Iran) (31%), Pakistan (48%), Gaza Strip (41%), Norway (26.2%), and China (4.5%) (20-26). These inconsistencies can be attributed to different factors including sample size, cultural methods, and local prevention strategy. Also, the colonization rate was almost equal in all the wards (20-25). However, the highest nasal colonization belonged to the staff of the gynecologic surgery ward (33.33%). We also found a high level of colonization in the staff of the NICU and pediatric ward (45%) which could increase the risk of nosocomial infection in children who may act as a source of infection to adults. Similar results are reported, accordingly (11, 22, 26).

Besides, out of the five isolates analysed phylogenetically, four strains showed high similarity representing the possibility of cross-infection between these staff in the hospital. Accordingly, the nasal infection can be spread throughout the community and lead to high morbidity (27).

With regard to previous researches, the antibiotic resistance rate of nasal *S. aureus* varies in different regions of the world (19-22). We found high resistance to  $\beta$ -lactam and macrolide antibiotics including penicillin (83.3%), cefazolin (75%), cephalexin (75%), and erythromycin (58.3%) according to disc diffusion assay, which is in compliance with other studies (21, 28). Furthermore, we observed a low resistance rate to ciprofloxacin, and a lack of resistance to vancomycin recommended for therapeutic choices. The result is similar to a previous report (19).

The isolates were also surveyed for *mecA* gene. Oxacillin-susceptible *S. aureus* which are PCR-positive for *mecA* firstly termed OS-MRSA in 2007. Cefoxitin is a potent inducer of *mecA* and more sensitive for screening MRSA (29). However, it may not yet detect heterogeneous resistance to methicillin (30). So, oxacillin or cefoxitin-susceptible is named OS-MRSA (31). The causative mechanism of OS-MRSA is complicated due to roles of many factors involved in the resistance to methicillin-like antibiotics in MRSA. The *mecA* gene and the promoter sequence placed upstream of this gene are critical. Moreover, the *bla* transcriptional regulatory system in staphylococcal cassette chromosome *mec* (SCC*mec*) and the genes involved in cell wall metabolism (*fem*) are decisive for MRSA resistance (29, 32, 33). In this study, all *S. aureus* isolates were surprisingly susceptible to oxacillin and cefoxitin phenotypically as similar to Norway health care workers, but in contrast to the previous reports (23, 25, 28, 34). However, these strains were genotypically *mecA* positive and showed a high rate of OS-MRSA (75% of the positive samples). This phenomenon, a challenging topic in laboratory diagnosis, may cause a life-threatening infection. Since the infected patients are susceptible to oxacillin and cefoxitin, they may be subjected to treatment with  $\beta$ -lactams, leading to antibiotic resistance (10, 11). Previous investigations have shown low to moderate prevalence rates of OS-MRSA (1-36%) (35) that are in disagreement with our report. For example, in a prior paper on

strains of nasal *S. aureus* isolated from nurses of Ardabil, Iran, 1.15% were colonized with OS-MRSA (36). Also, 17.7% of nasal *S. aureus* isolated from patients and healthcare workers in Africa were *mecA* positive, and susceptible to oxacillin (37). In Brazil, the prevalence of OS-MRSA in patients was reported at 33.7% (38).

Furthermore, a limitation of our study is that the most prevalent genotype of OS-MRSA accorded to the classification of the staphylococcal cassette chromosome *mec* (*SCCmec*) should be detected to identify multidrug resistance in this area thoroughly.

### Conclusions

As such, our survey indicated a low frequency of nasal *S. aureus*, but the high rate of *mecA* positive gene among OS-MRSA, which can be considered as a remarkable concern. It also showed underscoring the requirement of genotypic assays along with phenotypic tests for precise identification and further treatment of MRSA. Finally, the high rate of OS-MRSA infection in our study warned that impressive control strategies should be strikingly administered to prevent  $\beta$ -lactams resistance and to inhibit the infection transmission in the hospital, especially in the surgery ward.

### Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of Fasa University of Medical Sciences (IR.FUMS.REC.1397.084) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Acknowledgements

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### Conflict of Interest

The authors declare there is no conflict of interest.

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