



# Molecular and Phenotypic Characterization of Methicillin-Resistant *Staphylococcus aureus* in Community and Hospital Acquired Infections in Bandar Abbas

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## ABSTRACT

**Backgrounds:** This study aimed to assess the molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from community-acquired (CA) and hospital-acquired (HA) infections in Bandar Abbas, southern Iran.

**Materials & Methods:** This descriptive cross-sectional study was conducted on 110 *S. aureus* strains isolated from 59 outpatients and 51 inpatients during 2018-2019. Antimicrobial susceptibility testing was performed using disc diffusion method. Epsilon meter test was used to measure vancomycin minimum inhibitory concentration (MIC). Cefoxitin disc (30 µg) was used to screen MRSA isolates. The presence of *mecA* gene was examined by PCR method. Staphylococcal cassette chromosome *mec* (SCCmec) types were detected in *S. aureus* isolates using multiplex-PCR. Chi-square and Fisher's exact tests were used to analyze the results.

**Findings:** Out of 110 isolates, 45 (40.9%) isolates carried the *mecA* gene: 20 (39.2%) isolates from inpatients and 25 (42.4%) isolates from outpatients. MRSA isolates showed the highest resistance to azithromycin (69.8%), tetracycline (60.4%), and clindamycin (32.1%), respectively. Vancomycin MIC against MRSA isolates ranged from 0.75 to 5 µg/mL. SCCmec type I, III, IV, and V were detected in 20 (44.4%), three (6.7%), 16 (35.5%), and six (13.3%) isolates, respectively.

**Conclusion:** The predominant SCCmec types were type I and type IV, which were detected in CA- and HA-MRSA isolates, respectively. No significant difference in the presence of SCCmec type III and antibiotic resistance was found between CA- and HA-MRSA isolates, indicating the possibility of cross-infection between these isolates. Developing appropriate treatment protocols to prevent the spread of MRSA infections in the community is currently an urgent need.

**Keywords:** *Staphylococcus aureus*, Methicillin resistance, Drug resistance, Iran, Penicillin-binding protein.

## CITATION LINKS

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## Introduction

*Staphylococcus aureus* is a Gram-positive, catalase-positive cocci found in clusters. Although *S. aureus* is a nonspore-forming organism, it is a predominant pathogen in hospital-acquired (HA) and community-acquired (CA) infections [1]. Methicillin resistant *S. aureus* (MRSA) has become a major health challenge due to carrying the staphylococcal cassette chromosome mec (SCCmec) elements and showing resistance to antibiotics, making it difficult to deal with infections caused by this microorganism [2]. SCCmec elements are composed of two major genetic complexes known as the *mec* and *ccr* gene complexes. The *mec* gene complex is composed of *mecA* and associated insertion sequences (*IS431*) and regulatory genes (*mecI* and *mecR1*), and the cassette chromosome recombinase (*ccr*) gene complex is composed of two recombinase genes (*ccrA* and *ccrB*) mediating site-specific integration and excision of SCCmec [3]. Since the emergence and spread of MRSA worldwide in the 1960s [4], health care centers have been widely exposed to infections caused by this bacterium; in addition, there have been increasing reports of MRSA infections in patients who have had no contact with hospital environments [5]. People at risk of developing community-acquired MRSA infections are populations with poor health indicators or specific populations such as athletes, injecting drug users, prisoners, homosexual men, and children in child care centers [6].

The acquisition of different SCCmec elements carrying genes responsible for resistance to beta-lactams, such as methicillin, macrolides, streptogramin, lincosamide, and tetracycline, could play an important role in the distribution of multidrug-resistant clones of this bacterium [4]. Given that MRSA is responsible for up to 60% of nosocomial infections in the ICU, broad-spectrum

antibiotics such as vancomycin are commonly used to treat them; however, several cases of vancomycin-resistant *Enterococcus* and *S. aureus* have been reported [7]. The global distribution of MRSA clones emphasizes the need for developing appropriate methods to identify the epidemiology of this bacterium. In addition to genotyping, identifying SCCmec types is an easy and interpretable method that could help differentiate between community-based and nosocomial infections caused by this bacterium [8]. The emergence of antibiotic-resistant strains, such as methicillin- and vancomycin-resistant *S. aureus* strains, challenges infection management and control strategies.

**Objectives:** The present study was conducted to determine the prevalence of SCCmec types I–V among MRSA isolates and evaluate vancomycin resistance in community- and hospital-acquired infections in Bandar Abbas, southern Iran.

## Materials and Methods

This descriptive cross-sectional study was performed from April 2018 to March 2018. Clinical samples were collected from patients admitted to Payambar-e-Azam hospital and patients referring to outpatient healthcare facilities in Bandar Abbas. The sampling method was the census of available samples of nosocomial and community-acquired infections. Infections that occur in patients within 48 hours of admission, three days after discharge, or 30 days after surgery are defined as hospital-acquired infections [7]. Infections diagnosed in people referring to outpatient facilities are considered as community-acquired infections [9].

**Identification of bacteria:** *S. aureus* isolates were confirmed by Gram staining and biochemical tests, including growth in blood agar medium, hemolysis test, catalase test, mannitol fermentation, as well as coagulase

and DNase tests.

**Evaluation of antibiotic resistance:**

Bacterial susceptibility to antibiotics was determined by disc diffusion method using the following antibiotic disks: azithromycin (15 mg), tigecycline (15 mg), gentamicin (10 mg), linezolid (30 mg), clindamycin (2 mg), ciprofloxacin (5 mg), and tetracycline (30 mg) (CLSI-2018-M100-S28).

**Screening for methicillin resistance:**

Resistance to cefoxitin disk (FOX, 30 µg) was evaluated by growth on Mueller–Hinton agar (Merck, Germany) according to the Clinical and Laboratory Standard Institute (CLSI) guidelines for MRSA strains detection (CLSI-2018-M100-S28).

**Determination of the minimum inhibitory concentration (MIC) of vancomycin in MRSA isolates:**

Epsilometer test (E-test) method was used to determine the minimum inhibitory concentration (MIC) of vancomycin in MRSA isolates. Vancomycin sensitive isolates were identified with MIC ≤ 2 µg/mL, intermediate isolates with MIC=4-8 µg/mL, and resistant isolates with MIC ≥

16 µg/mL, respectively.

**Preservation of isolates:** After collecting the bacterial isolates, these isolates had to be preserved for subsequent procedures. For this purpose, after mixing the pure colonies of the isolates in trypticase soy broth (TSB) culture medium containing 50% glycerol, they were stored in a freezer at -80 °C (-112 °F).

**DNA extraction:** DNA was extracted by tissue buffer method. About 20 µL of buffer containing 0.25% SDS (sodium dodecyl sulfate) and 0.05 M NaOH was dissolved in 200 mL of deionized water. The bacterial colony solution was poured into an Eppendorf. The Eppendorf was placed at 95 °C for 10 min and centrifuged at 13000 g for 1 min, and then to which 180 µL of deionized water containing buffer was added [10].

**MRSA screening for the presence of the *mecA* gene:** All 110 *S. aureus* strains isolated from nosocomial and community-acquired infections were examined for the presence of the *mecA* gene. PCR reactions were performed using a SensoQuest lab thermocycler according to the method

**Table 1)** List of primers used to identify SCCmec type I-IV genetic elements

| Name           | Primer Sequence (5'→3')     | Length | Target            | I | II | III | IV | V |
|----------------|-----------------------------|--------|-------------------|---|----|-----|----|---|
| <i>mecA</i> F  | GTAGAAATGACTGAACGTCCGATAA   | 310    | <i>mecA</i>       |   |    |     |    |   |
| <i>mecA</i> R  | CCAATTCCACATTGTTTCGGTCTAA   |        |                   |   |    |     |    |   |
| B              | ATTGCCTTGATAATAGCCYTCT      | 937    | <i>ccrA2-B</i>    |   | ×  |     | ×  |   |
| α3             | TAAAGGCATCAATGCACAAACACT    |        |                   |   |    |     |    |   |
| <i>ccrC</i> F  | CGTCTATTACAAGATGTTAAGGATAAT | 518    | <i>ccrC</i>       |   |    | ×   |    | × |
| <i>ccrC</i> R  | CCTTTATAGACTGGATTATTCAAATAT |        |                   |   |    |     |    |   |
| 1272F1         | GCCACTCATAACATATGGAA        | 415    | <i>IS1272</i>     | × |    |     | ×  |   |
| 1272R1         | CATCCGAGTGAAACCCAAA         |        |                   |   |    |     |    |   |
| 5R <i>mecA</i> | TATACCAAACCCGACAACACTAC     | 359    | <i>mecA-IS431</i> |   |    |     |    | × |
| 5R431          | CGGCTACAGTGATAACATCC        |        |                   |   |    |     |    |   |

**Table 2)** Antibiotic resistance patterns of HA-MRSA and CA-MRSA isolates

|                      | MSSA<br>65(59%) |           | MRSA<br>45(41%) |           | Total<br>110 |
|----------------------|-----------------|-----------|-----------------|-----------|--------------|
|                      | HA31(48%)       | CA34(52%) | HA20(44%)       | CA25(55%) |              |
| <b>Cefoxitin</b>     | 4(13%)          | 4(12%)    | 19(95%)         | 25(100%)  | 52(47%)      |
| <b>Tigecycline</b>   | 4(13%)          | 2(6%)     | 2(10%)          | 1(4%)     | 9(8%)        |
| <b>Tetracycline</b>  | 11(35%)         | 17(50%)   | 7(35%)          | 19(76%)   | 54(49%)      |
| <b>Gentamicin</b>    | 2(6%)           | 2(6%)     | 2(10%)          | 4(16%)    | 10(9%)       |
| <b>Azithromycin</b>  | 13(42%)         | 25(73%)   | 11(55%)         | 19(76%)   | 68(62%)      |
| <b>Clindamycin</b>   | 4(13%)          | 6(18%)    | 7(35%)          | 9(36%)    | 26(24%)      |
| <b>Linezolid</b>     | 7(23%)          | 0(0%)     | 1(5%)           | 0(0%)     | 8(7%)        |
| <b>Ciprofloxacin</b> | 3(10%)          | 2(6%)     | 2(10%)          | 2(8%)     | 9(8%)        |

**Table 3)** Demographic characteristics of patients and frequency distribution of various genotypes in HA-MRSA and CA-MRSA isolates

|                          | HA-MRSA<br>20(39.2%) | CA-MRSA<br>25(42.4%) | Total MRSA<br>45(40.9%) | P-Value                    |
|--------------------------|----------------------|----------------------|-------------------------|----------------------------|
| <b>Age</b>               |                      |                      |                         |                            |
| ≤ 50 years old           | 13(65%)              | 21(84%)              | 34(75.5%)               | .1406                      |
| > 50 years old           | 7(35%)               | 4(16%)               | 11(24.4%)               |                            |
| <b>Sex</b>               |                      |                      |                         |                            |
| Male                     | 13(65%)              | 6(24%)               | 19(42.2%)               | HA-MRSA=0.9<br>CA-MRSA=0.4 |
| Female                   | 7(35%)               | 19(76%)              | 26(57.8%)               |                            |
| <b>Source</b>            |                      |                      |                         |                            |
| Blood                    | 3(15%)               | 0(0%)                | 3(6.7%)                 | <.001                      |
| Wound & abscess          | 10(50%)              | 0(0%)                | 10(22.2%)               |                            |
| Tracheal tube discharge  | 6(30%)               | 0(0%)                | 6(13.3%)                |                            |
| Urine                    | 1(5%)                | 25(100%)             | 26(57.8%)               |                            |
| <b>MIC of Vancomycin</b> |                      |                      |                         |                            |
| MIC of vancomycin        | 0.75-5 µg/mL         | 1-4 µg/mL            | 0.75-5 µg/mL            |                            |
| <b>Scmec Type</b>        |                      |                      |                         |                            |
| Scmec type I             | 5(25%)               | 15(60%)              | 20(44%)                 | .02                        |
| Scmec type II            | 0(0%)                | 0(0%)                | 0(0%)                   | <.00                       |
| Scmec type III           | 3(15%)               | 0(0%)                | 3(7%)                   | .06                        |
| Scmec IV                 | 12(60%)              | 4(16%)               | 16(35%)                 | .002                       |
| Scmec V                  | 0(0%)                | 6(24%)               | 6(13%)                  | .02                        |

described by Chongtrakool et al. (2018) [10]. Gene-specific primers were used to identify the *mecA* gene.

**SCCmec typing:** Different SCCmec types were studied by multiplex-PCR assay using specific primers for SCCmec types I-V. The primers used to identify SCCmec type I-IV genetic elements are listed in Table 1.

**Statistical analysis:** Data were analyzed using SPSS software by employing Fisher's exact test to study the association between variables. A *p*-value of < .05 was considered as significant.

### Findings

A total of 110 *S. aureus* strains isolated from 51 (46%) inpatients and 59 (54%) outpatients were assessed. Among the patients, 44 (40%) cases were male, and 66 (60%) cases were female. The mean age of the patients was 35 ±2 years, ranging from a minimum of one year to a maximum of 84 years. The highest frequency of both hospital- and community-acquired infections was observed in the age group of 21 to 60 years. Hospital-acquired *S. aureus* isolates were collected from different hospital wards and clinical specimens as follows: 17 (33.3%) isolates from tracheal samples, 15 (29%) isolates from wound samples, 11 (22 %) isolates from blood samples, three (6%) isolates from urine samples, two (4%) isolates from bronchoalveolar lavage (BAL) samples, one (2%) isolate from chaldean samples, one (2%) isolate from secretion samples, and one (2%) isolate from cerebrospinal fluid samples. All community-acquired *S. aureus* isolates were collected from urine samples of outpatients.

**Antibiotic resistance pattern:** Among 110 *S. aureus* isolates, the highest resistance was observed to azithromycin (68, 62%) and tetracycline (50, 49%), and the highest sensitivity was observed to linezolid (92, 84%), tigecycline (91, 82%), and

ciprofloxacin (91, 82%), respectively.

None of the community-acquired *S. aureus* isolates were resistant to linezolid. Antibiotic resistance patterns of HA-MRSA and CA-MRSA isolates are shown in Table 2.

### Determination of vancomycin MIC:

Vancomycin MIC was measured in 45 MRSA isolates carrying *mecA* gene using E. test method. The MIC of vancomycin was determined to be 2.27±1.08 µg/mL. The MIC range was from 0.75 to 5 µg/mL. Mean and exponential values were calculated as 2 and 1.5 µg/mL, respectively. According to the results, 37 (82%) isolates were vancomycin sensitive (MIC less than 2 µg/mL), and eight (18%) isolates showed intermediate resistance (MIC between 4 and 8 µg/mL). None of the isolates were identified as vancomycin resistant.

**Screening for methicillin resistance:** In this study, 52 (47%) *S. aureus* isolates were resistant to ceftioxin, of which 45 isolates carrying the *mecA* gene were confirmed as MRSA. Therefore, the prevalence of MRSA isolates was 41%, of which 20 (44%) and 25 (56%) isolates were HA-MRSA and CA-MRSA, respectively.

### SCCmec genotyping of MRSA isolates:

Among 45 MRSA isolates carrying the *mecA* gene, the predominant SCCmec type was type I (20, 44%), followed by type IV (16, 35%), type V (6, 13%), and type III (3, 7%). The frequency distribution of SCCmec types in HA-MRSA and CA-MRSA isolates is summarized in Table 3.

### Discussion

In the present study, antibiotic resistance patterns and SCCmec genotypes of HA-MRSA and CA-MRSA isolates were investigated. According to the present study results, 45 (41%) isolates carried the *mecA* gene, this finding is relatively lower than those reported in other studies in Iran, including 59% [11], 61% [12], and 79% in burn ward patients [13]. The prevalence of MRSA strains

in various studies has been reported to vary from 0.6% in the Netherlands to 66.8% in Japan [4].

In this study, 20 (39.2%) isolates were HA-MRSA, which is consistent with European studies results (49%) [14]. In a study by Zeinali et al. (2011), 39% of *S. aureus* isolates were HA-MRSA, and 61% were CA-MRSA, which are consistent with the present study results [15].

Some studies have found a relationship between gender and the prevalence of CA-MRSA [16]. In the present study, there was no significant relationship between the frequency of MRSA isolates and patients' gender. The present study showed that out of the 20 HA-MRSA isolates, nine (45%) and seven (35%) strains were isolated from clinical wound and tracheal aspirate samples. The former is consistent with the role of MRSA in causing wound infection in hospitalized patients. In a study by Nikbakht et al. (2017), 40% of MRSA isolates were isolated from wound samples [17]. Since multiple antibiotic and heavy-metal resistance genes could be included in SCCmec [5], as expected, the antibiotic resistance patterns of MRSA and MSSA isolates in our study were different. However, this difference in resistance patterns was not significant except for gentamicin, to which MRSA isolates showed higher resistance. Type IV SCCmec does not carry many antibiotic resistance genes; therefore, strains carrying SCCmec type IV are usually sensitive to antibiotics other than beta-lactams. On the other hand, HA-MRSA strains usually carry large SCCmec types, such as type I, II, or III, and a variety of antibiotic resistance genes. Therefore, HA-MRSA isolates are resistant not only to beta-lactams but also to different groups of antibiotics [18].

High resistance of MRSA isolates to antibiotics azithromycin (30, 67%) and tetracycline (26, 58%) has also been reported

in other studies in Iran [17, 19]. In this study, MRSA isolates showed the lowest resistance to linezolid (1, 2%). There was no evidence of linezolid resistance in CA isolates, consistent with the results of other studies in Iran [17, 19, 20]. Furthermore, initial empirical treatment with vancomycin, cotrimoxazole, or clindamycin is recommended in cases of high prevalence of CA-MRSA [6]. In the present study, the minimum growth inhibitory concentration of vancomycin was measured in MRSA strains. In line with previous studies, eight (18%) strains were identified as VISA (vancomycin intermediate resistant *S. aureus*). Since vancomycin treatment for MRSA also has its own limitations, including the emergence of VISA strains and treatment failure in endocarditis and bacteremia cases, attempts have been made to introduce new antibiotics [22, 23]. According to previous studies, one of these antibiotics is a broad-spectrum antibiotic called tigecycline [21, 22], which has been shown to be highly active against CA-MRSA (96%) and HA-MRSA (90%) isolates.

The present study results showed that among 45 MRSA isolates, the predominant SCCmec type was type I (20, 44%), followed by type IV (16, 35%), which were related to CA-MRSA and HA-MRSA isolates, respectively. While SCCmec type IV is generally carried by CA-MRSA, its relatively small size leads to its widespread dissemination in community and hospital settings [18]. The distribution of SCCmec elements varies in different regions and changes over time. An epidemiological study on HA-MRSA isolates in Japan showed a high prevalence of SCCmec type II, followed by SCCmec type IV [23]. Furthermore, studies have shown that most CA-MRSA isolates carry SCCmec types IV [24] and V [25]. In line with the present study results, Ebadi and Khaliliazad (2017) in their study in Larestan reported that type I (32.1%), type IV (28.6%), type V (14.3%), and type III (7.1%)

were the most predominant SCCmec types, respectively [26].

However, in another study by Havaei et al. (2017), CA-MRSA isolates harbored SCCmec type IV, while HA-MRSA isolates harbored type I (22.2%) and type III (2.2%) [27].

Fassihi et al. (2017) also examined SCCmec typing in HA- and CA-MRSA isolates in Kerman. Consistent with the present study results, type IV was more prevalent in strains isolated from hospitals [28].

In another study in 2016, the most frequent SCCmec type among HA-MRSA isolates was SCCmec type III (77%) [11]. Goudarzi et al. (2018) detected four different SCCmec types among MRSA isolates, including type III (38.9%), type II (31.1%), type IV (28.9%), and type I (1.1%). The most prevalent SCCmec type in HA-MRSA was type III (35, 38.9%) [12]. In another study in Denmark, SCCmec type IV was the most prevalent SCCmec type among both HA and CA MRSA isolates [8]. San Sit et al. (2017) showed that 59% of MRSA strains were HA-MRSA that carried SCCmec type II, III, IV, and V, while 31% were CA-MRSA strains carrying SCCmec III, IV, and V [29].

## Conclusion

The high prevalence of MRSA isolates highlights the need for developing rapid methods with high sensitivity to distinguish MRSA from MSSA. In this study, there was a significant difference in the distribution of SCCmec types between CA-MRSA and HA-MRSA isolates, except for SCCmec type III. However, there was no significant difference between the two groups of isolates in terms of antibiotic resistance, which could indicate the possibility of cross-infection between these isolates. Monitoring this possibility requires the use of reliable typing methods. Finally, given the reported sensitivity of MRSA isolates to linezolid and tigecycline, the design of clinical trial studies to

evaluate the therapeutic efficacy of these new antibiotics seems necessary.

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**Authors' contribution:** Conceptualization: AK; data curation: TD, AK; formal analysis: TD, AK; funding acquisition: AK; investigation: TD, AK; methodology: AK, TD; project administration: TD, AK; supervision: AK; writing of the original draft: TD, AK; writing-review and editing: TD, AK.

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