Clonal dissemination of *Staphylococcus aureus* isolates causing nosocomial infections, Tehran, Iran

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**ABSTRACT**

**Objectives:** In the current research, the prevalence of *Staphylococcus aureus* clones and genes encoding antimicrobial resistance and toxins were examined among 120 *S. aureus* strains from nosocomial infections in Tehran, Iran.

**Materials and Methods:** Antimicrobial susceptibility was examined, based on disk diffusion and PCR method to identify resistance and toxin-encoding genes. Based on the polymorphisms in SCCmec, agr, spa, and MLST, the isolates were typed.

**Results:** Among 120 *S. aureus* isolates, 85 (70.8%) were methicillin resistant *S. aureus* (MRSA), and 35 (29.2%) were methicillin sensitive *S. aureus* (MSSA). The tested isolates contained resistance genes, including *ant(4')-Ia* (90%), *aac(6')-Ileaph(2')* (90%), *aph(3')-Ia* (30%), *erm(A)* (26.7%), *erm(B)* (10.8%), *erm(C)* (11.7%), *msr(A)* (40.8%), *msr(B)* (14.2%), *tet(M)* (45.8%), and *mupA* (8.3%). The MRSA strains were clustered into six different clones. The most common genotypes included ST239-SCCmec III/t037 (23.3%), ST239-SCCmec III/t388 (22.5%), ST22-SCCmec IV/t790 (8.3%), ST15-SCCmec IV/t1084 (7.5%), ST585-SCCmec III/t173 (5%), and ST239-SCCmec III/t124 (4%), respectively. ST182/t196 (8.3%) and ST123/t171 (5%) belonged exclusively to MSSA strains. Overall, 10 (66.7%) and 5 (33.3%) out of 15 isolates with pvl genes were attributed to clones ST22-SCCmec IV/t790 and ST15-SCCmec IV/t1084, respectively. ST22-SCCmec IV/t790, ST239-SCCmec III/t037, and ST15-SCCmec IV/t1084, were related to high-level mupirocin-resistant phenotypes.

**Conclusion:** The genetic diversity of *S. aureus* was confirmed in our hospitals, and ST239-SCCmec III/t037 showed a relatively high prevalence in our study. It seems that a assessment of resistance and virulence genes in different *S. aureus* molecular types is necessary for proper antibiotic consumption.

**Introduction**

*Staphylococcus aureus* is characterized as a common nosocomial pathogen, responsible for various diseases, such as food poisoning, osteomyelitis, wound infections, and even fatal conditions, such as endocarditis (1). Over the past few decades, it has been well-documented that the pathogen's resistance potential to antimicrobial agents, especially methicillin, may lead to its persistence in the hospital and community (2). In 1961, the first case of methicillin-resistant *S. aureus* (MRSA) occurred in the UK (3). The prevalence of this infection has steadily increased since then, as confirmed in several studies, raising major concerns about the global increase in its prevalence, as well as its associated mortality and morbidity in the healthcare setting, especially intensive care units (ICUs) due to MRSA infections (1-3).

Resistance to methicillin is attributed to β-lactamase expression or changes in the structure of mecA gene-encoded penicillin-binding protein-2. Generally, mecA gene (21-67 kbp) is recognized as a staphylococcal cassette chromosome mec (SCCmec). Generally, SCCmec is categorized into 11 types with respect to mec genes and ccr gene complexes (4). Identification of SCCmec type among *S. aureus* clinical isolates can be useful in molecular typing of MRSA strains (5).

Based on previous findings, SCCmec I-III and IV-V are respectively responsible for the most common hospital-acquired and community-acquired MRSA (HA-MRSA and CA-MRSA, respectively) infections. HA- and CA-MRSA strains can be distinguished with respect to some genotypic, phenotypic, and epidemiological characteristics, as well as virulence factors (4, 5).

The emergence and prevalence of MRSA infections containing multidrug-resistant (MDR) genes have significantly limited the availability of antibiotics over the past decades. In addition, the growing emergence of MDR-MRSA strains poses a major global health concern (1). Wide resistance to β-lactams, besides other antibiotics, including aminoglycosides, lincosamides, and macrolides, has been shown in MRSA strains (6).

Aminoglycosides play a key role in serious anti-staphylococcal therapies. According to the previous researches, resistance to aminoglycosides is attributed...
to aminoglycoside-modifying enzymes (AMEs) including aminoglycoside nucleotidyltransferases, aminoglycoside phosphotransferases and aminoglycoside acetyltransferases (7). mupA and mupB genes are responsible for resistance to mupirocin which is used to treat various types of skin diseases caused by S. aureus (8). Resistance to macrolides as protein synthesis inhibitors is mediated by msr genes activating efflux pumps and erm genes modifying the ribosomal binding site (9). Thus, in spite of new antibiotics introduction, the emergence and dissemination of antibiotic resistance genes, MRSA infections treatment is still a great dilemma worldwide.

This study was conducted to identify antibiotic resistance patterns and the carriage of resistance and virulence genes as well as major MRSA clones by MLST, spa, SCCmec and agr techniques in clinical samples taken from patients in Tehran, Iran.

Materials and Methods

Sampling, MRSA isolation and antibacterial susceptibility testing

This cross-sectional study included 368 clinical samples from wound, blood, and urine specimens during April-December 2016. Ethics Committee of Shahid Beheshti University of Medical Sciences approved the implementation of this study (IR.ESBSMU. SM.REC.1395.157). All patients signed written informed consent forms.

After the rapid transfer of the specimens to the laboratory, S. aureus identification was performed, based on the conventional biochemical tests. S. aureus identification was confirmed based on the PCR assay for nacI gene (10). According to the Clinical and Laboratory Standards Institute (CLSI) standards, resistance to methicillin was examined with oxacillin and ceftoxitin (1 and 30 µg, respectively) disks in Mueller-Hinton agar plates (Merck; Germany) containing 4% sodium chloride (11). For further molecular analysis, confirmed isolates were stored in Tryptic Soy Broth with 15% glycerol.

Table 1. Primer and oligo sequences used in present research

| Gene     | Primer            | Primer sequence (5´→3´) | Length (bp) | Reference |
|----------|-------------------|-------------------------|-------------|-----------|
| meA      | F                 | AGA TAA TGG TAT GTC GAA ATT AG | 398 (10)    |
| R        | A TG TAT GTC GAA TGG TAT TGC |               |
| tst-1    | F                 | TTA TCG TAA GGC CTT TGT TG | 398 (10)    |
| R        | TAA AGG TAC TTT TAT TGG AGT AAG |               |
| nucle     | F                 | GCG ATT GAT GGT GAT CTT GGT | 270 (12)    |
| R        | AGC CAA GCC TTT AGC AGG AAG TAA AGC |               |
| luk-PV    | F                 | TTC ACT ATT TGT AAG AGT GCC AGC ACT | 180 (12)    |
| R        | TAC TAA TGA ATT TTT TTA TGG TAA GGC CTT TGT AG |               |
| etb       | F                 | AGA AGC AAA AGA ATA CAG CG | 226 (13)    |
| R        | GYT TTT GCC TGC TTT TTC TGT |               |
| eta       | F                 | GCA GGT GAT GAT TTA GCA TT | 93 (13)     |
| R        | AGA TCT CTT CTT TGG TGC |               |
| tet(M)    | F                 | AGT GGA GGC ATT ACA GAA | 158 (13)    |
| R        | CAT ATC TCG TGC LCT GCT GTA |               |
| aph(3’)-IIIa | F         | CTT GAT GCA AAA ATG CCC GCG | 269 (14)    |
| R        | TCA TAC TCT TCT GAC CAA A |               |
| ant(4’)-la | F                 | AAT CGG TAG AAG CCC GA A | 135 (14)    |
| R        | GCA GTC GCC ATT GCT A |               |
| aac(6’)-le/aph(2) | F       | CCA AGA GCA AAT AGG GCA TAC C | 222 (14)    |
| R        | CAC ACT ATC AAG ACC ACT |               |
| erm(B)    | F                 | CTA TCT GAT TGT TGA AGA AGC ATT | 141 (15)    |
| R        | GTC TAC TCT TGG TTT AGC ATC AAM |               |
| erm(A)    | F                 | CTA TCT TCT TGG ATC AGG GTA | 139 (15)    |
| R        | CTA CAC TCT GGT CAT GAA A |               |
| mcr(B)    | F                 | CTH CAT ATC CAT AAT AAT TAT CCA ATC | 595 (16)    |
| R        | AAG TTA TAT GAA TAG ATG GGT CTT TTT |               |
| mcr(A)    | F                 | GCC ACA ATA AGA GGT TTT AAA GG | 940 (16)    |
| R        | AAG TTA TAT GAA TAG ATG GGT CTT TTT |               |
| mupA      | F                 | CCA TGG AGC ACT ATG GCA | 1158 (17)   |
| R        | CCA TGG AGC ACT ATG GCA |               |

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SCCmec typing, multiplex PCR amplification was performed with specific primers (4). The controls comprised of the MRSA strains, i.e., ATCC 10442, N315, 85/2082, MW2, and WIS (attributed to types I, II, III, IV, and V, respectively).

**Multiplex PCR amplification for agr typing**

In addition, for agr typing, multiplex PCR amplification was carried out, using forward (Pan) and reverse (agr1 to agr4) primers for the amplification was carried out, using forward (Pan) and reverse (agr1 to agr4) primers for the agr typing, multiplex PCR amplification for agr typing and V, respectively).

85/2082, MW2, and WIS (attributed to types I, II, III, IV, and V, respectively). After spa typing groups as agr reverse (agr1 to agr4) primers for the agr groups as agr reverse (agr1 to agr4) primers for the agr types, the Ridom SpaServer database was searched. The specific oligonucleotide primers are listed in Table 1.

**spa typing**

On the other hand, spa gene was detected according to a study by Harmsen and colleagues (19). After the positive spa PCR products were purified, DNA sequencing was carried out in both strands (Macrogen; South Korea). Chromas 1.45 (Australia) was used to edit the sequences. To assign the sequences to specific spa types, the Ridom SpaServer database was searched.

**MLST technique**

Via amplification and sequencing, MLST was carried out on S. aureus isolates. The internal fragments of housekeeping genes were used to identify the allelic profiles; these genes included gmk, arcC, aroE, glpF, pta, yqiL, and tpi. The isolate was assigned a sequence type (ST) after comparing the sequences with the S. aureus MLST database.

**Results**

**Sampling and antibiotic susceptibility**

In this study, out of 368 samples obtained from various clinical specimens, 120 isolates (83 (69.2%) obtained from men and 37 (30.8%) from women) were identified as S. aureus. These isolates originated from wound (60%), blood (20.8%) and urinary tract infections (19.2%). Of the 120 S. aureus clinical isolates obtained from the hospitalized patients, 85 (70.8%) were MRSA and 35 (29.2%) were methicillin susceptible S. aureus (MSSA).

None of the isolates were susceptible to all of the antimicrobial agents tested regarding in vitro antimicrobial susceptibility tests. All isolates were susceptible to vancomycin, among which 55 (45.8%), 48 (40%) and 17 (14.2%) isolates had a MIC of 0.5, 1 and 2 μg/ml, respectively.

Among the 35 MSSAs, no mupirocin resistance was detected, whereas 30 MRSA isolates (35.3%) were mupirocin resistant. Of these mupirocin resistant isolates, 14 (46.7%) and 16 (53.3%) had high and low resistance levels, respectively. All the high-level mupirocin-resistant (HLMUPR) isolates were collected from wound samples. Patients diagnosed with mupirocin-susceptible and -resistant MRSA infections were not significantly different in terms of age and gender (P= 0.145 and 0.128, respectively). The frequency of resistance for MRSA and MSSA isolates to different antibacterial agents are presented in Table 2.

Of the 120 S. aureus isolates, 87.5% (105/120) were defined as MDR. The predominant multiple drug resistance profile among the MDR isolates were resistance to 9 and 7 antibiotics found in 75 (62.5%) and 12 (10%) isolates, respectively. Distribution of resistance profile and different clinical sample in S. aureus isolated from nosocomial infections are presented in Figure 1.

**The distribution of resistance genes**

In addition, the frequency of antibiotic resistance genes was measured. The genes included ant(4′)-Ia (90%), aac(6′)-le-aph(2′) (80%), aph(3′)-IIIa (30%), erm(A) (26.7%), erm(B) (10.8%), erm(C) (11.7%), msr(A) (40.8%), msr(B) (14.2%), tet(M) (45.8%), and mupA (8.3%) (Figure 2). The MRSA strains contained ant(4′)-Ia, aac(6′)-le-aph(2′), and meca genes. Other

![Figure 1. Resistance pattern of *Staphylococcus aureus* obtained from clinical samples](image)

**Table 2.** The frequency of antimicrobial resistance in 120 *Staphylococcus aureus* isolates obtained from clinical specimens

| Antibiotics          | MSSA (n=35) n (%) | MSSA (n=85) n (%) | All (n=120) n (%) |
|----------------------|-------------------|-------------------|------------------|
| penicillin           | S                  | R                  | S                | R                | S                  | R                  |
| 2 (5.7)              | 33 (94.3)          | 6 (7.1)            | 79 (92.9)        | 10 (6.7)         | 112 (93.3)         |
| kanamycin            | 17 (48.6)          | 18 (51.4)          | 10 (11.8)        | 75 (88.2)        | 27 (22.5)          |
| gentamicin           | 21 (60)            | 14 (40)            | 9 (10.6)         | 76 (89.4)        | 30 (25)            |
| ciprofloxacin        | 23 (65.7)          | 12 (34.3)          | 12 (14.1)        | 73 (85.9)        | 35 (29.2)          |
| tetracycline         | 21 (60)            | 14 (40)            | 15 (17.6)        | 70 (82.4)        | 36 (30)            |
| amikacin             | 27 (77.1)          | 8 (22.9)           | 11 (12.9)        | 74 (87.1)        | 38 (31.7)          |
| tobramycin           | 29 (82.9)          | 6 (17.1)           | 20 (23.5)        | 65 (76.5)        | 49 (40.8)          |
| erythromycin         | 29 (82.9)          | 6 (17.1)           | 21 (24.7)        | 64 (75.3)        | 50 (41.7)          |
| clindamycin          | 30 (85.7)          | 5 (14.3)           | 32 (37.6)        | 53 (62.4)        | 62 (51.7)          |
| ceftriaxone          | 30 (85.7)          | 5 (14.3)           | 35 (41.2)        | 50 (58.8)        | 65 (54.2)          |
| trimethoprim-sulphamethoxazole | 33 (94.3) | 2 (5.7)   | 53 (62.4)        | 32 (37.6)        | 86 (71.7)          |
| mupirocin            | 35 (100)           | 0 (0)              | 55 (64.7)        | 30 (35.3)        | 90 (75)            |
| quinupristin-dalfopristin | 31 (88.6) | 4 (11.4) | 74 (87.1)        | 11 (12.9)        | 105 (87.5)         |
| linzolid             | 35 (100)           | 0 (0)              | 85 (100)         | 0 (0)            | 120 (100)          |
| teicoplanin          | 35 (100)           | 0 (0)              | 85 (100)         | 0 (0)            | 120 (100)          |
| vancomycin           | 35 (100)           | 0 (0)              | 85 (100)         | 0 (0)            | 120 (100)          |

![Image](image)
detected antibiotic resistance genes included tet(M) (64.7%), msr(A) (57.6%), aph(3’)-Ile encoding gene, Lane 6 PCR product of erm(A) encoding gene, Lane 7 PCR product of msr(B) encoding gene, Lane 8 PCR product of mupA encoding gene, and Lane 9 PCR product of tet(M) encoding gene. B) Lane M, DNA Ladder; lane 1 negative control, Lane 2 the 323 bp PCR product of agr type III, lane 3 the 575 bp PCR product of agr type II, lane 4 the 441 bp PCR product of agr type I, lane 5 the 518 bp PCR product of SCCmec Type IV. C) Lane M, DNA ladder; lane 1-5, variable PCR product of spa. D) Lane M, DNA Ladder; Lane 1 the 270 bp PCR product of nucA gene, Lane 2 the 93 bp PCR product of eta gene, Lane 3 the 226 bp PCR product of etb gene, Lane 4 the 398 bp PCR product of ogtf-1 gene, Lane 5 the 180 bp PCR product of luk-PV gene, Lane 6 the 583 bp PCR product of mecA gene.

Figure 2. A) Lane M, 100-bp DNA Ladder (Fermentas, UK); Lane 1 PCR product of aac(6’)-le/aph(2’) encoding gene, Lane 2 PCR product of aac(6’)le/aph(2’) encoding gene, Lane 3 PCR product of erm(A) encoding gene, Lane 4 PCR product of erm(B) encoding gene, Lane 5 PCR product of erm(C) encoding gene, Lane 6 PCR product of msr(A) encoding gene, Lane 7 PCR product of msr(B) encoding gene, Lane 8 PCR product of mupA encoding gene, and Lane 9 PCR product of tet(M) encoding gene. B) Lane M, DNA Ladder; lane 1 negative control, Lane 2 the 323 bp PCR product of agr type III, lane 3 the 575 bp PCR product of agr type II, lane 4 the 441 bp PCR product of agr type I, lane 5 the 518 bp PCR product of SCCmec Type IV. C) Lane M, DNA ladder; lane 1-5, variable PCR product of spa. D) Lane M, DNA Ladder; Lane 1 the 270 bp PCR product of nucA gene, Lane 2 the 93 bp PCR product of eta gene, Lane 3 the 226 bp PCR product of etb gene, Lane 4 the 398 bp PCR product of ogtf-1 gene, Lane 5 the 180 bp PCR product of luk-PV gene, Lane 6 the 583 bp PCR product of mecA gene.

Distribution of SCCmec types
According to SCCmec typing, 66 (77.6%) and 19 (22.4%) MRSA isolates contained SCCmec types III and IV, respectively. No isolate harbored SCCmec type V, II, or I. Based on the multiplex PCR, isolates positive for PVL were attributed to SCCmec type IV, while tst gene was found in MRSA isolates from SCCmec III and IV. The HA-MRSA origin was emphasized by the presence of SCCmec type III.

Frequency of agr types
agr typing indicated that type I was the predominant agr type present in 91 isolates (75.8%), followed by type III which was present in 20 isolates (16.7%). agr type II was detected in 9 isolates (7.5%). Among 35 MSSA isolates, 20 and 15 harboured agr types I and III respectively (Figure 2).

spa typing
spa typing was performed for all S. aureus isolates.
Table 3. Distribution of MRSA molecular types isolated from nosocomial infections

| Molecular types | spa class | Type of toxin | Genotypic resistance patterns (No.%) | Phenotypic resistance patterns (No.%) | No (%) |
|----------------|-----------|---------------|-------------------------------------|--------------------------------------|-------|
| ST239-SCCmecIII/1037 | I | mecA (28;100), aph(3')-Ia (20;71.4), mupA (28;60), tet(M) (28;60) | PG, GM, K, CIP, AK, T, TN, E, CD (18;64.3) | 55 (28.3) |
| | I | mecA (27;100), aph(3')-Ia (10;37), erm(A) (12;44.4), ermA (8;29.6), mup(A) (12;44.4), aac(6')-Ib-aph(3') (27;100), mup(A) (12;44.4), tet(M) (27;100), tet(M) (12;44.4) | PG, GM, K, CIP, AK, T, CRO, MUP, TS (8;28.6) | 53 (28.2) |
| ST239-SCCmecIII/1388 | I | mecA (27;100), aph(3')-Ia (20;71.4), mupA (28;60), tet(M) (28;60) | PG, GM, K, CIP, AK, T, TN, E (20;71.4) | 52 (27.7) |
| ST239-SCCmecIII/1037 | I | mecA (28;100), aph(3')-Ia (20;71.4), mupA (28;60), tet(M) (28;60) | PG, GM, K, CIP, AK, T, TN, E, CD (18;64.3) | 55 (28.3) |
| ST239-SCCmecIII/1388 | I | mecA (27;100), aph(3')-Ia (10;37), erm(A) (12;44.4), ermA (8;29.6), mup(A) (12;44.4), aac(6')-Ib-aph(3') (27;100), mup(A) (12;44.4), tet(M) (27;100), tet(M) (12;44.4) | PG, GM, K, CIP, AK, T, CRO, MUP, TS (8;28.6) | 53 (28.2) |
| ST239-SCCmecIII/1037 | I | mecA (28;100), aph(3')-Ia (20;71.4), mupA (28;60), tet(M) (28;60) | PG, GM, K, CIP, AK, T, TN, E (20;71.4) | 52 (27.7) |

Figure 3. Distribution of spa types in methicillin resistant S. aureus (MRSA) and methicillin sensitive S. aureus (MSSA) strains isolated from nosocomial infections

spa typing discriminated eight different types: t037 (23.3%), t388 (22.5%), t713 (16.8%), t924 (8.3%), t790 (8.3%), t196 (8.3%), t084 (7.5%) and t171 (5%) (Figure 2). All the spa types except t196 and t171 were found in MRSA strains. Distribution of spa types among methicillin resistance and methicillin sensitive strains are presented in Figure 3. The most prevalent spa type among MSSA strains was t713 (11.7%, 14/120), followed by t196 (8.3, 10/120), t171 (5%, 6/120) and t924 (4.2%, 5/120), respectively.

MLST

Apparently, 120 isolates belonged to six different STs including ST239 (65 strains), ST22 (10 strains), ST182 (10 strains), ST15 (9 strains), ST585 (20 strains) and ST123 (6 strains). ST239, ST585, ST182 and ST123 were found in MSSA strains. It should be noted that of these STs, ST182 and ST123 belonged exclusively to MSSA strains. In conclusion, MRSA strains are clustered into six different groups. ST239-SCCmec III/t037 was found to be the most prominent MRSA clone identified in this study. Distribution of MRSA clones isolated from nosocomial infections are presented in Figure 4.

Fifteen PVL-carrying strains in our study belonged to ST239-SCCmec III/1037 (15 isolates, 80.3%) and ST239-SCCmec III/1388 (10 isolates, 55.6%) clones. Among the isolates under study, 58 (48.3%) isolates harboring tst-I were distributed in ST239-SCCmec III/t037 (15 isolates, 25.9%), ST239-SCCmec III/t388 (11 isolates, 19%), ST15-SCCmec IV/t084 (8 isolates, 13.3%), ST585-SCCmec III/t171 (6 isolates, 10.3%), ST239-SCCmec III/t924 (4 isolates, 8.6%), and ST22-SCCmec IV/t790 (3 isolates, 5.2%) clones. Among examined isolates, nine isolates (7.5%) were found to carry the eta gene. The eta positive isolates were distributed in ST15-SCCmec IV/t084 (4 isolates, 44.4%), ST22-SCCmec IV/t790 (3 isolates, 33.3%) and ST239-SCCmec III/t037 (2 isolates, 22.2%) clones. The etb gene was detected in ST585-
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SCCmec III/t713 (66.7%, 2/3) and ST22-SCCmec IV/ t790 (33.3%, 1/3) clones. MRSA clones resistance profile varied. Resistance to mupirocin was detected in all the MRSA clones with the exception of ST85-SCCmec III/t713 clone. Interestingly, mupirocin resistant MSSA isolates belonged to ST182/spa type t196. HLMUPR-MRSA strains were detected in ST22-SCCmec IV/t790 (42.8%, 6/14), ST15-SCCmec IV/t084 (28.6%, 4/14) and ST239-SCCmec III/t037 (28.6%, 4/14) clones.

cMLSB phenotype was detected in ST239-SCCmec III/ t037 (43.5%, 20/46), ST239-SCCmec III/t388 (43.5%, 20/46), ST85-SCCmec III/t713 (4.3%, 2/46), ST15- SCCmec IV/t084 (4.3%, 2/46), ST239-SCCmec III/t924 (2.2%, 1/46) and ST22-SCCmec IV/t790 (2.2%, 1/46) while iMLS phenotype distributed among 3 major clones ST239-SCCmec III/t388 (55.6%, 5/9), ST22- SCCmec IV/t790 (33.3%, 3/9) and ST15-SCCmec IV/ t084 (11.1%, 1/9). Of 12 MSSA isolates with cMLSB phenotype, 5 isolates belonged to ST182/t196, 4 isolates belonged to ST239/t924, 2 isolates belonged to ST85/t713 and 1 isolate belonged to ST123/t171. All the iMLS phenotype among MSSA strains belonged to ST85/t713. Characteristics of MRSA clones are presented in Table 3.

Discussion

In consistent with the results of a multicenter study made by Ko et al. (20), a relatively high resistance to gentamicin (75%), amikacin (68.3%), kanamycin (77.5%), and tobramycin (59.2%) was reported in this study which was higher than the resistance rate reported by Goudarzi et al. (12). In contrast to the findings of Marghaki et al.’s study (6) who reported high frequency of aac(6’)/aph(2’’) gene (40.3%) in comparison with other AIME genes, in the present study, ant(4’)-la (90%) was the most frequent AIME gene in S. aureus isolates. However, in this study, aph(3’)-IIIa gene frequency rate was higher than the study reported by Ida et al. (8.9%) (21) and Marghaki et al. (15.7%) (6).

Mupirocin as an important agent in the control of MRSA outbreaks and eradicating MRSA colonization was used for the treatment of different types of staphylococcal skin infections. Long period widespread use of mupirocin may lead to the emergence of mupirocin resistance S. aureus strains (8). In this study, 30 isolates (25%) presented mupirocin resistance phenotype and 14 (11.7%) isolates were confirmed as HLMUPR-MRSA which is relatively lower than study in Iran (40%) (22) and higher than study in India (5%) (23) and Jordan (2.6%) (24). However, as a result of proper mupirocin prescription in clinic, in our study mupirocin resistant S. aureus isolates were found to be lower. We detected the resistance gene mupA in 10 isolates (8.3%) which is lower than 12.6% (25) and 25% in Iran (22).

In accordance with the results of our previous study (25) a high tetracycline resistance was seen in the present work (70%). In consistent with others (25), in our study the tet(M) gene that may cause tetracycline resistance was detected in 55 (45.8%) isolates.

In this study, 58 isolates (48.3%) presented cMLSB phenotype while the frequency of iMLSB phenotype was 10% (12/120). In consistent with our findings, Schreckenberger et al. (7%) reported low frequency of iMLS phenotype among S. aureus isolates as well (26). According to the literature, there are discrepant rates of inducible clindamycin resistance in different geographic area. Rashid Nezhad et al. performed a study in seven Iranian teaching hospitals (25). They found that the frequency of cMLSB, iMLSB and MLSb phenotypes was 52.6%, 12.6%, and 5.3% respectively. Similarly, Fiebelkorn et al. in USA (27) reported that of 114 S. aureus isolates resistance to erythromycin, 34% and 29% indicated constitutive and inducible resistance pattern respectively. In a Canadian survey (28), the frequency of iMLSB and cMLSB phenotypes was found to be 64.7% and 35.3% respectively. In current work, the frequency of constitutive resistance was found to be higher than inducible resistance which is in line with the findings reported by Rashidi Nezhad et al (25).

As previously mentioned, resistance to macrolides is encoded by genes often carried on plasmids (erm(C)) or transposons (erm(A) and erm(B)) and msr genes expressing active efflux pumps mainly msr(A) (9). Our results revealed the frequency of msr(A), erm(A), msr(B), erm(C), and erm(B) genes to be 40.8%, 26.7%, 14.2%, 11.7%, and 10.8% respectively. In contrast to Rashidi Nezhad’s study (25) who reported erm(A) gene as the predominant gene among the isolates with inducible phenotype and erm(C) among the isolates with the constitutive phenotype, our finding revealed that the msr(A) gene was the most common gene among strains with the constitutive phenotype (35; 29.2%), followed by erm(A) (21; 17.5%), erm(C) (11; 9.2%), erm(B) (10; 8.3%), msr(B) (10; 8.3%) while erm(A) (4; 3.3%), erm(B) (2; 1.7%), erm(C) (1; 0.83%), msr(A) (5; 4.2%) and msr(B) (1; 0.83%) were much more common among the isolates with inducible phenotype. It is worth mentioning that the frequency of erm and msr genes depends on the bacterial species as well as the geographic region in which the study is carried on.

Type III was the most frequently found SCCmec (77.6%), according to the results of SCCmec typing, associated with an MDR pattern among MRSA isolates. This is in consistent with the results reported by Japioni and colleagues (10). The nosocomial origin of the samples was confirmed by the high frequency of SCCmec type III.

A significant relationship was found between the expression of virulence factors and specific agr locus. In consistent with our previous study, the most common agr types were agr type I (75.8%), type III (16.7%), and type II (7.5%), respectively. Goudarzi et al. (12) reported agr type III to be the most frequent type of SCCmec in Iran. According to several studies, the frequency of toxin and adhesion molecules was higher in isolates harboring agr type I gene in comparison with those harboring agr type II; this is in agreement with our study. Therefore, it can presume that regulation of staphylococcal adhesion molecules and toxins is associated with agr type I.

According to the spa typing results, spa t1037 was recognized as the most common spa type (23.3%). This spa type was reported from Saudi Arabia, China, Iran as well as among HA-MRSA isolates found in Europe, America and other regions of Asia (12, 29, 30).

In present study spa type t388 was estimated at 22.5%. This spa type has also been reported in a study in Iran (31). Similarly, in a study performed in Taiwan, Ho et al described spa type t388 in MRSA strains recovered
from blood cultures in different medical centers (32). It seems that the prevalence of t388 is progressively increased and has been successfully established in our healthcare settings. In our study, the frequency of t713 and t924, earlier reported in UAE and Iran (12), were found to be 16.8% and 8.3% respectively.

In contrast with previous study conducted in Iran (12), in this study, low frequency of t790 and t084 spa types among our isolates, was also demonstrated. For the first time we are reporting t196, and t171 spa types in MSSA strains from Iran.

Using various MRSA typing methods, the isolates were attributed to six different clones, namely ST239-SCCmec III/t388, ST22-SCCmec IV/t790, ST239-SCCmec III/t037, ST15-SCCmec IV/t084, ST585-SCCmec III/t713, and ST239-SCCmec III/t924. In line with previous study from Iran (12), ST239-SCCmec III/t037 clone is currently more frequent in our hospitals (23.3%). The review of the literature reveals that the multiresistant ST239 clone is responsible for at least 90% of HA-MRSA infections in Europe, United States, and some Asian countries, including Kuwait and Malaysia (29). Therefore, the presence of ST239-SCCmec III/t037 in our healthcare setting might be attributed to neighboring regions.

ST239-SCCmec III/t388 (22.5%) was the second most commonly detected MRSA clone. A similar result was reported by Ohadian Moghadam et al from Iran in which major universal MRSA clones were described as ST239, ST291, and ST30 (31).

On the other hand, the third most commonly detected clone was ST22-SCCmec IV/t790 (8.3%). This clone was associated with high resistance to mupirocin, carrying resistance genes, including mecA, msr(A), ant(4)’-la, msr(B), tet(M), and mupA. In our study, all ST22-SCCmec IV/t790 strains contained pvl genes. There are reports of S. aureus ST22 harboring pvl gene from Iran (12), England (33), Saudi Arabia (30), and Kuwait (34). In this regard, a study on PVL-positive MDR-MRSA isolates by Ellington et al (33) from England reported ST5, ST22, ST772, ST80, ST8, and ST59 strains, as later confirmed by Nadig and colleagues (35).

Based on the findings, in clinical MRSA strains, ST15-SCCmec IV/t084 (7.5%) was the fourth most commonly detected clone. The low frequency of this clone has been previously reported in 16 European countries (36). PVL-carrying ST15 isolates were identified in a study by Rasigade and colleagues on 211 S. aureus strains from 19 different countries (37). Five (4.2%) ST15-SCCmec IV/t084 isolates carried pvl genes in our study. Previously, PVL-positive ST15 was reported by Japoni-Nejad and colleagues in Iran (10). In contrast to several studies in which ST15 was reported to be prevalent among CA- and HA-MSSA isolates, all ST15 isolates belonged to MRSA strains in our study. We reported the presence of ST585-SCCmec III/t713 in 5% of isolates. In another study by Goudarzi et al the molecular features of MRSA isolates were identified, and ST585-SCCmec III/t713 was reported in 12% of blood samples from bacteremia patients (12).

To sum up, the present findings showed that MRSA isolates have various genetic backgrounds in our hospitals and involve six major clones. Certain molecular types were associated with some resistance and virulence genes (e.g., eta with ST22-SCCmec IV/t790, ST15-SCCmec IV/t084, and ST239-SCCmec III/t037; mupA with ST15-SCCmec IV/t084 and ST22-SCCmec IV/t790; pvl with ST15-SCCmec IV/t084 and ST22-SCCmec IV/t790; etb with ST585-SCCmec III/t713 and ST22-SCCmec IV/t790). The presence of eight different spa types, i.e., t037, t388, t713, t924, t790, t196, t084, and t171, was also confirmed. For the first time in Iran, STs 182 and 123, as well as spa types t196 and t171, were detected, which might be indicative of the emergence of new clones. Further studies on other neighboring regions, focusing on the emergence of new circulating clones, are necessary to reach an overall understanding of dynamic MRSA clones in Iran and the Middle East.

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Conflicts of Interest

The authors declare that they have no conflicts of interest with the content of this article.

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