New update on molecular diversity of clinical *Staphylococcus aureus* isolates in Iran: antimicrobial resistance, adhesion and virulence factors, biofilm formation and SCCmec typing

Mahtab Tabandeh¹ · Hami Kaboosi¹ · Mojtaba Taghizadeh Armaki² · Abazar Pournajaf³ · Fatemeh Peyravii Ghadikolaii⁴

Received: 27 April 2021 / Accepted: 10 January 2022 / Published online: 21 January 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

**Background** *Staphylococcus aureus* is often considered as a potential pathogen and resistant to a wide range of antibiotics. The pathogenicity of this bacterium is due to the presence of multiple virulence factors and the ability to form biofilm. SCCmec types I, II and III are mainly attributed to HA-MRSA, while SCCmec types IV and V have usually been reported in CA-MRSA infections.

**Methods and results** In this study, we performed a cross-sectional study to determine the antimicrobial resistance, adhesion and virulence factors, biofilm formation and SCCmec typing of clinical *S. aureus* isolates in Iran. *S. aureus* isolates were identified using microbiological standard methods and antibiotic susceptibility tests were performed as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Inducible resistance phenotype and biofilm formation were determined using D-test and tissue culture plate methods, respectively. Multiplex-PCRs were performed to detect adhesion and virulence factors, antibiotic resistance genes, biofilm formation and SCCmec typing by specific primers. Among 143 clinical samples, 67.8% were identified as MRSA. All isolates were susceptible to vancomycin. The prevalence of cMLSB, iMLSB and MS phenotypes were 61.1%, 22.2% and 14.8%, respectively. The TCP method revealed that 71.3% of isolates were able to form biofilm. The predominant virulence and inducible resistance genes in both MRSA and MSSA isolates were related to *sea* and *ermC* respectively. SCCmec type III was the predominant type.

**Conclusions** Data show the high prevalence rates of virulence elements among *S. aureus* isolates, especially MRSA strains. This result might be attributed to antibiotic pressure, facilitating clonal selection.

**Keywords** *Staphylococcus aureus* · Virulence factor · Antibiotic resistance · Biofilm · SCCmec typing · Iran

Introduction

*Staphylococcus aureus* (*S. aureus*) is one of the most common causes of healthcare and community-acquired infections and so responsible for a wide variety of illnesses, from soft and skin tissue infections (SSTIs) to life-threatening infections such as septicemia, toxic shock, hospital- and community-acquired pneumonia (HAP and CAP) and endocarditis [1]. *S. aureus* clinical isolates often promote infections by expressing various exotoxins such as heat-stable staphylococcal enterotoxins (SEs), staphylokinase (SAK), toxic shock syndrome toxin-1 (TSST-1), capsular polysaccharides, lipase, exfoliative toxins (ETA and ETB), hemolysins (α, β, γ, δ) and leukocidins (Panton-Valentine leukocidin; PVL, LukE/D) [2]. From the clinical point of view, indwelling medical devices or catheter-related infections...
such as central venous catheters (CVC), are at risk of *S. aureus*-related infection. The ability to form a stable biofilm is one of the most crucial factors in *S. aureus* pathogenicity and biofilm-associated *S. aureus* infections are often resistant to antibiotic therapy and innate host immune system [3]. Biofilm formation requires polysaccharide intercellular adhesin (PIA), which is encoded and regulated by the intercellular adhesion (*ica*) operon. This operon includes an N-acetylglucosamine transferase (*icaA* and *icaB*), a predicted exporter (*icaC*), and a deacetylase (*icaD*) [1, 4].

Multidrug-resistant *S. aureus* (MDRSA), is becoming a serious global concern, as a common cause of nosocomial- and community-acquired infections [5]. In recent years, methicillin-resistant *S. aureus* (MRSA), which is now the most common MDR, has emerged with the acquisition of Staphylococcal Cassette Chromosome *mec* (SCCmec) elements, which carry a *mecA* gene that encodes a penicillin-binding protein (PBP2a or PBP2′) with a low affinity to β-lactams [6]. MRSA is spread worldwide and is common cause of health care (HAIs)-and community-acquired (CAIs) infections. SCCmec determinants are classified into various types based on the combination of *ccr* and *mec* genes complexes, which includes 5 and 8 *mec* and *ccr* classes, respectively. To date, at least 13 types of SCCmec elements have been recognized and all SCCmec types have individual characteristics. In general, SCCmec type I, II, and III are distributed in the hospital-associated MRSA (HA-MRSA) and type IV and V are present in the community-acquired MRSA (CA-MRSA) [7].

The Mupirocin—a topical ointment that broadly used for SSTIs and nasal decolonization of MRSA—is effective on the isoleucyl-tRNA synthetase (IleRS) which is encoded by *ileS* gene, interfering with protein synthesis. According to the minimal inhibitory concentration (MIC), two mupirocin-resistant phenotypes have been identified; MIC 8-256 µg/ml (low-level resistant-LLR or LMR) and MIC ≥ 512 µg/ml (high-level resistant-HLR or HMR). A point mutation in *ileS*-1 gene (*mupL*) led to LLR isolates, while HLR is usually mediated by a conjugate plasmid-borne *ileS*-2 (*mupA*) gene which encodes a new IleRS that is not bound by mupirocin [8].

Macrolides, lincosamides, and streptogramins (MLS) resistance genes are responsible for resistance to these antibiotics in *Staphylococcus* infections. The MLS resistance phenotypes cMLSb (constitutive resistance to macrolide-lincosamide-streptogramin B), iMLSb (inducible resistance to macrolide-lincosamide-streptogramin B), M/MSb (resistance to macrolide/macrolide-streptogramin B), and LSA/b (resistance to lincosamide-streptogramin A/streptogramin B) were determined in *Staphylococcus* isolates [2].

Aminoglycosides are a class of bactericidal broad-spectrum antimicrobials that bind to the A-position of 16S rRNA in 30S ribosomal small subunit and inhibit protein synthesis. Aminoglycoside-modifying enzymes (AMEs) and 16S ribosomal RNA (16S rRNA) methylation are two important mechanisms for antibiotic inactivation in the *S. aureus* [9]. Based on their functions, AMEs are generally categorized into three types: AAC (aminoglycoside acetyltransferase), APS (aminoglycoside phosphotransferase), and ANT (aminoglycoside nucleotidyltransferase). Numerous AMEs, counting variants of acetyltransferases AAC(3)-I, AAC(3)-II, AAC(3)-III, AAC(6′)-I, AAC(6′)-II, and AAC(6′)-III, the phosphotransferases APH(3′)-I, APH(3′)-II, and APH(3′)-VI, and the nucleotidyltransferases ANT(3)-I, ANT(4′)-I, and ANT(2′′)-I have been known so far in *S. aureus* [10, 11].

The aim of this study was a new update on molecular diversity of antimicrobial resistance, adhesion and virulence factors, biofilm formation and SCCmec typing of clinical *S. aureus* isolates in Iran.

### Materials and methods

#### Clinical sampling and laboratory identification

A total of 143 non-duplicative clinical samples were collected from admission patients referred to teaching therapeutic hospitals (Shahid Beheshti & Ruhani Hospitals, Babol, Iran) in a period of 8 months from September 2019 to April 2020. The samples were transported to the microbiology laboratory in Brain-Heart Infusion Broth (Merck Co., Germany). Each sample was cultured on Mannitol Salt Agar (supplemented with 7.5% sodium chloride) (Merck Co., Germany) and incubated at 37 °C for 24 h. All *S. aureus* colonies were identified based on routine biochemical and microbiological standard tests [12].

#### Antimicrobial susceptibility test

Antimicrobial susceptibility was determined using the agar disk diffusion method on Mueller-Hinton agar plates (Merck Co., Germany) as described by the Clinical and Laboratory Standards Institute (CLSI document M100-S14) [13]. The test antimicrobials were used as follows; Clindamycin (CD; 2 µg), Erythromycin (ERY; 15 µg), Gentamicin (GM; 10 µg), Vancomycin ( VAN; 30 µg), Ciprofloxacin (CIP; 5 µg), Tetracycline (TET; 30 µg), Mupirocin (MUP; 5 µg), Rifampicin (RIF; 2 µg), Cefoxitin (FOX; 30 µg), and Co-trimoxazole (SXT; 5 µg) (MAST Diagnostics, Merseyside, UK). Mupirocin MIC was determined by the E-test strip method (AB Biodisk, Solna, Sweden) on the Mueller-Hinton agar Petri dish according to the manufacturers' guidelines. *S. aureus* ATCC 29,213 was used as positive quality control.
**Inducible resistance phenotype**

The Inducible resistance phenotype was recognized using the double disk test including, Clindamycin (CD; 2 µg) and Erythromycin (ERY; 15 µg) disks applied 20 mm separately [10, 11]. After an incubation time of 24 h at 35 °C, a flattening inhibition zone adjacent to the ERY disk representing an inducible type (D-shaped zone) of MLSB resistance (IR), whereas no-susceptibility to both ERY and CD was mentioned as a constitutive type (CR). The nonappearances of a D-shaped zone in ERY-resistant and CD-susceptible isolates were interpreted as the M/MSB efflux phenotype [14].

**Quantitative biofilm production assay**

In Brief, pure colonies were inoculated in 10 mL of 1% glucose-rich tryptic soy broth (TSBglu), incubated at 37 °C for 24 h in a stationary growth phase and diluted 1:100 with fresh medium. Each well of sterile 96 well-flat bottom polystyrene tissue culture microtiter plates (Falcon® 3046, Lincoln Park, NJ) was full with 200 µL aliquots of diluted cultures. Sterile TSBglu broth was used as a negative control. All plates were incubated at 37 °C for 24 h and then, substances of all wells were gradually removed by tapping the petri. The wells were washed three times with 0.3 mL of phosphate buffer saline (PBS, pH 7.2) to remove loosely attached and floating “planktonic” microorganisms. Biofilm formed by adherent “sessile” isolates in petri was immobile with sodium acetate (NaA) and stained with crystal violet (0.1% w/v). Extra dye was removed by washing with sterile deionized water and plates were kept for drying. Adherent *S. aureus* cells frequently formed biofilm on the sides of the wells and were regularly stained with crystal violet (CV; 1%). The investigation of biofilm formation was assessed by adding the 200 µL of 95% CH₃−CH₂−OH (ethanol) to decolorize the wells. The optical density (OD) of stained adherent isolates was measured with a micro ELISA auto-reader (Bio-Tek Instruments, USA) at a wavelength of 570 nm (OD570 nm). Biofilm formation was recorded as follows: non-biofilm forming (A570 < 1); weak (1 < A570 < 2); ++, moderate (2 < A570 < 3); ++++, strong (A570 > 3) [1, 15].

**Multiplex-polymerase chain reactions (M-PCRs)**

M-PCRs reactions were performed for detection of virulence, resistance and biofilm corresponding genes. Chromosomal DNA was extracted from the pure colonies using the Bacterial Genomic DNA Extraction kit (TaKaRa Biotechnology Co., Ltd, Dalien, China). The DNA concentration and purity were evaluated using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, UK) and then kept at −20 °C until further use. The details of the primers used in this study are shown in Table 1. The process of M-PCR reactions in the final volume of 25 µL was performed according to Table 2 in an Eppendorf MasterCycle Gradient Thermocycler (Eppendorf, Hamburg, Germany). M-PCRs products were electrophoresed in a 1% agarose/0.5 x TBE (45 mM-Tris-borate, 1 mM-EDTA) gel stained with 0.1 µL/mL Gel Red™ (Biotium, USA), then photographed under an UV trans-illuminator (Tanon, China).

**Data analysis**

SPSS version 18.0 for Windows (SPSS Inc., Chicago, USA) was used for statistical analysis. P ≤ 0.05 was considered as a statistical significance.

**Results**

In this cross-sectional study, clinical samples were collected from 143 patients, 51.7% (n = 74) female and 48.3% (n = 96) male with the mean age of 61.4 ± 1.1 years (range from 14 to 98 years). *S. aureus* isolates were collected from several clinical samples including, wound (n = 38; 26.6%), pus (n = 31; 21.7%), blood (n = 27; 18.9%), skin lesion (n = 19; 13.3%), bronchoalveolar lavage (n = 11; 7.7%), sputum (n = 9; 6.3%), intra-tracheal tube (n = 6; 4.2%), joint fluid (n = 1; 0.7%), and cerebrospinal fluid (CSF) (n = 1; 0.7%). The majority of *S. aureus* was isolated from different wards of the hospital as follows: ICU (n = 38; 26.6%), NICU (n = 26; 18.2%), internal medicine (n = 19; 13.3%), surgery (n = 15; 10.5%), urology (n = 14; 9.9%), hemodialysis (n = 14; 9.9%), ENT (Ear, Nose and Throat) (n = 7; 4.9%) hematology-oncology (n = 6; 4.2%), gynecology (n = 2; 1.4%), neurosurgery (n = 1; 0.7%) and orthopedics (n = 1; 0.7%). Of the 143 *S. aureus* strains, 67.8% (n = 97) were resistant to 30 µg-FOX disk phenotypically considered as MRSA isolates and confirmed by mecA-gene amplification using PCR. As shown in Table 3, the resistance rate in MRSA strains was higher than MSSA (P-value ≤ 0.05) (Table 3). The resistance rate on MRSA strains showed that 91.7%, 87.6%, 84.5%, 83.5%, 78.3%, 67.8%, 55.7% and 31.9% of isolates were resistant to RIF, MUP, respectively. MUP MIC E-test showed that, of 31 MUP-resistant MRSA strains, 16.1% (n = 5) and 83.9% (n = 26) were MuL and MuR respectively. Also, all MSSA isolates were susceptible to RIF and MUP. Among the 143 *S. aureus* isolates, 78.3% (n = 112) of the isolates were resistant to at least 3 different antibiotic classes and therefore considered as MDR. The frequency of MDR strains in MRSA and MSSA isolates was 75.3% (n = 73/97) and 45.6% (n = 21/46), respectively. The result of inducible resistance test showed that 39.2% (n = 56) of the isolates were resistant to both CD and ERY. Indeed these 56 isolates had four different resistance phenotypes, n = 33 (58.9%) strains had resistant phenotype...
| Gene type                         | Encoded protein | Target gene | Primer sequences (5′ → 3′) | Ampli-con size (bp) | References |
|----------------------------------|-----------------|-------------|-----------------------------|---------------------|------------|
| Virulence association genes      | Collagen-binding protein | can        | F = 5′-GTCAAGACGATATTAGACACCAG-3′<br>R = 5′-AATCATGTAATGTGCAC TTTGCCACTG-3′ | 423 | [16]       |
|                                  |                 |             | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 292 | [17]       |
|                                  |                 |             | F = 5′-ACATCGTAAATGTA GGGCGAAC-3′<br>R = 5′-TTCGCACGTTTGTTG TTTGCCAC-3′ | 205 |           |
|                                  |                 | clfA        | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 292 | [17]       |
|                                  |                 | clfB        | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 205 |           |
|                                  | Fibronectin–binding proteins A/B | fnbA       | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 643 |           |
|                                  |                 | fnbB        | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 524 |           |
|                                  |                 | fib         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 404 |           |
|                                  |                 | eno         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 302 |           |
|                                  | Hemolysin-encoding genes | hla        | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 209 | [18]       |
|                                  |                 | hlb         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 309 |           |
|                                  |                 | hld         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 111 | [18]       |
|                                  |                 | hlg         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 937 |           |
|                                  | Toxic shock syndrome toxin-1 | tsst-1     | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 350 | [19]       |
|                                  |                 | pvl         | F = 5′-ATTGGGCTGTTCA GTGCT-3′<br>R = 5′-CGTTCTCCTGTTGCA ATTTG-3′ | 433 |           |
### Table 1 (continued)

| Gene type                  | Encoded protein | Target gene | Primer sequences (5′ → 3′)                                      | Ampli-con size (bp) | References |
|----------------------------|-----------------|-------------|-----------------------------------------------------------------|---------------------|------------|
| Staphylococcal enterotoxin A/B/C/D | **sea**         |             | F=5′-TTGCGAAAAAAGTCT GAATGC-3′                                 | 552                 | [20]       |
|                            |                 |             | R=5′-ATTAACGGAAGGTTG TGTAGAAGTA-3′                             |                     |            |
|                            | **seb**         |             | F=5′-GTATGGTGTTGTAAC TGAGC-3′                                 | 164                 | [21]       |
|                            |                 |             | R=5′-CCAAATAGTGACGAG TTAGG-3′                                 |                     |            |
|                            | **sec**         |             | F=5′-GACATAAAGCTAGG AATT-3′                                   | 257                 | [21]       |
|                            |                 |             | R=5′-AAATCGGAAATAC TATCC-3′                                   |                     |            |
|                            | **sed**         |             | F=5′-CTAGTTTGGTAATATCTC CT-3′                                 | 317                 |            |
|                            |                 |             | R=5′-TAATGCTATATCTTATAG GG-3′                                 |                     |            |
| Exfoliative toxin A/B      | **eta**         |             | F=5′-GCAGGTTGTGATT TA GCATT-3′                                | 93                  | [22]       |
|                            |                 |             | R=5′-AGATGTCCTATITTGTGC TG-3′                                 |                     |            |
|                            | **etb**         |             | F=5′-ACAAAGCAAAGAATA CAGCG-3′                                 | 226                 |            |
|                            |                 |             | R=5′-GTTTTTGCTGCTTTCTCT TG-3′                                 |                     |            |
| Resistance encoding genes  | Resistance to methicillin | **mecA**    | F=5′-AAAATCGATGGTAAG GTTGGC-3′                                | 533                 | [23]       |
|                            |                 |             | R=5′-AGTTCTGGAGTACC GATTTGC-3′                                |                     |            |
| Resistance to mupirocin    | **ileS-2 (mupA)** |             | F=5′-TATATTATGCGATGGAAG GTTGG-3′                              | 456                 |            |
|                            |                 |             | R=5′-AATAAAATCAGCTGG AAAAGTGTTTG-3′                           |                     |            |
| Aminoglycoside-enzymes modifying | **APH(3′)-I**  |             | F=5′-ATGTCGCAATITCCA CCGGAAACG-3′                             | 816                 | [24]       |
|                            |                 |             | R=5′-TCGAGAAAACCTACG CATC AGCACATCA-3′                         |                     |            |
|                            | **APH(3′)-IIIa** |             | F=5′-CTTAGTCGAAAAATA CGCGTGC-3′                               | 296                 |            |
|                            |                 |             | R=5′-TCATACTCTTCCGAG CAAA-3′                                  |                     |            |
|                            | **aac(6′)/aph(2′)** |             | F=5′-GAAGTGACGCAG AGAAGA-3′                                   | 491                 | [25]       |
|                            |                 |             | R=5′-ACATGGCAAGCTCTA GGA-3′                                   |                     |            |
|                            | **ANT(4′)-Ia**  |             | F=5′-AATCGGATGAAG CCCA-3′                                     | 135                 | [26]       |
|                            |                 |             | R=5′-GCACCTGCATATTGCTA-3′                                     |                     |            |
| Resistance to vancomycin   | **vanA**        |             | F=5′-GGCAAGTCAGGTTGAAGATG-3′                                  | 713                 |            |
|                            |                 |             | R=5′-ATCAAGCGGTCATTGCTA-3′                                    |                     |            |
| Inducible resistance       | **ermA**        |             | F=5′-TATCTTATCGGTTGAGAAG GGATT-3′                             | 139                 | [14]       |

*References:* [20], [21], [22], [23], [24], [25], [26]
to cMLS$_B$ (resistant to both ERY and CD), $n=12$ (21.4%) isolates had the resistant phenotype to inducible resistance MLS$_B$ (iMLS$_B$; resistant to ERY and susceptible to CD), $n=8$ (14.3%) isolates had the MS resistance phenotype (susceptible to ERY and resistant to CD) and finally, $n=3$ (5.4%) isolates were susceptible to ERY and resistant to CD. All

| Table 1 (continued) |
|----------------------|
| Gene type | Encoded protein | Target gene | Primer sequences (5' → 3') | Ampli-con size (bp) | References |
|------------|-----------------|-------------|-----------------------------|---------------------|------------|
| **ermB** | | | $R=5'$-CTACACTTGGCTTGGATGAAA-3' | | |
| | | | $F=5'$-CCGTTTACGAAAATTGGAACGGTAAAGGGGC-3' | | |
| | | | $R=5'$-GAATCGGAGCTTGGATGTGGC-3' | | |
| **ermC** | | | $F=5'$-ATCTTTGAAATCGGGTCAGG-3' | | |
| | | | $R=5'$-CAAACCCGTATTCCACGATTG-3' | | |
| **ereA** | | | $F=5'$-AACACCCTGGAACCGGAGGACCG-3' | | |
| | | | $R=5'$-CTTCACATCGCATTGCGTCA-3' | | |
| **Biofilm-genes** | **Biofilm-encoding genes** | **icaA** | $F=5'$-GATTATGTAAATGTGCTTGGA-3' | 770 | [27] |
| | | | $R=5'$-ACTACTGTCGCGTTGATAAT-3' | | |
| | | **icaB** | $F=5'$-AGAATCGTGAAATGTTAGAAATT-3' | 900 | [28] |
| | | | $R=5'$-TCTAAATTTTTTCATGGAATCCGT-3' | | |
| | | **icaC** | $F=5'$-CATGAAAAATATGGAGGTTG-3' | 1000 | [28] |
| | | | $R=5'$-TCAAACTGATTTCCGCACCG-3' | | |
| | | **icaD** | $F=5'$-ACGCTATCCGAGGATAGCATCAAAGATAC-3' | 381 | [16] |
| **SCCmec typing** | **Type II & IV** | **ccrA2-B** | $F=5'$-ATTGCCTTTGATAATGCTCCYTCT-3' | 937 | [29] |
| | | | $R=5'$-TAAAGGCATCAATCACAACACCT-3' | | |
| | **Type III & V** | **ccr** | $F=5'$-CGTCTATTACAGAGTTAGGATTAAAGTAT-3' | 518 | |
| | | | $R=5'$-CATTTTAGACTGGATTTTCAAAAATAT-3' | | |
| | **Type I & IV** | **IS1272** | $F=5'$-GCCACTCATAACATAAGGAA-3' | 415 | |
| | | | $R=5'$-CATCCGAGTTAAAACCAAAACCAA-3' | | |
| | **Type V** | **mecA-IS431** | $F=5'$-TATAACCAAACCGACAACTAC-3' | 359 | |
| | | | $R=5'$-CGGCTACAGTGAATAACCATCC-3' | | |
iMLSB$_B$ strains, except two isolates, belonged to the MRSA. The prevalence of cMLSB, iMLSB and MS phenotypes in the MRSA isolates were 81.8% (n = 27), 83.3% (n = 10) and 62.5% (n = 5), respectively. So, 11.1% (n = 6), 3.7% (n = 2) and 5.5% (n = 3) of MSSA isolates have cMLSB, iMLSB and MS phenotypes, respectively. The TCP method revealed that 71.3% (n = 102/143) of isolates were able to form biofilm, including strong (n = 69/102, 67.6%), moderate (n = 21/102, 20.6%) and weak (n = 12/102, 11.7%). Blood and wound isolates have the highest proportion for strong biofilm phenotype (n = 21/27; 77.7%, and n = 27/38; 71.1%). Biofilm formation in MRSA isolates was far greater than MSSA (P value ≤ 0.05). In MRSA isolates, 84.1% (n = 58/69), 80.9% (n = 17/21) and 58.3% (n = 7/12) of isolates had a strong, moderate and weak phenotype, respectively. But, in the MSSA strains 15.1% (n = 11/69), 19% (n = 4/21) and 41.6% (n = 5/12) had strong, moderate and weak biofilm formation phenotype, respectively.

The molecular distribution of virulence-related genes was significantly higher in MRSA strains, especially in the isolates collected from ICU (P-value ≤ 0.05). The most prevalent virulence-related gene was sea in both MRSA and MSSA isolates. tss-t, sec and sed genes were present only in MRSA strains. 8.2% and 2.3% of MRSA and MSSA isolates were positive for pvl gene, respectively. The frequencies of inducible-resistance encoding genes in the MRSA strains were 21.6%, 16.5%, 44.3% and 9.3% for ermA, ermB, ermC and ereA, respectively. Such as MRSA strains, in MSSA isolates ermC gene was the predominant gene. vanA gene was not detected in both MRSA and MSSA isolates. So, distribution of AMEs genes in MRSA strains were 33%, 62.8%, 24.7% and 85.6% for APH(3′)-I, APH(3′)-IIIa, aac(6′)/aph(2″) and ANT(4′)-Ia, respectively. The prevalence of the ica genes in the MRSA strains was as follows: icaA (84.5%), icaB (70.1%), icaC (74.2%) and icaD (81.4%). Of 97 MRSA isolates, 55.7% (n = 54) and 44.3% (n = 43) were HA-MRSA and CA-MRSA, respectively. SCCmec type III was the most predominant type. In general, among 97 MRSA isolates, 34% (n = 33), 23.7% (n = 23), 18.5% (n = 18), 11.3% (n = 11) and 6.2% (n = 6) were belonged to the SCCmec type III, I, IV, II, and V, respectively; though, 6.4% (n = 6) isolates were non typable. SCCmec type I was only found in the blood strains, types IV and V were mostly observed from wound, BAL and sputum and type III were found in all clinical samples (Table 4).

**Discussion**

In the current study, a high prevalence of MRSA (67.3%) was found, especially in samples obtained from ICU and NICU wards. These data are in agreement with Mir et al. [16], but do not agree with Darban-Sarokhalil et al. [30]. In a study directed by Kateete et al., all isolates were found to be MRSA [31]. These conflicts could be attributed to the sample types (burn vs. other samples), year of study, geographic location (Uganda vs. Iran), level of hygiene, different protocols in infection control, irrational antibiotic administration, and laboratory method for determination of methicillin-resistant isolates. In line with our study, Guardabassi et al. indicated that the 30 μg Fox disk diffusion method is preferred to most of the other recommended tests such as; oxacillin disc diffusion and oxacillin screen agar tests and it is currently an accepted method for recognition of MRSA isolates by Clinical and Laboratory Standards Institute strategies [32].

The resistance rate in MRSA isolates is significantly higher than in the MSSA (P ≤ 0.05), which is consistent with the study of Solgi et al. [33], Mir et al. [16] and Pour-najaf et al. [24]. Among antibiotics used for MRSA strains, CIP showed the least anti-staphylococcal activity and VAN was the most effective. According to the study performed by Solgi et al. [33], VAN is still the best option in the treatment of patients with MRSA infection. In comparison with other studies, there has been an increase in resistance to antimicrobial in MRSA isolates. The resistance rates in the MRSA strains were as follows: CIP (91.7%), GM (87.6%), SXT (84.5%), ERY (83.5%), TET (76.3%), RIF (62.8%), CD (55.7%) and VAN (0.0%). So, 78.3% of our isolates were considered MDR. This could be due to the continuous and empirical usage of broad-spectrum antimicrobials and the lack of an appropriate antibiotic treatment strategy. According to our data, although 14.4% of MRSA strains were resistant to MUP, it’s still recommended as an option in the removal of MSSA nasal colonization. These data are in agreement with Chaturvedi et al. [34] and Antonov et al. [35] studies. Interestingly, among 14.4% MUP-resistant MRSA strains, only 8.2% were positive for ileS-2 gene. These data are consistent with Solgi et al. [33] and McNeil et al. [36] studies. Solgi et al. [33] declare that low-level MUP resistance may be occurred due to another responsible gene such as; mapL/D/W/O/T. Contrary to our study, Mir et al. [16], showed that 85.6% of the isolates were resistant to MUP. On the other hand, Chen et al. reported a high frequency of MUP-resistant MRSA isolates in burn centers [37]. This topical bacteriostatic antimicrobial is mainly used for prophylaxis against *S. aureus* nasal carriage and other skin diseases. Its target is the bacterial isoleucyl transfer ribonucleic acid synthetase. The long-term use of MUP, mostly for the decolonization of nasal carriage, burns, diabetic foot, bedsores and other skin lesions could be related to the development of resistance to MUP [33]. D-test revealed that the prevalence of cMLSB, iMLSB and MS resistance phenotypes were 61.1%, 22.2% and 14.8%, respectively. This data has also been described by Solgi et al. [33], Khodabandeh et al. [14] and Gupta et al.
Table 2  M-PCRs conditions and cycles in the present study

| Reaction set | Amplified genes | Reaction compounds | M-PCR program | Cycles of amplification |
|--------------|-----------------|--------------------|---------------|-------------------------|
| S1           | Cna/clfA/fnbA/fnbB/tst-1 | 1.0 µL of template DNA, 12.1 µL of CinnaGen PCR Master Mix, 0.8 µL of each primer, and 10.3 µL of ddH2O. | Initial denaturation at 94 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 56 °C for 60 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 30 |
| S2           | clfB/fib/eno/mecA | 0.9 µL of template DNA, 10.6 µL of CinnaGen PCR Master Mix, 1.0 µL of each primer, and 11.5 µL of ddH2O | Initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 45 s, annealing at 55 °C for 50 s, extension at 72 °C for 60 s and a final extension at 72 °C for 6 min. | 32 |
| S3           | hla/hlb/hld/lg/pvl | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 45 s, annealing at 55 °C for 50 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 31 |
| S4           | Sea/seb/sec/sec/leS-2 | 0.8 µL of template DNA, 11.6 µL of CinnaGen PCR Master Mix, 1.0 µL of each primer, and 10.6 µL of ddH2O. | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 35 s, annealing at 56 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 35 |
| S5           | eta/etb | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 55 s, annealing at 56 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 31 |
| S6           | APH(3')-I/APH(3')-IIa/aac(6')/aph(2'/Y)ANT(4')-la/vanA | 1.0 µL of template DNA, 12.5 µL of CinnaGen PCR Master Mix, 1.0 µL of each primer, and 9.5 µL of ddH2O. | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 55 s, annealing at 56 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 31 |
| S7           | ermA/ermB/ermC/ereA | 1.0 µL of template DNA, 12.3 µL of CinnaGen PCR Master Mix, 0.9 µL of each primer, and 9.9 µL of ddH2O. | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 55 s, annealing at 56 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 33 |
| S8           | IcaA/icaB/icaC/icaD | 0.8 µL of template DNA, 11.7 µL of CinnaGen PCR Master Mix, 0.9 µL of each primer, and 10.7 µL of ddH2O. | Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 55 s, annealing at 56 °C for 45 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. | 33 |
| S9           | ccrA2-B/ccr/IS1272/mecA-IS431 | 0.9 µL of template DNA, 12.2 µL of CinnaGen PCR Master Mix, 0.8 µL of each primer, and 10.3 µL of ddH2O. | Initial denaturation at 94 °C for 5 min, denaturation at 95 °C for 45 s, annealing at 55 °C for 60 s, extension at 72 °C for 60 s and a final extension at 72 °C for 10 min. | 32 |
Table 3 Antimicrobial resistance profile in MRSA and MSSA strains

| S. aureus isolates | No. (%) of antimicrobial resistance pattern |
|-------------------|-------------------------------------------|
|                   | ERY | CD  | GM  | CIP | TET | MUP | RIF | SXT | VAN |
| MRSA (n = 97, 67.8%) |     |     |     |     |     |     |     |     |     |
| S                 | 16 (16.5) | 42 (43.3) | 12 (12.4) | 7 (7.2) | 23 (23.7) | 83 (85.6) | 36 (37.1) | 15 (15.5) | 97 (100%) |
| I                 | 0 (0.0) | 1 (1) | 0 (0.0) | 1 (1) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| R                 | 81 (83.5) | 54 (55.7) | 85 (87.6) | 89 (91.7) | 74 (76.3) | 14 (14.4) | 61 (62.8) | 82 (84.5) | 0 (0.0) |
| MSSA (n = 46, 32.2%) |     |     |     |     |     |     |     |     |     |
| S                 | 19 (41.3) | 13 (28.3) | 33 (71.7) | 36 (78.3) | 29 (63) | 46 (100) | 46 (100) | 40 (86.9) | 46 (100%) |
| I                 | 0 (0.0) | 1 (2.2) | 0 (0.0) | 1 (2.2) | 2 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| R                 | 27 (58.7) | 32 (69.6) | 13 (28.3) | 9 (19.6) | 15 (32.6) | 0 (0.0) | 0 (0.0) | 6 (13) | 0 (0.0) |

In our studies, isolates collected from blood and wound from patients hospitalized in ICU had the highest ability in biofilm formation. With this regard, 71.3% of the isolates were able to form a biofilm, including strong (67.6%), moderate (20.6%) and weak (11.7%). In MRSA isolates, 84.1%, 80.9% and 58.3% had a strong, moderate and weak phenotypes, respectively. So, 84.1%, 19% and 41.6% of MSSA strains had strong, moderate and weak biofilm phenotype, respectively. These data are in contrast with Avila-Novoa et al. [15] and Gowrishankar et al. [28]. One reason for this discrepancy is the source of the samples (food and pharyngitis samples vs. our clinical samples). Contrary to our study, Ghasemian et al. [27] declare that the prevalence of icaA, icaB, icaC and icaD were 73% (n = 16), 63.6% (n = 14), 73% (n = 16) and 73% (n = 16), respectively. So, they showed that there was no significant difference between MRSA and MSSA strains for the presence of icaADBC operon. In the present study, biofilm formation in MRSA isolates was far greater than MSSA. In line with Kord et al. study, the highest and lowest ica gene was icaA and icaD, respectively [46].

In our study, all MRSA isolates were harbored sea gene. The seb, sec and sed were detected in 17.5%, 5.2% and 3.1% of MRSA isolates. Sec and sed were not found in MSSA isolates. In a study performed by Mehrotra et al. [22], among 107 strains collected from nasal swabs from healthy humans, 19.6% (n = 21), 24.3% (n = 26), 5.6% (n = 6), 7.5% (n = 8) and 1.9% (n = 2) were positive for sea, tst, seb, sec, and sed, respectively. This contrast may be related to the source of the sample (anterior nasal swabs vs. clinic samples). Sabouni et al. [47] showed that of 133 S. aureus isolates 48% (n = 64) were MRSA. The frequency of virulence-encoded genes was 40.6%, 19.6%, 12.8%, 11.3%, 9%, 4.5% and 3% for sea, tst, eta, etb, sed and sec, respectively. In contrast with the present study, among MSSA isolates, seb and tst were the more prevalent toxins in comparison with MRSA isolates. In our samples, none of the MSSA isolates were positive for tst-I gene. Mir et al. [16] showed that the frequency of hla, [38], but have conflict with Seifi et al. [39], Adhikari et al. [11], Ruiz-Ripa et al. [40] and Deotale et al. [10] studies. These conflicts may be related to the year of study, topographical locations and surveillance strategies, as well as limitation in drug prescription. The rate of inducible resistance varies from hospital to hospital and even from patient to patient. In agreement with Solgi et al. [33], and Gupta et al. [38], the frequency of iMLSB phenotype was higher than iMLSB, but in another study, the frequency of iMLSB phenotype showed to be higher than cMLSB. Therefore, notice of regional frequency of MLSB resistant isolates is very important for microbiology laboratories to choose to perform D-test regularly. In concordance with Khodabandeh et al. [14], ermC was the predominant gene on both MRSA and MSSA isolates. The prevalence of ermA, ermB, ermC and ereA in the MRSA isolates were 21.6%, 16.5%, 44.3% and 9.3%, respectively. ereA gene was only found in the MRSA isolates which were collected from ICU. The combination of ermA/ermB ermC genes was detected in only two MRSA isolates collected from blood samples. So, 26.1%, 15.2% and 23% of MSSA isolates were positive for ermA, ermB and ermC, respectively. Our findings contradict the study conducted by Ghanbari et al. [41] and Saribas et al. [42]. This discrepancy could be due to genetic variation and the spread of a single clone in our area. Distribution of AMEs genes in our samples were as follows: aph(3′)-Ia (33%), aph(3′)-IIa (62.8%), aac(6′)/aph(2′) (24.7%) and ant(4′)-Ia (85.6%) in the MRSA isolates and aph(3′)-Ia (36.9%), aph(3′)-IIa (45.6%), aac(6′)/aph(2′) (13%) and ant(4′)-Ia (26.1%) in MSSA. M-PCR showed that 8.2% (n = 8/97), 12.4% (n = 12/97), and 33% (n = 32/97) of MRSA isolates carried simultaneously aph(3′)-IIa/aac(6′)/aph(2′), aph(3′)-IIa/aph(3′)-IIa, and aph(3′)-IIa/ant(4′)-Ia genes, respectively. Only 2.1% (n = 2/97) isolates were positive for all AMEs tested genes. These results are inconsistent with the studies of Khorasavi et al. [43] and Goudarzi et al. [44]. In agreement with our study, the ant(4′)-Ia was the most prevalent gene in Yadegar et al. [45]. As a result, according to other studies, the AMEs gene in the MRSA strains was higher than MSSA, which could be due to the ability of these strains to acquire resistant genetic elements.

According to our study, the prevalence of ant(4′)-Ia was the most prevalent toxins in comparison with MRSA isolates. In agreement with our study, the ant(4′)-Ia was the most prevalent gene on both MRSA and MSSA isolates. The prevalence of ermA, ermB, ermC and ereA in the MRSA isolates were 21.6%, 16.5%, 44.3% and 9.3%, respectively. ereA gene was only found in the MRSA isolates which were collected from ICU. The combination of ermA/ermB ermC genes was detected in only two MRSA isolates collected from blood samples. So, 26.1%, 15.2% and 23% of MSSA isolates were positive for ermA, ermB and ermC, respectively. Our findings contradict the study conducted by Ghanbari et al. [41] and Saribas et al. [42]. This discrepancy could be due to genetic variation and the spread of a single clone in our area. Distribution of AMEs genes in our samples were as follows: aph(3′)-Ia (33%), aph(3′)-IIa (62.8%), aac(6′)/aph(2′) (24.7%) and ant(4′)-Ia (85.6%) in the MRSA isolates and aph(3′)-Ia (36.9%), aph(3′)-IIa (45.6%), aac(6′)/aph(2′) (13%) and ant(4′)-Ia (26.1%) in MSSA. M-PCR showed that 8.2% (n = 8/97), 12.4% (n = 12/97), and 33% (n = 32/97) of MRSA isolates carried simultaneously aph(3′)-IIa/aac(6′)/aph(2′), aph(3′)-IIa/aph(3′)-IIa, and aph(3′)-IIa/ant(4′)-Ia genes, respectively. Only 2.1% (n = 2/97) isolates were positive for all AMEs tested genes. These results are inconsistent with the studies of Khorasavi et al. [43] and Goudarzi et al. [44]. In agreement with our study, the ant(4′)-Ia was the most prevalent gene in Yadegar et al. [45]. As a result, according to other studies, the AMEs gene in the MRSA strains was higher than MSSA, which could be due to the ability of these strains to acquire resistant genetic elements.
hlb, hld, hlg, tst and pvl genes was 92.8%, 34.7%, 89.8%, 11.9%, 10.7%, and 0.5% respectively. In line with our study, hla gene had the highest frequency among isolates (94.4% for MRSA and 89.8% for MSSA). Exfoliative toxin A and B were detected in the 11.3% and 6.2% of MRSA isolates, respectively. In the MSSA strains, the etA gene was present in 4.3% and etB in 6.5% of isolates. These data are similar to the results reported by Sila et al. [48]. Also to support Sabouni et al. study [47], etA gene was higher among MRSA isolates. However, no significant relationship was observed in the presence of etB between MRSA and MSSA strains. The etA and etB genes are more common in samples collected from Skin and soft tissue lesions [47].

Bacterial adherent to the target cell is the primary stage of infection. At this stage, attachment of S. aureus is facilitated by microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) including, fnbA and fnbB (encoding fibronectin-binding proteins A and B), fib (encoding fibrinogen-binding protein), clfA and clfB (encoding clumping factors A and B), and eno (encoding laminin-binding protein) [49]. The prevalence of the MSCRAMMs-encoding genes in the MRSA isolates was as follows: eno (84.5%), fib (75.3%), clfB (66%), clfA (56.7%), cna (52.3%), fnbA (15.5%) and fnbB (13.4%). These data are similar to Mir et al. study [16]. As a result, the frequency of MSCRAMMs genes in MRSA strains was higher than in MSSA, which could be due to the high pathogenicity of the strains. The distribution of these genes in the samples collected from the ICU was much higher than the samples of other units. Also, the strains collected from the wound, sputum, blood, and BAL samples, had the

| Table 4 Distribution of virulence, resistance and biofilm genes in MRSA and MSSA isolates |
|---------------------------------|-----------------|-----------------|
| Gene type                      | Gene            | MRSA (n = 97)   | MSSA (n = 46)   |
| Virulence-related genes        |                 |                 |
| cna                            | 51 (52.3%)      | 28 (60.7%)      |
| clfA                           | 55 (56.7%)      | 19 (41.3%)      |
| clfB                           | 64 (66%)        | 31 (67.4%)      |
| fnbA                           | 15 (15.5%)      | 17 (37%)        |
| fnbB                           | 13 (13.4%)      | 21 (45.7%)      |
| fib                            | 73 (75.3%)      | 25 (54.3%)      |
| eno                            | 82 (84.5%)      | 33 (71.3%)      |
| hla                            | 88 (90.7%)      | 39 (84.7%)      |
| hld                            | 80 (82.5%)      | 39 (84.7%)      |
| hlg                            | 6 (6.2%)        | 13 (28.3%)      |
| tsst-I                         | 5 (5.2%)        | 0 (0.0%)        |
| Pvl                            | 8 (8.2%)        | 1 (2.3%)        |
| sea                            | 97 (100%)       | 41 (89.1%)      |
| seb                            | 17 (17.5%)      | 2 (4.3%)        |
| sec                            | 5 (5.2%)        | 0 (0.0%)        |
| sed                            | 3 (3.1%)        | 0 (0.0%)        |
| eta                            | 11 (11.3%)      | 2 (4.3%)        |
| etb                            | 6 (6.2%)        | 4 (6.5%)        |
| Resistance encoding genes      |                 |                 |
| ileS-2                         | 8 (8.2%)        | 0 (0.0%)        |
| APH(3')-I                      | 32 (33%)        | 17 (36.9%)      |
| APH(3')-IIIa                   | 61 (62.8%)      | 21 (45.6%)      |
| aac(6')/aph(2')                | 24 (24.7%)      | 6 (13%)         |
| ANT(4')-Ia                     | 83 (85.6%)      | 12 (26.1%)      |
| vanA                           | 0 (0.0%)        | 0 (0.0%)        |
| ermA                           | 21 (21.6%)      | 12 (26.1%)      |
| ermB                           | 16 (16.5%)      | 7 (15.2%)       |
| ermC                           | 43 (44.3%)      | 23 (50%)        |
| ereA                           | 9 (9.3%)        | 0 (0.0%)        |
| Biofilm genes                  |                 |                 |
| icaA                           | 82 (84.5%)      | 36 (78.3%)      |
| icaB                           | 68 (70.1%)      | 12 (26.1%)      |
| icaC                           | 72 (74.2%)      | 18 (39.1%)      |
| icaD                           | 79 (81.4%)      | 42 (91.3%)      |
highest frequency of these genes, respectively. Mir et al. reported that various molecules such as collagen, fibronectin and other factors are present in the burn wound [16]. *S. aureus* encodes many MSCRAMMs that precisely interact with host cells and it enables the microbe to colonize on the burn wounds. One of the most important virulence factors in *S. aureus* infections, particularly in the skin and soft-tissue infections is the Panton-Valentine Leukocidin (PVL). This cytotoxin has been known as a virulence factor related to tissue necrosis such as necrotizing pneumonia (NP). M-PCR showed that 6.3% (n = 9/143) of *S. aureus* carried *pvl* gene. In contrast with Mir et al. [16] and Mkrtchyan et al. [50] studies, 8% of MRSA strains were positive for *pvl* gene, all of which were CA-MRSA. In our isolates, 55.7% and 44.3% of *mecA*-positive strains were HA-MRSA and CA-MRSA, respectively. In various studies directed by Rodrigues et al. [51] and Teare et al. [52], the prevalence of *pvl* gene was 14.6% and 2% respectively. Surprisingly, in concordance with Mir et al. [16] only one MSSA isolate (2.3%) was positive for *pvl* gene.

According to our result, the frequency rate of types I, II, III, IV, and V of SCCmec was 23.7%, 11.3%, 34%, 18.5% and 6.2%, respectively. In line with Mariem et al. [53] and Ghanbari et al. [41], SCCmec typing did not show 100% type ability and had poor discriminatory power, as 6.4% of MRSA strains were non typable. Overall, 75% (n = 6 of 8) of *pvl*-positive MRSA strains belonged to the SCCmec type IV and V. In agreement with Taherriad et al. [54], Ghanbari et al. [41] and Moosavian et al. [55], the most common SCCmec type was type III. However, Jamshidi et al. [56] and Boye et al. [29] reported type IV as the most predominant type. This contrast may be related to the patients included in the study, multiple sclerosis (MS) cases vs. various *Staphylococcus* infections and geographical locations.

**Conclusions**

We determined the high prevalence of virulence elements and raised rate of antimicrobial resistance in our samples. MRSA strains also have a high ability to form biofilm. In addition, SCCmec type III was recognized as the predominant type. These data recommend that efficient control procedures must be considered to prevent the transmission of MRSA isolates among patients in hospital units, especially in the ICU.

**Acknowledgements** We appreciate the sincere collaboration of the Microbiology Department, Ayatollah Amoli branch of Islamic Azad University and Department of Microbiology, School of Medicine, Babol University of Medical Sciences for assistance in the conduct of the study.

**Author contributions** HK and MTA conceived and designed the experiment; AP and MT conducted the study and collected the samples. AP, MT and FPG performed the experiments and analyzed the data. All authors contributed to paper writing. The authors have read and approved the final manuscript.

**Funding** This study was supported by the Ayatollah Amoli Branch, Islamic Azad University, Amol, Mazandaran province, Iran. The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Data availability** All original data and materials are available upon request from the corresponding author.

**Declarations**

**Conflict of interest** The authors declare that they have no conflicts of interest relevant to this study.

**Ethical approval** This study was approved by the Research Ethics Committee of Babol Branch, Islamic Azad University (approved ID: IR.IAU.BABOL.REC.1399.035) and performed in accordance with the Declaration of Helsinki. The participants signed the informed consent forms from each patient, and the Research Ethics Committee of Babol Branch, Islamic Azad University approved the study protocol.

**References**

1. Khandan Del A, Kaboosi H, Jamalli A, Peyravii Ghadikolaii F (2019) Prevalence and expression of *psmA* gene in biofilm-producing *Staphylococcus aureus* clinical isolates. Jundishapur J Microbiol 12(8):e89610. https://doi.org/10.5812/jjm.89610
2. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661. https://doi.org/10.1128/CMR.00134-14
3. Antunes AL, Bonfanti JW, Perez LR, Pinto CC, Freitas AL, Macedojr AJ, Barth AL (2011) High vancomycin resistance among biofilms produced by *Staphylococcus* species isolated from central venous catheters. Mem Inst Oswaldo Cruz 106(1):51–55. https://doi.org/10.1590/s0074-02762011000100008
4. Lade H, Park JH, Chung SH, Kim HJ, Kim JM, Joo HS, Kim JS (2019) Biofilm formation by *Staphylococcus aureus* clinical isolates is differentially affected by glucose and sodium chloride supplemented culture media. J Clin Med 8(11):1853. https://doi.org/10.3390/jcm811185
5. Gurung RR, Maharjan P, Chhetri GG (2020) Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. Future Sci OA 6(4):FSO464. https://doi.org/10.2144/fsoa-2019-0122
6. IWG-SCC (2009) International working group on the classification of staphylococcal cassette chromosome elements, classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 53(12):4961–4967. https://doi.org/10.1128/AAC.00579-09
7. Huang YH, Tseng SP, Hu JM, Tsai JC, Hsueh PR, Teng LJ (2007) Clonal spread of SCCmec type IV methicillin-resistant *Staphylococcus aureus* between community and hospital. Clin Microbiol Infect 13(7):717–724. https://doi.org/10.1111/j.1469-0691.2007.01718.x
8. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW (2013) Prevalence of mupirocin resistance among invasive coagulate-negative staphylococci and methicillin-resistant *Staphylococcus aureus* (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring mupA. J Antimicrob Chemother 68(8):1714–1717. https://doi.org/10.1093/jac/dkt085

9. Sabzezali F, Goudarzi M, Goudarzi H, Azimi H (2017) Distribution of aminoglycoside resistance genes in coagulase-negative staphylococci isolated from hospitalized patients. Arch Pediatr Infect Dis 5(3):e57297. https://doi.org/10.5812/pedinfect.57297

10. Deotale V, Mendiratta DK, Raut U, Narang P (2010) Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian J Med Microbiol 28(2):124–126. https://doi.org/10.4103/0255-0857.62488

11. Adhikari RP, Shrestha S, Barakoti A, Amatya R (2017) Identification of the classical enterotoxin genes of *Staphylococcus aureus*. Int J Pept Res Ther 26:2411–2418. https://doi.org/10.1007/s10989-017-1007/8

12. Baron EG, Finegold SM (1999) Diagnostic Microbiology, 8th edn. The CV Mosby Company, St. Louis

13. Patra KP, Vanchieri JA, Bocchini JA Jr (2011) Adherence to CLSI recommendations for testing of *Staphylococcus aureus* isolates in Louisiana hospitals: report of a clinical failure and results of a questionnaire study. J Clin Microbiol 49(8):3019–3020. https://doi.org/10.1128/JCM.00944-11

14. Khodabande M, Abdolzaheri MR, Alvandi-Maneshe A, Gholami M, Bibalan MH, Pouriran R, Rahmani S (2019) Analysis of resistance to macroline-lincosamide-streptogramin B among mecA-positive *Staphylococcus aureus* isolates. Osong Public Health Res Perspect 10(1):25–31. https://doi.org/10.24171/j.phr.2019.10.1.06

15. Avila-Novoa MG, Igüérez-Moreno M, Solís-Velázquez OA, González-Gómez JP, Guerrero-Medina PJ, Gutiérrez-Lomelí M (2018) Biofilm formation by *Staphylococcus aureus* isolated from food contact surfaces in the dairy industry of Jalisco, Mexico. J Food Quality 2018:1–8. https://doi.org/10.1155/2018/1746139

16. Mir Z, Nofeh Farahani N, Abbasian S, Alinejad F, Sattarzadeh T, Abbaszadeh H, Sharifi B, Haeili M (2016) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from the main burn center of Tehran, Iran. Iran J Basic Med Sci 19(3):209–212. https://doi.org/10.12980/JPJTB.4.2014C423

17. Tristan A, Ying L, M, Etienne J, Vandenesch F, Lina G (2003) Prevalence of mupirocin resistant *Staphylococcus aureus* strains and their antibiotic resistance profiles. Asian Pac J Trop Biomed 31:209–212. https://doi.org/10.1371/journal.pone.0183607

18. Chaturvedi P, Singh AK, Singh AK, Shukla S, Agarwal L (2014) Prevalence of mupirocin resistant *Staphylococcus aureus* isolates among patients admitted to a tertiary care hospital. N Am J Med Sci 6(8):403–407. https://doi.org/10.4103/1947-2714.139293
35. Antonov NK, Garzon MC, Morel KD, Whittier S, Planet PJ, Lauren CT (2015) High prevalence of mucopeptide resistance in Staphylococcus aureus isolates from a pediatric population. Antimicrob Agents Chemother 59(6):3350–3356. https://doi.org/10.1128/AAC.00709-15

36. McNeil JC, Hulten KG, Kaplan SL, Mason EO (2011) Mupirocin resistance in Staphylococcus aureus causing recurrent skin and soft tissue infections in children. Antimicrobial Agents Chemotherapy 55(5):2431–2433. https://doi.org/10.1128/AAC.01587-10

37. Chen X, Yang HH, Huangfu YC, Wang WK, Liu Y, Ni XY, Han LZ (2012) Molecular epidemiologic analysis of Staphylococcus aureus isolated from four burn centers. Burns 38(5):738–742. https://doi.org/10.1016/j.burns.2011.12.023

38. Gupta V, Datta P, Rani H, Chander J (2009) Inducible clindamycin resistance in Staphylococcus aureus: a study from North India. J Postgrad Med 55(3):176–179. https://doi.org/10.4103/0022-3859.57393

39. Seifi N, Kahani N, Askari E, Mahdipour S, Naderi NM (2012) Inducible clindamycin resistance in Staphylococcus aureus isolates recovered from Mashhad, Iran. Iran J Microbiol 4(2):82–86

40. Ruiz-Ripa L, Alcalá L, Simón C, Gómez P, Mama OM, Rezusta A, Zarazaga M, Torres C (2019) Diversity of Staphylococcus aureus clones in wild mammals in Aragon, Spain, with detection of MRSA ST130 mecC in wild rabbits. J Appl Microbiol 127(1):284–291. https://doi.org/10.1111/jam.14301

41. Ghanbari F, Ghajavand H, Havaei R, Jami MS, Khademi F, Heydari L, Shahin M, Havaei SA (2016) Distribution of MRSA ST130 mecC in wild rabbits. J Appl Microbiol 127(1):284–291. https://doi.org/10.1111/jam.14301

42. Gupta V, Datta P, Rani H, Chander J (2009) Inducible clindamycin resistance in Staphylococcus aureus isolates recovered from Mashhad, Iran. Iran J Microbiol 4(2):82–86

43. Antonov NK, Garzon MC, Morel KD, Whittier S, Planet PJ, Lauren CT (2015) High prevalence of mucopeptide resistance in Staphylococcus aureus isolates from a pediatric population. Antimicrob Agents Chemother 59(6):3350–3356. https://doi.org/10.1128/AAC.00709-15

44. Kord M, Ardebili A, Jamalan M, Jahanbakhsh R, Behnampour N, Ghaemi EA (2018) Evaluation of biofilm formation and presence of ica genes in Staphylococcus epidermidis clinical isolates. Ospolg Public Health Res Perspect 9(4):160–166. https://doi.org/10.24171/j.phrp.2018.9.4.04

45. Sabouni F, Mahmoudi S, Bahador A, Pourakbari B, Sadeghi RH, Ashtiani MT, Nikmanesh B, Mamishi S (2014) Virulence factors of Staphylococcus aureus isolates in an Iranian referral children’s hospital. Ospolg Public Health Res Perspect 5(2):96–100. https://doi.org/10.1016/j.jphrp.2014.03.002

46. Sita J, Sauer P, Kolar M (2009) Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, pantovalent-leukocidin and tsst-1 between methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus at the university hospital in Olomouc. Biomed Pap Med Fac Univ Palacký Olomouc Czech Repub 153(3):215–218. https://doi.org/10.5507/bp.2009.036

47. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirluff ME (2011) Staphylococcus aureus biofilms: properties, regulation, and roles in human disease. Virulence 2(5):445–459. https://doi.org/10.4161/viru.2.5.17724

48. Rodrigues MV, Fortaleza CM, Riboli DF, Rocha RS, Rocha C, da Cunha MeD (2013) Molecular epidemiology of methicillin-resistant Staphylococcus aureus in a burn unit from Brazil. Burns 39(6):1242–1249. https://doi.org/10.1016/j.burns.2013.02.006

49. Teare L, Shelley OP, Millership S, Kears A (2010) Outbreak of pantovalent-leucocidin-positive meticillin-resistant Staphylococcus aureus in a regional burns unit. J Hosp Infect 76(3):220–224. https://doi.org/10.1016/j.jhin.2010.04.023

50. Mariem BJ, Ito T, Zhang M, Jin J, Li S, Ilhem BB, Adnan H, Han X, Hiramatsu K (2013) Molecular characterization of methicillin-resistant pantovalent-leucocidin-positive staphylococcus aureus clones disseminating in Tunisian hospitals and in the community. BMC Microbiol. https://doi.org/10.1186/1471-2180-13-2

51. Taherirad A, Jahanbakhsh R, Shakeri F, Anvary S, Ghaemi EA (2016) Staphylococcal cassette chromosome mec types among methicillin-resistant Staphylococcus aureus in northern Iran. Jundishapur J Microbiol 9(8):e33933. https://doi.org/10.5812/jjm.33933

52. Moosaviani M, Shahin M, Navidifar T, Torabipour M (2017) Typing of staphylococcal cassette chromosome mec encoding methicillin resistance in Staphylococcus aureus isolates in Ahvaz. Iran New Microbes New Infect 21:90–94. https://doi.org/10.1016/j.jmmi.2017.11.006

53. Jamshidi Y, Pourmand MR, Pakbaz Z, Pourmand A, Rahimi Foroushani A, Sahraian MA (2019) SCCmec genotypes of methicillin-resistant Staphylococcus aureus in nasal carriage of multiple sclerosis patients in Iran. Iran J Public Health 48(12):2270–2276

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.