



Original Article

## Genetic Variability of Methicillin Resistant *Staphylococcus Aureus* Strains Isolated from Burns Patients

Mehdi Goudarzi <sup>a,\*</sup>, Nobumichi Kobayashi <sup>b</sup>, Ali Hashemi <sup>a</sup>, Maryam Fazeli <sup>c</sup>, Masoumeh Navidinia <sup>d</sup><sup>a</sup> Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran<sup>b</sup> Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan<sup>c</sup> Department of Virology, Pasteur Institute of Iran, Tehran, Iran<sup>d</sup> School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

### ABSTRACT

#### Article history:

Received: March 14, 2019

Revised: March 25, 2019

Accepted: March 27, 2019

#### Keywords:

burn, coagulase, methicillin-resistant *Staphylococcus aureus*, minimum inhibitory concentration, *Staphylococcus aureus*

**Objectives:** *Staphylococcus aureus* is a nosocomial pathogen that provides a major challenge in the healthcare environment, especially in burns units where patients are particularly susceptible to infections. In this study, we sought to determine molecular types of *S. aureus* isolates collected from burns patients, based on staphylococcal protein A and coagulase gene polymorphisms.

**Methods:** Antibiotic susceptibility testing of 89 *S. aureus* strains isolated from burn wounds of patients was assessed using the Kirby-Bauer disk diffusion method. Strains were characterized by *spa* typing, *coa* typing, and resistance and toxin gene profiling.

**Results:** A total of 12 different *spa* types were identified with the majority being t790 (18%). Panton-Valentine leucocidin encoding genes were identified in *spa* types t044 (5.6%), t852 (2.2%) and t008 (2.2%). The most commonly detected antibiotic resistance gene was *ant* (4')-Ia (60.7%). Ten different *coa* types were detected and the majority of the tested isolates belonged to *coa* III (47.2%). All the high-level mupirocin-resistant and low-level mupirocin resistant strains belonged to *coa* type III.

**Conclusion:** The present study illustrated that despite the high frequency of *coa* III and *spa* t790 types, the genetic background of *S. aureus* strains in Iranian burns patients was diverse. The findings obtained are valuable in creating awareness of *S. aureus* infections within burns units.

<https://doi.org/10.24171/j.phrp.2019.10.3.08>  
pISSN 2210-9099 eISSN 2233-6052

©2019 Korea Centers for Disease Control and Prevention. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Burns patients are at an increased risk of colonization and subsequent infections by nosocomial pathogens due to the disruption of skin protective barrier and reduction of immune responses, which can lead to poor clinical outcomes and increased morbidity and mortality rates [1]. More than 70% of deaths in burns patients results from infection with nosocomial microorganisms. Based on the literature, many pathogens are responsible for infection in burns patients, but it is well established that *Staphylococcus aureus*, in particular, methicillin-resistant *S. aureus* (MRSA) strains, are one of the

most common nosocomial pathogens of burn wounds [2,3]. They could originate from the patient or transmitted easily by direct (i.e. contaminated hands or droplet) and indirect contact (i.e. airborne infection). Many of the published data in Iran indicates that multidrug-resistant (MDR) bacteremia and wound infections are the most important causes of mortality in burns patients [2,4,5]. Our previous study showed that there is a high rate of infection with MRSA in burns patients, which is a serious threat for the individual, and also a hazard to public health. Infection with MDR MRSA in burns patients may increase the economic burden of the healthcare system, and causes limitations to the therapeutic options available for

\*Corresponding author: Mehdi Goudarzi  
Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran  
E-mail: [goudarzim@yahoo.com](mailto:goudarzim@yahoo.com)

treatment [1-4].

A major concern with managing MRSA infections in burns patients is the lack of awareness of the molecular and resistance patterns of MRSA. Various molecular typing techniques have been employed for *S. aureus* isolates include pulsed-field gel electrophoresis, staphylococcal cassette chromosome *mecA* (SCC*mec*) typing, the accessory gene regulator (*agr*) typing, the *mec*-associated hypervariable region (*dru*), multilocus sequence typing, coagulase (*coa*) typing, and staphylococcal protein A (*spa*) typing [6]. Although the pulsed-field gel electrophoresis technique is a standard method, it is acknowledged that polymerase chain reaction based methods such as *coa* and *spa* typing due to its cost-benefit, rapidity and high throughput ability could be an effective method for routine typing of MRSA isolates [7,8].

The coagulase protein is genetically and antigenically divergent and is an important virulence factor for *S. aureus*. Sequence analysis of staphylocoagulase (SC) showed 6 regions including a signal sequence, N-terminal D1 region and D2 region, central region, 27-amino acid-repeat region and C-terminal sequence. Ten main types of SC have been described as a result of sequence diversity in the SC region [8-10].

The staphylococcal protein A (*spa*) gene contains 3 distinct regions including Fc, X and C regions; typing is based on the number of tandem repeats and the sequence variation in region X of the protein A gene [7]. The distribution of *spa* types of MRSA strains isolated from different geographical areas of the world, show different patterns [11].

In addition, *coa* typing is also simple to perform, easily interpreted, and requires minimal laboratory skills and equipment, and therefore could be a useful addition to *spa* typing for genotyping of *S. aureus*, including MRSA strains [8,9]. In Iran, there is very little information about the genetic diversity of *S. aureus* isolated from burns patients, therefore, the present study aimed to determine the resistance pattern, carriage of resistance determinants, and molecular characteristics of MRSA isolates collected from burns patients based on *coa* and *spa* gene polymorphism analysis.

## Materials and Methods

### 1. Sampling and data collection

In the current cross-sectional study, 89 *S. aureus* isolates were investigated from the burn wounds of patients during a 12-month study starting from October, 2016 to November, 2017. The Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran) also approved this study (IR.SBMU.MSP.REC.1396.878). Swab sticks and scalpel blades were used for sampling burn wounds with pus and hemorrhaging

tissues. Specimens were submitted to the laboratory within 4 hours of collection and were processed immediately. Standard biochemical tests, such as growth on mannitol salt agar, colony morphology on blood agar, and coagulase, DNase, and catalase production assays, were used to identify *S. aureus* isolates. A PCR assay targeting the *S. aureus*-specific *nuc* gene was performed to provide definitive identification of *S. aureus* isolates [1]. *S. aureus* isolates were stored at -70°C in Tryptic Soy Broth (TSB, Merck co., Germany) containing 20% glycerol until molecular analysis.

### 2. Antibacterial susceptibility testing and MRSA screening

*S. aureus* isolates were tested for *in vitro* susceptibility to ampicillin, amikacin, gentamicin, clindamycin, erythromycin, tetracycline, linezolid, teicoplanin, rifampicin, and quinupristin-dalfopristin (Mast Diagnostics Ltd, Merseyside, UK) by the disk diffusion method, based on the direction of the Clinical and Laboratory Standards Institute (CLSI) guide [12]. The minimum inhibitory concentration (MIC) for vancomycin and mupirocin was determined by the broth microdilution method in accordance with the CLSI recommendation. The MIC cutoff points for vancomycin based on the CLSI criteria were as follows: resistant,  $\geq 16$   $\mu\text{g}/\text{mL}$ ; intermediate, 4-8  $\mu\text{g}/\text{mL}$ ; and susceptible,  $\leq 2$   $\mu\text{g}/\text{mL}$ . Strains were considered to demonstrate low-level mupirocin resistance (LLMUPR) if the MIC value was between 8-256  $\mu\text{g}/\text{mL}$ , and high-level mupirocin resistance (HLMUPR) if the MIC value was  $\geq 512$   $\mu\text{g}/\text{mL}$ . MDR isolates are defined by resistance to 3 or more unique antibiotic classes [1,13]. ATCC29213 and ATCC25923 (*S. aureus*) were considered as the reference strains for quality control purposes. For MRSA screening, phenotypic growth was investigated around the cefoxitin disc (30  $\mu\text{g}$ ) placed on Mueller-Hinton agar plates (Merck, Germany), containing 4% NaCl. PCR was applied for genotypic amplification of *mecA* genes [12].

### 3. DNA preparation

Genomic DNA from 24-hour cultures of *S. aureus* isolates were extracted using the InstaGene Matrix kit (BioRad, Hercules co., CA, USA) based on the manufacturer's protocols. Briefly, for each sample, lysostaphin (Sigma-Aldrich co., USA) was added at a final concentration of 15 $\mu\text{g}/\text{mL}$  for cell wall lysis. After extraction, the purity of DNA was assessed using a nanometer.

#### 3.1. Detection of resistance and toxin encoding genes

PCR was performed to determine the presence of resistance (*mupA*, *mecA*, *erm(A)*, *vanA*, *msr(A)*, *mupB*, *erm(B)*, *vanB*, *msr(B)*, *erm(C)*, *tet(M)*, *aac(6')-Ie/aph(2'')*, *aph(3')-IIIa*, and *ant(4)-Ia*,) and toxin (*etb*, *eta*, *pvl*, *tst*) encoding genes as described by Rashidi et al [13] and Goudarzi et al [1].

#### 4. Typing of the *S. aureus* protein A locus

PCR amplification was carried out with specific primers for *spa* typing as suggested by Harmsen and colleagues [7]. DNA fragments containing the *spa* gene were purified using the QIAGEN PCR purification kit, and were subjected to DNA sequence analysis and nucleotide sequences on both strands were determined using an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer Co., Foster City, CA). Sequence-editing was performed using the Chromas software (Version 1.45, Australia). The edited sequences were assigned to particular *spa* types according to the guidelines described by a Ridom SpaServer database (<http://www.spaserver.ridom.de>).

#### 5. *Coa* typing

Four sets of multiplex PCR reactions were used for assigning SC types (I-X) according to the procedure of Hirose et al [5]. Set A contained primers for identifying SC types I, II, III, IVa, IVb, Va, and VI, while set B contained primers for identifying SC types VII, VIII, and X. Set 3 was used for identifying SC types IX and Vb. SC types IVa and IVb were distinguished using set 4 primers [8].

## Results

### 1. Sampling and antibiotic susceptibility

There were 89 *S. aureus* strains obtained from burns patients. All the strains under study were confirmed as MRSA due to the presence of the *mecA* gene as well as phenotypically. The results of antimicrobial susceptibility showed that all the isolates were susceptible to linezolid, teicoplanin, and vancomycin, but resistant to ampicillin. The highest rate of resistance was observed to erythromycin (86.5%), followed by gentamicin (84.2%), tetracycline (65.2%), amikacin (55.1%), clindamycin (51.7%), rifampicin (33.7%), and quinupristin-dalfopristin (25.8%).

The results of the microdilution method showed that 28.1% were resistant to mupirocin. Of these, 12 (13.5%) and 13 (14.6%) were HLMUPR and LLMUPR, respectively. The results of mupirocin MIC showed that 10 had MIC values of 16 µg/mL (40%), 3 had MIC of 32 µg/mL (12%), 11 (44%) had MIC ≥ 512 µg/mL, and 1 (4%) had MIC ≥ 1,024 µg/mL.

The MICs of vancomycin showed 31 (34.8%) isolates had MIC of 0.5 µg/mL, 18 (20.3%) had MIC of 1 µg/mL, 23 (25.8%) had MIC of 2 µg/mL and 17 (19.1%) had MIC of 8 µg/mL. Five different resistant phenotypes were identified among our MRSA isolates. The predominant resistance pattern included resistance to 4 antibiotics (52.8%), followed by 6 antibiotics (15.7%), 8 antibiotics (13.5%), 5 antibiotics (11.3%), and 7 antibiotics (6.7%), simultaneously.

### 2. Antimicrobial resistance and toxin genes

All MRSA strains in the current study harbored at least 2 antibiotic resistance genes. The most commonly detected antibiotic resistance genes were *ant* (4')-Ia (60.7%), *aac* (6')-Ie/aph (2") (55.1%), *aph* (3')-IIIa (46.7%), *tet*(M) (43.8%), *erm*(A) (29.2%), *erm*(C) (23.6%), *erm*(B) (20.2%), *msr*(B) (18%), *msr*(A) (13.5%), and *mupA* (13.5%). *vanA*, *vanB* and *mupB* genes were not detected among tested isolates. Analyzing toxin genes revealed that the most prevalent gene was *tst* (14; 15.7%), followed by *pvl* (9; 10.1%), *eta* (2; 2.2%) and *etb* (2; 2.2%).

### 3. *spa* typing

A total of 12 different *spa* genotypes were identified. t790 accounting for 18% (16/89 each) was found to be the predominant *spa* type, followed by t064 (13.5%; 12), t030 (11.3%; 10), t044 (10.1%; 9), t852 (9%; 8), t223 (7.9%; 7), t421 (6.7%; 6), t008 (6.7%; 6), t019 (6.7%; 6), t021 (4.5%; 4), t005 (3.4%; 3), and t10795 (2.2%; 2). PVL (*lukS-lukF*)-encoding genes were identified in 5 strains (55.6%, 5/9) with *spa* types t044, 2 strains with t852 (22.2%, 2/9), and 2 strains with t008 (22.2%, 2/9), while *tst* was identified among *spa* types t790 (64.3%, 9/14) and t223 (35.7%, 5/14). Of the 13 LLMUPR strains, 11 strains belonged to t790 (84.6%), and 2 strains (15.4%) to t008. *spa* types t064 (10 isolates; 83.3%) and t008 (2 isolates; 16.7%) were observed among 12 HLMUPR-MRSA strains.

### 4. SC typing

In the present study, 10 different types were detected and the most common *coa* type was type III (42; 47.2%) with *spa* types t790 (16; 18%), t064 (10; 11.2%), t030 (10; 11.2%) and t008 (6; 6.8%). The *coa* type II was observed in 13 isolates (14.6%) with *spa* types t019 (5; 5.6%), t044 (4; 4.5%) and t021 (4; 4.5%). The *coa* type VIII with *spa* types t852 (5; 5.6%) and t044 (5; 5.6%), were detected in 10 isolates (11.2%). From the 9 isolates with *coa* type I (10.1%), 4 isolates belonged to t223 (4.5%), 3 isolates to t852 (3.4%), and 2 isolates to t005 (2.2%). Both *coa* type IVb with *spa* type t421 and *coa* type VI with *spa* types t223 (3.4%) and t421 (1.1%) were detected in 4 isolates (4.5%). *coa* type IVa were identified in 2 isolates with *spa* type t10795 (2.2%). Two isolates with *spa* type t064 (2.2%) and 1 isolate with *spa* type t421 (1.1%) were found in strains with *coa* type X (3.4%).

PVL-encoding genes were distributed among *coa* types I (1 isolate; 1.1%), II (3 isolates; 3.4%), III (2 isolates; 2.2%), and VIII (3 isolates; 3.4%), while *tst* was identified among *coa* types I (2 isolates; 2.2%), III (9 isolates; 10.1%), VI (3 isolates; 3.4%). *eta* and *etb* were detected in *spa* types t019 (2.2%) and t030 (2.2%), respectively. Surprisingly, all the LLMUPR and HLMUPR-MRSA strains belonged to *coa* type III. The characteristics of the 89 MRSA strains isolated from burns patients have been summarized in Table 1.

Table 1. Characteristics of the MRSA strains isolated from burns patients.

SC type	spa type	Toxin genes (No.:%)	Antibiotic resistance profile (No.:%)	Antibiotic resistance genes (No.:%)	Total n (%)
I	t223	<i>tst</i> (2;50)	AP, CD, E, AK, GM, T (2;50) AP, CD, E, AK, GM, RP,T (2;50)	<i>MecA</i> (4;100), <i>erm(C)</i> (4;100), <i>msr(B)</i> (2;50), <i>erm(A)</i> (3;75), <i>ant (4')-Ia</i> (4;100), <i>aac (6')-Ie/aph (2'')</i> (3;75)	4 (4.5)
	t852	<i>pvl</i> (1;33.3)	AP, CD, E, AK, GM (2;66.7) AP, E, AK, GM, SYN (1;33.3)	<i>MecA</i> (3;100), <i>ant (4')-Ia</i> (3;100), <i>aac (6')-Ie/aph (2'')</i> (3;100), <i>aph (3')-IIIa</i> (3;100)	3 (3.4)
	t005	-	AP, CD, E, AK, GM, RP, T (1;50) AP, E, T, SYN (1;50)	<i>MecA</i> (2;100), <i>erm(A)</i> (2;100), <i>msr(B)</i> (1;50), <i>tet(M)</i> (2;100), <i>ant (4')-Ia</i> (2;100),	2 (2.2)
II	t019	<i>eta</i> (2;40)	AP, GM, CD, E (1;20) AP, GM, RP, T (1;20) AP, CD, E, AK, GM, T (3;60)	<i>MecA</i> (5;100), <i>tet(M)</i> (4;80), <i>ant (4')-Ia</i> (3;60), <i>aac (6')-Ie/aph (2'')</i> (4;80), <i>aph (3')-IIIa</i> (2;40)	5 (5.6)
	t044	<i>pvl</i> (3;75)	AP, CD, E AK, GM (3;75) AP, E, T, SYN (1;25)	<i>MecA</i> (4;100), <i>tet(M)</i> (3;75), <i>ant (4')-Ia</i> (4;100), <i>aac (6')-Ie/aph (2'')</i> (3;75), , <i>aph (3')-IIIa</i> (4;100)	4 (4.5)
	t021	-	AP, E, AK, GM, SYN (2;50) AP, CD, E, AK, GM, RP, T (2;50)	<i>MecA</i> (4;100), <i>ant (4')-Ia</i> (4;100), <i>aac (6')-Ie/aph (2'')</i> (3;75), <i>aph (3')-IIIa</i> (2;50), <i>erm(A)</i> (2;50) <i>erm(B)</i> (4;100),	4 (4.5)
III	t790	<i>tst</i> (9;56.3)	AP, T, E, MUP (4;25) AP, E, GM, T, SYN, MUP (3;18.8) AP, CD, E, AK, GM, RP, T, MUP (4;25) AP, CD, E, AK, GM, RP, T (1;6.5) AP, E, T, SYN (4;25)	<i>MecA</i> (16;100), <i>erm(C)</i> (12;75) <i>msr(A)</i> (10;62.5), <i>ant (4')-Ia</i> (12;75), <i>aac (6')-Ie/aph (2'')</i> (10;62.5), <i>aph (3')-IIIa</i> (9;56.3), <i>tet(M)</i> (14;87.5)	16 (18)
	t064	-	AP, CD, E, AK, GM, RP, T, MUP (8;80) AP, E, GM, T, SYN, MUP (2;20);	<i>MecA</i> (10;100), <i>erm(C)</i> (5;50) <i>erm(A)</i> (8;80), <i>ant (4')-Ia</i> (9;90), <i>aac (6')-Ie/aph (2'')</i> (5;50), <i>aph (3')-IIIa</i> (3;30), <i>tet(M)</i> (9;90), <i>mupA</i> (10;100)	10 (11.2)
	t008	<i>pvl</i> (2;33.3)	AP, E, AK, GM, SYN (2;33.3) AP, T, E, MUP (4;66.7)	<i>MecA</i> (6;100), <i>msr(B)</i> (5;83.3) <i>msr(A)</i> (2;33.3), <i>erm(A)</i> (5;83.3), <i>ant (4')-Ia</i> (4;66.7), <i>aac (6')-Ie/aph (2'')</i> (5;83.), <i>aph (3')-IIIa</i> (4;66.7), <i>mupA</i> (2;33.3)	6 (6.7)
	t030	<i>etb</i> (2;20)	AP, GM CD, E (6;60) AP, GM RP, T (2;20) AP, CD, E, AK, GM T (2;20)	<i>MecA</i> (10;100), <i>aac (6')-Ie/aph (2'')</i> (8;80), <i>aph (3')-IIIa</i> (6;60), <i>erm(A)</i> (6;60), <i>msr(B)</i> (8;80), <i>tet(M)</i> (6;60)	10 (11.2)
IVa	t10795	-	AP, CD, E, AK, GM (2;100)	<i>MecA</i> (2;100), <i>ant (4')-Ia</i> (2;100),	2 (2.2)
IVb	t421	-	AP, GM CD, E (2;50) AP, GM, RP, T (1;25) AP, CD, E, AK, GM (1;25)	<i>MecA</i> (4;100), <i>erm(B)</i> (3;75), <i>ant (4')-Ia</i> (4;100)	4 (4.5)
			Va	t005	-
VI	t223	<i>tst</i> (3;100)	AP, CD, E, AK, GM, T (2;66.7) AP, CD, E, AK, GM (1;33.3)	<i>MecA</i> (3;100), <i>aph (3')-IIIa</i> (3;100)	3 (3.4)
	t421	-	AP, CD, E, AK, GM (1;100)	<i>MecA</i> (1;100), , <i>aac (6')-Ie/aph (2'')</i> (1;100), <i>erm(B)</i> (1;100),	1 (1.1)
VII	t019	-	AP, GM, RP, T (1;100)	<i>MecA</i> (1;100), <i>erm(B)</i> (1;100),	1 (1.1)
VIII	t852	<i>pvl</i> (1;20)	AP, E, AK, GM, SYN (3;60) AP, GM, RP, T (2;40)	<i>MecA</i> (5;100), , <i>aac (6')-Ie/aph (2'')</i> (4;80), <i>aph (3')-IIIa</i> (5;100), <i>erm(B)</i> (4;80),	5 (5.6)
	t044	<i>pvl</i> (2;40)	AP, E, AK, GM, SYN (2;40) AP, GM, RP, T (3;60)	<i>MecA</i> (5;100), <i>erm(B)</i> (5;80),	5 (5.6)
X	t064	-	AP, E, AK, GM, SYN (2;100)	<i>MecA</i> (2;100), <i>ant (4')-Ia</i> (2;100)	2 (2.2)
	t421	-	AP, GM, RP, T (1;100)	<i>MecA</i> (1;100), <i>ant (4')-Ia</i> (1;100)	1 (1.1)

AP = ampicillin; CD = clindamycin; E = erythromycin; AK = amikacin; GM = gentamicin; MRSA = methicillin-resistant *Staphylococcus aureus*; MUP = mupirocin; RP = rifampicin; SYN = quinupristin-dalfopristin; T = tetracycline.



## Discussion

Several studies have revealed different findings of resistance rate of *S. aureus* isolated from burns patients which may be linked to various bacterial detection methods [10]. Although the prevalence rate of mupirocin-resistant *S. aureus* strains depends on various geographic areas and/or patient populations, a relatively high prevalence of mupirocin-resistant *S. aureus* strains (28.1%) was found in this study, which was similar to our previously reported rate from burns patients (28.3%) [1]. These results however, show a different prevalence of mupirocin-resistant *S. aureus* strains than reported by Abbasi-Montazeri et al (6%) [5], Shahsavan et al (40%) [14] and Saderi et al (2.7%) [15] in Iran. A higher prevalence rate of mupirocin resistance was detected among the isolates examined in this study, compared with that found in Greece (2%) [16], Egypt (17.8%) [17], and France (2.1%) [18]. Given the many factors affecting mupirocin resistance, the high rate of mupirocin resistance among *S. aureus* strains may be related to type and volume of our clinical samples, geographic or socioeconomic factors, infection control policies in healthcare and burn centers, and unrestricted policies in the use of mupirocin. Likewise, the present study showed that the frequency of HLMUPR (13.5%) was lower than LLMUPR (14.6%). The findings of a study in Egypt showed that 61.5% of mupirocin resistance isolates had the HLMUPR pattern, while low-level resistance was reported in 38.5% of mupirocin-resistant isolates [17].

Antimicrobial susceptibility testing revealed a high prevalence rate of resistance to erythromycin (86.5%), gentamicin (84.2%), tetracycline (65.2%), amikacin (55.1%), and clindamycin (51.7%). This finding is similar to that reported by Ko et al from 12 Asian countries [19]. *S. aureus* isolates with resistance to vancomycin have emerged in many parts of the world and their prevalence is increasing [20,21], and in accordance with other studies [1,5,13], all the isolates in our study were susceptible to vancomycin.

The predominant resistance genes in this study were *ant*(4')-*Ia* (60.7%), *aac*(6')-*Ie/aph*(2'') (55.1%), and *aph*(3')-*IIIa* (46.7%). Similarly, other researchers showed that aminoglycoside resistance genes are dominant in *S. aureus* clinical isolates [4,13,22]. In addition, in line with other studies, the prevalence of *ant*(4')-*Ia* gene was higher than that of the 2 other aminoglycoside resistance genes, *aac*(6')/*aph*(2'') and *aph*(3')-*IIIa* [13]. In a study conducted by Ardic et al [23] to determine aminoglycoside modifying enzyme genes in MRSA strains isolated in Turkey, 50 staphylococci from in-patients were assessed. They reported the majority of the isolates carrying *aac*(6')-*Ie/aph*(2'') (60.5%) and followed *ant*(4')-*Ia* (24%) and *aph*(3')-*IIIa* (8%). This increased rate among the isolates tested could be related to the source of the isolated strains (burn

wound), inappropriate use of aminoglycoside in the treatment of serious staphylococcal infections, and horizontal gene transfer among the strains.

The *tet*(M) gene was the 4<sup>th</sup> most detected antibiotic resistance gene among our isolates, and was detected in 43.8% of the strains. The frequency of *tet*(M) in the study of Rashidi et al on 105 *S. aureus* strains collected from an intensive care unit was 56.8% [13]. In the Emaneini et al study, this frequency was reported as 32.4% [4].

In the present study, 13.5% of isolates carried the *mupA* gene and were confirmed as HLMUPR MRSA. This finding is lower than the rates reported by Abbasi-Montazeri et al from Iran (34%) [5], González-Domínguez et al from Spain (27.2%) [24], and is higher than India (5%) [25] and Korea (1.8%) [26]. In general, variations in resistance genes were not unexpected, as these genes are subjected to a high, but variable selective pressure.

The findings in the present study provide insight into the epidemiology of *coa* and *spa* types in Iran. There were 89 MRSA strains isolated from burns patients with 10 *coa* genes identified by multiplex PCR, similar to the results of Afrogh et al [10], who showed 9 different patterns of *coa* genes among staff nose, and patients' clinical samples in Iran. The same results were reported by Janwithayanuchit et al [27] from Thailand, and Omar et al [28] from Egypt. Our study has reported that types III, II, I and VIII were the most common, and accounted for 47.2%, 14.6%, 10.1% and 10.1% of the isolates, respectively. Hirose et al [8], using the same primer, showed 7 different types of *coa* gene. They showed that the majority of MRSA isolates belonged to *coa* type II (91.9%), followed by type VII (3.9%), type I (1.7%), type Iva (1.1%), types III and X (0.6%) and type VI (0.2%). There were 2 MRSA isolates that were untypeable. The difference in coagulase types was attributed to geographical variation.

The present study reported the existence of t790 as the predominant *spa* type in 18% of isolates which were all *coa* type III. The low frequency of t790 in the study was in line with Udo's study from Kuwait, 2016 [21], and a study conducted by Goudarzi et al [1] who analyzed *S. aureus* strains isolated from burns patients in Iran (13.2%). More than half of *spa* t790 isolates in this study were carrying the *tst* gene (56.3%). LLMUPR was detected in 11 strains with t790 (12.4%). Although, virulence markers and drug resistance patterns in t790 isolates may vary, resistance to mupirocin and *tst* carriage in t790 isolates has been reported by several investigators [1,20].

In the current study, the second most frequent *spa* type was t064 (13.5%), distributed in *coa* types III (11.2%) and X (2.3%). All these strains were carrying the *mupA* gene and confirmed as HLMUPR strains. In agreement with our study, mupirocin resistance in t064 strains has been reported previously from Nigeria [29], Kuwait [21] and Ireland [20].

The third most common *spa* type was t030 detected in 11.3% of isolates, that all belonged to *coa* type III. This finding contrasts with our previous study in burns patients that reported t030 as one of the most common *spa* types amongst the tested isolates (24.5%) [1]. Similarly, the authors of a study of MRSA in Kuwait reported a low prevalence of *spa* type t030 (2.7%) [21].

Another *spa* type in our study was t044 (10.1%; 9 isolates), that of those with 5 of those isolates found to be positive for the *pvl* encoding gene (55.6%). Of 9 isolates with the t030 *spa* type, 4 isolates belonged to *coa* type II (44.4%) and 5 isolates to *coa* type VIII (55.6%). A review performed by Asadollahi and colleagues in 2018, to determine distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and -susceptible *S. aureus*, indicated that t044, distributed in 11 countries, was one of the most common *spa* types [11]. This *spa* type was also previously reported [30].

In agreement with studies performed in Kuwait [31], and Saudi Arabia [32], PVL-positive, multi-resistant *spa* type t852 isolates (9%) were found in our survey, pointing to their spread in the Gulf Persian Cooperative Council countries.

In this study, the *spa* type t223 was found in 7.9% of isolates for the first time in Iran. The resistance and virulence profiles of the t223 isolates (PVL-negative, *tst*-positive) was similar to t223 isolates reported in *S. aureus* strains isolated from children and parents in the Gaza Strip [33], and also t223 recovered from healthy individuals in Jordan [34], which may suggest the origin of *spa* type t223.

The t421 isolates was detected in small numbers, similar to Wang et al [35], who revealed that out of 99 MRSA isolates classified as ST239, 92 were ST239-*spa* t037 and 4 were ST239-*spa* t421.

In the present study, *spa* type t008 was detected at an incidence of 6.7%. The majority of the t008 MRSA isolates were resistant to mupirocin, as has been reported in t008 isolates obtained in Kuwait, the USA, and many European countries [20,21].

The *spa* type t019 was observed among 6 isolates (6.7%) with 5 isolates belonging to SC II and 1 isolate to SC VII. Two isolates harbored the *eta* encoding gene and belonged to SC II. *spa* type t019 has also been reported in the United States, and certain Asian and European countries like Egypt, Japan, Poland, and Taiwan [20]. The findings of the present study are in line with a multicenter study conducted during a 6-year period in 17 countries, that reported a low frequency of *spa* type t019 (3.6%) among tested isolates [36].

In line with our findings that showed the existence of *spa* type t021 in 4.5% of isolates, several researchers in Lebanon, Ireland, Romania, and Portugal reported a low frequency of the t021 among MRSA clinical isolates in comparison to other *spa* types, but not to the same extent [20].

t10795 with SC type IVa accounted for 2.2% of all strains. This was supported by Boswihi's report from Kuwait [21] that *spa* type t10795, was one of the *spa* types associated with the ST772-V, Bengal Bay clone, and was detected in small numbers. The Bengal Bay clone was previously reported in Italy, UK, Kuwait, Malaysia, Saudi Arabia, and UAE [21,35,37].

## Conclusion

There were 10 different SCs and the 12 *spa* types in the present study suggesting infection in burns patients is caused by *S. aureus* strains harboring different variants of the *coa* and *spa* genes. The present study showed the prevalence of *coa* type III and *spa* type t790 with a high level of MDR in the burns unit, which highlights the special attention for systematic surveillance and antimicrobial stewardship programs for *S. aureus* infections within burns patients.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

This work was supported by the Research Department of the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant No.: 13322).

## References

- [1] Goudarzi M, Bahramian M, Tabrizi MS, et al. Genetic diversity of methicillin resistant *Staphylococcus aureus* strains isolated from burn patients in Iran: ST239-SCmec III/t037 emerges as the major clone. *Microb Pathog* 2017;105:1-7.
- [2] Emameini M, Beigverdi R, van Leeuwen WB, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* isolated from burn patients: a systematic review and meta-analysis. *J Glob Antimicrob Resist* 2018;12:202-6.
- [3] Motallebi M, Jabalameli F, Asadollahi K, et al. Spreading of genes encoding enterotoxins, haemolysins, adhesin and biofilm among methicillin resistant *Staphylococcus aureus* strains with staphylococcal cassette chromosome mec type IIIA isolated from burn patients. *Microb Pathog* 2016;97:34-7.
- [4] Emameini M, Bigverdi R, Kalantar D, et al. Distribution of genes encoding tetracycline resistance and aminoglycoside modifying enzymes in *Staphylococcus aureus* strains isolated from a burn center. *Ann Burns Fire Disasters* 2013;26(2):76-80.
- [5] Abbasi-Montazeri E, Khosravi AD, Feizabadi MM, et al. The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. *Burns* 2013;39(4):650-4.
- [6] Deurenberg RH, Vink C, Kalenic S, et al. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2007;13(3):222-35.
- [7] Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant

- Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 2003;41(12):5442-8.
- [8] Hirose M, Kobayashi N, Ghosh S, et al. Identification of Staphylocoagulase Genotypes I-X and Discrimination of Type IV and V Subtypes by Multiplex PCR Assay for Clinical Isolates of *Staphylococcus aureus*. *Jpn J Infect Dis* 2010;63(4):257-63.
- [9] Sakai F, Takemoto A, Watanabe S, et al. Multiplex PCRs for assignment of Staphylocoagulase types and subtypes of type VI Staphylocoagulase. *J Microbiol Methods* 2008;75(2):312-7.
- [10] Afrough P, Pourmand MR, Sarajian AA, et al. Molecular investigation of *Staphylococcus aureus*, coa and spa genes in Ahvaz hospitals, staff nose compared with patients clinical samples. *Jundishapur J Microbiol* 2013;6(4):1-7.
- [11] Asadollahi P, Farahani NN, Mirzaii M, et al. Distribution of the most prevalent spa types among clinical isolates of methicillin-resistant and susceptible *Staphylococcus aureus* around the world: A review. *Front Microbiol* 2018;9:163.
- [12] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 27th Informational Supplement (M100-S27). 2017.
- [13] Nezhad RR, Meybodi SM, Rezaee R, et al. Molecular characterization and resistance profile of methicillin resistant *Staphylococcus aureus* strains isolated from hospitalized patients in intensive care unit, Tehran-Iran. *Jundishapur J Microbiol* 2017;10(3):e41666.
- [14] Shahsavvan S, Emaneini M, Khoshgnab BN, et al. A high prevalence of mupirocin and macrolide resistance determinant among *Staphylococcus aureus* strains isolated from burnt patients. *Burns* 2012;38(3):378-82.
- [15] Saderi H, Owlia P, Habibi M. Mupirocin resistance among Iranian isolates of *Staphylococcus aureus*. *Med Sci Monit* 2008;14(10):BR210-3.
- [16] Petinaki E, Spiliopoulou I, Kontos F, et al. Clonal dissemination of mupirocin-resistant staphylococci in Greek hospitals. *J Antimicrob Chemother* 2004;53(1):105-8.
- [17] Barakat GI, Nabil YM. Correlation of mupirocin resistance with biofilm production in methicillin-resistant *Staphylococcus aureus* from surgical site infections in a tertiary centre, Egypt. *J Glob Antimicrob Resist* 2016;4:16-20.
- [18] Desroches M, Potier J, Laurent F, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant *Staphylococcus aureus* (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring mupA. *J Antimicrob Chemother* 2013;68(8):1714-7.
- [19] Ko KS, Lee J-Y, Suh JY, et al. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *J Clin Microbiol* 2005;43(1):421-6.
- [20] Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 2011;6(4):e17936.
- [21] Boswihi SS, Udo EE, Al-Sweih N. Shifts in the clonal distribution of methicillin-resistant *Staphylococcus aureus* in Kuwait hospitals: 1992-2010. *PLoS One* 2016;11(9):e0162744.
- [22] Ida T, Okamoto R, Shimauchi C, et al. Identification of aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. *J Clin Microbiol* 2001;39(9):3115-21.
- [23] Ardic N, Sareyyupoglu B, Ozyurt M, et al. Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant staphylococci. *Microbiol Res* 2006;161(1):49-54.
- [24] González-Domínguez M, Seral C, Potel C, et al. Genotypic and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) clones with high-level mupirocin resistance. *Diagn Microbiol Infect Dis* 2016;85(2):213-7.
- [25] Gadepalli R, Dhawan B, Mohanty S, et al. Mupirocin resistance in *Staphylococcus aureus* in an Indian hospital. *Diagn Microbiol Infect Dis* 2007;58(1):125-7.
- [26] Park SH, Kim JK, Park K. *In vitro* antimicrobial activities of fusidic acid and retapamulin against mupirocin- and methicillin-resistant *Staphylococcus aureus*. *Ann Dermatol* 2015;27(5):551-6.
- [27] Janwithayanuchit I, Ngam-Ululert S, Paungmoung P, et al. Epidemiologic study of methicillin-resistant *Staphylococcus aureus* by coagulase gene polymorphism. *Science Asia* 2006;32:127-32.
- [28] Omar NY, Ali HAS, Harfoush RAH, et al. Molecular typing of methicillin resistant *Staphylococcus aureus* clinical isolates on the basis of protein A and coagulase gene polymorphisms. *Int J Microbiol* 2014;2014:650328.
- [29] O'Malley SM, Emele FE, Nwaokorie FO, et al. Molecular typing of antibiotic-resistant *Staphylococcus aureus* in Nigeria. *J Infect Public Health* 2015;8(2):187-93.
- [30] Mirzaii M, Emaneini M, Jabalameli F, et al. Molecular investigation of *Staphylococcus aureus* isolated from the patients, personnel, air and environment of an ICU in a hospital in Tehran. *J Infect Public Health* 2015;8(2):202-6.
- [31] Udo EE, Al-Lawati B-H, Al-Muharmi Z, et al. Genotyping of methicillin-resistant *Staphylococcus aureus* in the Sultan Qaboos University Hospital, Oman reveals the dominance of Pantone-Valentine leucocidin-negative ST6-IV/t304 clone. *New Microbes New Infect* 2014;2(4):100-5.
- [32] Monecke S, Skakni L, Hasan R, et al. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. *BMC Microbiol* 2012;12:146.
- [33] Al Laham N, Mediavilla JR, Chen L, et al. MRSA clonal complex 22 strains harboring toxic shock syndrome toxin (TSST-1) are endemic in the primary hospital in Gaza, Palestine. *PLoS One* 2015;10(3):e0120008.
- [34] AL-Bakri AG, AL-Hadithi H, Kasabri V, et al. The epidemiology and molecular characterization of methicillin-resistant staphylococci sampled from a healthy Jordanian population. *Epidemiol Infect* 2013;141(11):2384-91.
- [35] Wang W-Y, Chieuh T-S, Sun J-R, et al. Molecular typing and phenotype characterization of methicillin-resistant *Staphylococcus aureus* isolates from blood in Taiwan. *PLoS One* 2012;7(1):e30394.
- [36] Tristan A, Bes M, Meugnier H, et al. Global distribution of Pantone-Valentine leucocidin-positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg Infect Dis* 2007;13(4):594-600.
- [37] Udo EE, Boswihi SS, Al-Sweih N. High prevalence of toxic shock syndrome toxin-producing epidemic methicillin-resistant *Staphylococcus aureus* 15 (EMRSA-15) strains in Kuwait hospitals. *New Microbes New Infect* 2016;12(July 2016):24-30.