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High prevalence of direct repeat unit types of 10di, 8 h and 8i among methicillin resistant *Staphylococcus aureus* strains with staphylococcal cassette chromosome *mec* type IIIA isolated in Tehran, Iran

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Abstract

Background: The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is a main concern in burn care centers worldwide. The some reports of MRSA in Iran suggested that MRSA with type SCC*mec* III is common among burn patients. The aim of this study was to determine the prevalence, virulence genes, and antimicrobial susceptibility of the direct repeat units (*dru*) types of MRSA with SCC*mec* IIIA isolated from burn wounds in a burn care center in Tehran, Iran.

Methods: In total, 165 *S. aureus* isolates were collected from clinical samples. In order to detect MRSA isolates, the *mec*A gene was amplified through the polymerase chain reaction (PCR) method. Antimicrobial susceptibility was tested using the disc agar diffusion test. Moreover, the PCR method was applied to determine SCC*mec* types, virulence genes, and antimicrobial resistance genes. The *dru* region was sequenced and thereby, *dru* types and *dru* repeats were identified. A similarity matrix was used to create minimum spanning tree (MST).

Results: The prevalence of MRSA was 69% (114 out of 165 isolates). Most of MRSA isolates (61 out of 114, 53.5%) were SCC*mec* type IIIA. All MRSA isolates were vancomycin-susceptible and more than 68% of MRSA isolates with SCC*mec* type IIIA were mupirocin resistant. The successful *dru* typing of isolates with SCC*mec* type IIIA revealed fourteen different *dru* types. There were two new *dru* types, namely dt10di and dt7aj. MST analysis indicated the presence of the three clusters of dt10di (cluster I), dt8i-dt8 h (cluster II), and dt11c-dt10ao-dt11dd-dt11a-dt10a (cluster III). There were significant differences between clusters I and II respecting antimicrobial resistance pattern and virulence genes.

Conclusion: Three main *dru* clusters are prevalent in the study setting. The main *dru* types in the setting are dt10di, dt8i, and dt8 h. *Dru* typing can be used to differentiate MRSA strains with SCC*mec* IIIA.

Keywords: Methicillin-resistant *Staphylococcus aureus*, SCC*mec* typing, *Dru* type, Virulence factor

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Background

Staphylococcus aureus (*S. aureus*) is one of the most common causes of infection among patients with severe burn wounds [1, 2]. Colonization of burn wounds with *S. aureus* can cause septicemia and substantially increase mortality rate [3]. The pathogenicity of *S. aureus* strains depends on different virulence factors such as Panton-Valentine leukocidin (PVL), staphylococcal enterotoxins, and hemolysins alpha, beta, gamma, and delta [4].

One of the major concerns in the treatment of infections is antimicrobial resistance, particularly to methicillin [5, 6]. Methicillin resistance is encoded by the *mec*A gene. In 2011, a second gene, *mec*C has been discovered that also causes methicillin/beta-lactam resistance. Both genes are situated on large, potentially mobile genetic elements, so-called SCC*mec* elements (staphylococcal cassette chromosome *mec*) [7, 8]. So far, thirteen main types of SCC*mec* have been identified [9]. These types differ from each other in size and genetic composition. Some studies reported that MRSA with SCC*mec* III is the most prevalent type of *S. aureus* in burn care centers in some countries of the world [2, 10–12]. The few reports of MRSA in Iran also suggested that MRSA with type SCC*mec* III is common among burn patients [13, 14]. *mecC* MRSA represent a recently recognised form of MRSA, encoding a divergent *mec* gene, which can colonise and cause disease in humans and a wide range of other host species [8].

A variable number of tandem repeats region including 40-bp of direct repeat units (*dru*) has been detected downstream to the *mec*A gene close to IS431 in the SCC*mec* element. The sequencing of this region can be used for detecting and subtyping methicillin-resistant *S. aureus* (MRSA) [15, 16]. A study reported that the *dru* typing of ST239 MRSA isolates provided the clearest distinction between SCC*mec* IIIA and III isolates [17]. *Dru* types are stable enough and hence, can be used in epidemiological analyses [16, 18].

Despite the high prevalence of MRSA with SCC*mec* III in burn care centers, there is limited information about its *dru* types. Therefore, the present study was conducted in a burn care center in Tehran, Iran, to determine the prevalence, virulence genes, and antimicrobial susceptibility of the *dru* types of MRSA with SCC*mec* IIIA isolated from burn wounds.

Materials and methods

Bacterial isolates

In total, 165 non-duplicate *S. aureus* isolates were collected using sterile swab from burn wound infections in a burn care center in Tehran, Iran. Sampling was done in four consecutive trimesters from June 2013 to June 2014. Isolates were primarily identified as *S. aureus* based on colony morphology, Gram staining, and catalase, coagulase, mannitol fermentation, and deoxyribonuclease tests [19]. Then, the identity of *S. aureus* isolates was confirmed through the amplification of the *fem*A gene based on the polymerase chain reaction (PCR) method and using primers explained in an earlier work [20]. After that, in order to identify MRSA isolates, the *mec*A gene was detected using specific primers [21, 22]. Finally, MRSA isolates were subjected to further testing.

Antimicrobial susceptibility tests

The antimicrobial susceptibility of MRSA isolates was tested via the disc diffusion method on Mueller-Hinton agar based on the guideline recommended by the Clinical and Laboratory Standards Institute (CLSI) [23]. The discs used in this study were cotrimoxazole 25 μg, erythromycin 15 μg, clindamycin 2 μg, mupirocin 5 μg, rifampin 5 μg, linezolid 30 μg, and quinupristin-dalfopristin 15 μg (MAST, Merseyside, England). The microbroth dilution method was also used to determine the antimicrobial minimum inhibitory concentration (MIC) of oxacillin and vancomycin (Sigma, Steinheim, Germany). The control strain was *S. aureus* ATCC 29213. Moreover, the PCR method was employed to amplify the *erm*A, *erm*C, *bla*Z, and *mup*A genes using specific primers [24].

SCC*mec* typing and detection of virulence genes

SCC*mec* types were determined through the Multiplex-PCR as described elsewhere [21, 25]. The PCR method was used to detect the genes encoding haemolysins (*hla, hlb*), toxic shock syndrome toxin (*tst*), exfoliative toxin A (*eta*), staphylococcal enterotoxins (*sea, seb* and *sec*), and Panton–Valentine leukocidin (*pvl*) among isolates with SCC*mec* type III [22, 26, 27].

Dru typing

Dru region was detected using the primers HVR1:59 ACTATTCCCTCAGGCGTCC 39 and HVR2:59 GGAG TTAATCTACGTCTCATC 39 [28]. The sequencing of all PCR products was performed on both strands through the same primers used in the primary PCR. The ChromasPro software (Technelysium Pty, Australia) was employed to analyze and align sequences. New repeats were confirmed through re-sequencing. Then, the nomenclature published by Goering et al. [16] (available at www.dru-typing.org) was used to detect and name *dru* repeats (dr, 40 bps) and *dru* types (dt, the combination of *dru* repeats). A minimum spanning tree (MST) was also created via the BioNumerics software v. 7.6.1 (Applied Maths, Austin, USA) and distance intervals were created using a bin distance of 1.0%. Clustering was done based on the distances among *dru* types. Accordingly, *dru* types, separated by a single MST distance,

were considered to be closely related to each other and hence, were assigned to an identical cluster.

Data analysis

Data were presented using the measures of descriptive statistics. Moreover, the Fisher's exact test was conducted for categorical comparisons. The level of significance was set at less than 0.05.

Results

Among 165 *S. aureus* isolates, 114 (69%) were MRSA. Most of MRSA isolates (61/114; 53.5%) were SCC*mec* type IIIA. Also, twenty (17.5%) were identified as SCC*mec* type V, two (1.7%) as SCC*mec* type I, and 31 (27.2%) as non-typable.

All MRSA isolates showed susceptibility to vancomycin ($MIC_{50} \leq 1$ μg/ml, $MIC_{90} \leq 2$ μg/ml), while most MRSA isolates (68%) were resistant to mupirocin.

All MRSA isolates were resistant to cefoxitin and more than 73% of them were resistant to erythromycin and clindamycin. Moreover, around 53% of MRSA isolates were resistant to mupirocin and trimethoprim-sulfamethoxazole. However, only a few of MRSA isolates showed resistance to rifampin (22%), quinupristin/dalfopristin (2%), and linezolid (2%). The MIC of oxacillin in 100% of MRSA isolates was higher than 64 μg/mL and all of isolates were susceptible to vancomycin. The most prevalent antimicrobial resistance gene was *blaZ* which was found in 85% of isolates followed by *ermA*, *mup* and *ermC* which were found in 65, 64 and 57% of isolates, respectively. The most prevalent genes encoding virulence factors in MRSA isolates were *hla* (61%), *hlb* (44%), *sea* (23%) and *seb* (2%), respectively. The *sec*, *eta*, *tst*, and *pvl* genes were not detected in any of MRSA isolates in this study.

As Table 1 shows, all *dru* types of SCC*mec* type III were successfully identified, which included fourteen different *dru* types with fifteen *dru* repeats. Among the identified *dru* types, two were new (dt10di and dt7aj). The most prevalent *dru* types among SCC*mec* type IIIA isolates were dt10di, dt8 h, and dt8i. Each minor *dru* type was observed only in one isolate.

MST analysis revealed three clusters of SCC*mec* type IIIA, namely dt10di (cluster I), dt8i-dt8 h (cluster II), and dt11c-dt10ao-dt11dd-dt11a-dt10a (cluster III) (Fig. 1). Analysis of *dru* types indicated that cluster I was the most prevalent *dru* cluster in the first nine months of the study (i.e. from June 2013 to February 2014), while cluster II was the most prevalent cluster in the last trimester of the study (i.e. from March to May 2014) (Table 1).

The results of antimicrobial susceptibility tests on clusters I and II indicated that resistance to rifampin in cluster II was significantly higher than cluster I ($P = 0.003$). Moreover, the results of virulence gene analysis illustrated the significantly higher prevalence of the virulence gene *hlb* in cluster II compared with cluster I ($P = 0.01$).

Analysis of antimicrobial susceptibility pattern indicated that 92% of isolates in cluster I were resistant to erythromycin, clindamycin, cotrimoxazole, and mupirocin. On the other hand, 44% of isolates in cluster II showed resistance to erythromycin, clindamycin, cotrimoxazole, rifampin, and mupirocin.

Analysis of the patterns of antimicrobial resistance gene also showed that the most prevalent patterns in clusters I and II were *ermA* + *ermC* + *blaZ* + *mup* and *ermA* + *ermC* + *blaZ*, respectively. Moreover, the greatest frequency of virulence gene patterns in these two clusters was respectively related to *hla* + *sea* and *hla* + *hlb*. However, the *sec*, *eta*, *tst*, and *pvl* genes were detected in none of the MRSA isolates.

Discussion

This study aimed to determine the prevalence, virulence genes, and antimicrobial susceptibility of the *dru* types of MRSA with SCC*mec* III isolated from burn wounds in a burn care center in Tehran, Iran. Study results illustrated that the prevalence of MRSA among patients with burn wounds was 69%. This prevalence rate is higher than the rates reported in earlier studies in Iran [20, 29, 30], except for a study in burn centers in Ahvaz which reported a prevalence rate of 80% [31]. Moreover, the prevalence of MRSA in the present study was higher than the rates reported in burn centers in the United States (32%), European countries (26%), and Australia (23%) [32–34]. This difference can be attributed to the differences in infection control policies used in different areas.

Our study also showed that a high proportion of MRSA isolates harbored SCC*mec* type IIIA. Similarly, studies in Iran and other Asian countries reported the high prevalence of SCC*mec* type IIIA [13, 35–37]. SCC*mec* types III and IIIA were also detected in Hungarian and Brazilian clones [36]. MRSA isolates with SCC*mec* types III can act as large reservoirs of enterotoxins and antimicrobial resistance and prevail in communities. Our previous survey shown that the *sea, hla, fib* and *icaA* were most frequent genes encoding virulence factors among MRSA with SCC*mec* type IIIA [4]. Therefore, accurate diagnosis and effective control strategies are essential to minimize their prevalence. Otherwise, they may become prevalent and cause serious consequences in a near future.

None of the MRSA isolates in the present study were resistant to vancomycin. Although MRSA non-resistance to vancomycin in our study supports the effectiveness of this antibiotic in managing MRSA, this antibiotic should be prescribed with great caution in order to prevent the emergence of vancomycin-resistant *S. aureus*.

Table 1 Antimicrobial resistance pattern, antibiotic resistance genes, virulence genes, and *dru* types in MRSA isolates with type III SCC*mec*

Sampling Time	Sample no.	*Dru* type	Cluster	Antibiotic resistance	Antibiotic resistance genes	Virulence genes
First trimester	1	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*sea*
	2	10di	I	E, CD, TS, MUP	*ermC, blaZ*	*hla, sea*
	3	11dd	III	E, CD, TS, SYN, MUP	*ermA, mup*	*hla, hlb*
	4	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	5	7aj	–	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, sea*
	6	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	7	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	8	14 k	–	E, CD, TS, MUP	*ermC, blaZ*	*sea*
	9	11c	III	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	10	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, hlb, seb*
	11	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, seb*
	12	11a	III	E, TS, MUP	*blaZ, mup*	–
	13	5 k	–	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	14	10di	I	E, CD, TS, MUP	*ermA, blaZ, mup*	*sea*
	15	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	16	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	–
	17	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
Second trimester	18	10di	I	E, CD, TS, MUP	*ermC*	–
	19	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	20	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	21	10di	I	E, CD, TS, MUP	*blaZ*	*sea*
	22	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	23	10di	I	E, CD, TS, MUP	*ermA, ermC, mup*	*hla, sea*
	24	10di	I	E, CD, TS, MUP	*ermC, blaZ*	*hla, sea*
	25	10di	I	E, CD, TS, MUP	*ermA, blaZ*	*Sea, seb*
	26	10ao	III	E, CD, MUP	*ermA, ermC, mup*	–
	27	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, hlb, sea*
	28	10di	I	SYN, MUP	*ermC, blaZ, mup*	*sea*
	29	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	30	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla*
	31	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*sea*
	32	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	33	8af	–	E, CD, RP, MUP	*ermC, blaZ, mup*	–
	34	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
Third trimester	35	10di	I	E, CD, TS, MUP	*ermC, blaZ*	–
	36	10di	I	E, CD, TS, MUP	*ermA, ermC, mup*	–
	37	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	38	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, hlb, sea*
	39	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, hlb, sea*
	40	10a	III	MUP	*ermA, ermC, blaZ, mup*	*hla*
	41	10di	I	E, CD, TS, MUP	*ermC, blaZ, mup*	*seb*
	42	10di	I	E, CD, TS, SYN, LZD, RP, MUP	*ermA, ermC, blaZ, mup*	–
	43	10di	I	E, CD, TS, MUP	*ermA, ermC*	*sea*

Table 1 Antimicrobial resistance pattern, antibiotic resistance genes, virulence genes, and *dru* types in MRSA isolates with type III *SCCmec (Continued)*

Sampling Time	Sample no.	*Dru* type	Cluster	Antibiotic resistance	Antibiotic resistance genes	Virulence genes
	44	10di	I	E, CD, TS, MUP	*ermA, ermC*	–
	45	10di	I	E, CD, TS	*ermA, ermC, blaZ, mup*	*hla, hlb*
	46	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, sea*
	47	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, sea*
	48	10di	I	E, CD, TS, MUP	*mup*	–
	49	10c	–	E	*blaZ*	*sea*
Forth trimester	50	8i	II	E, TS, MUP	*ermA, ermC, blaZ, mup*	*hla, hlb*
	51	8 h	II	–	*blaZ, mup*	*hla*
	52	6d	–	–	*ermA, blaZ, mup*	*hla, hlb*
	53	8i	II	CD	*ermA, ermC, mup*	*hla, hlb*
	54	8i	II	E, CD, TS, MUP	*ermA, ermC, blaZ*	*hla, hlb, sea*
	55	8 h	II	E, TS, RP, MUP	*blaZ*	*hlb, sea*
	56	8 h	II	E, CD, TS, RP, MUP	*ermA, ermC, blaZ, mup*	*sea*
	57	8 h	II	E, CD, TS, RP, MUP	*ermC*	–
	58	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	59	8i	II	E, CD, TS, RP, MUP	*ermA, ermC, blaZ*	*hla, hlb*
	60	10di	I	E, CD, TS, MUP	*ermA, ermC, blaZ, mup*	–
	61	8 h	II	E, CD, TS, RP, MUP	*ermC*	–

FOX: Cefoxitin, E: Erythromycin, CD: Clindamycin, TS: trimethoprim/ Sulfamethoxazole, RP: Rifampicin,
SYN: quinupristin/dalfopristin, LZD: Linezolid, MUP: Mupirocin

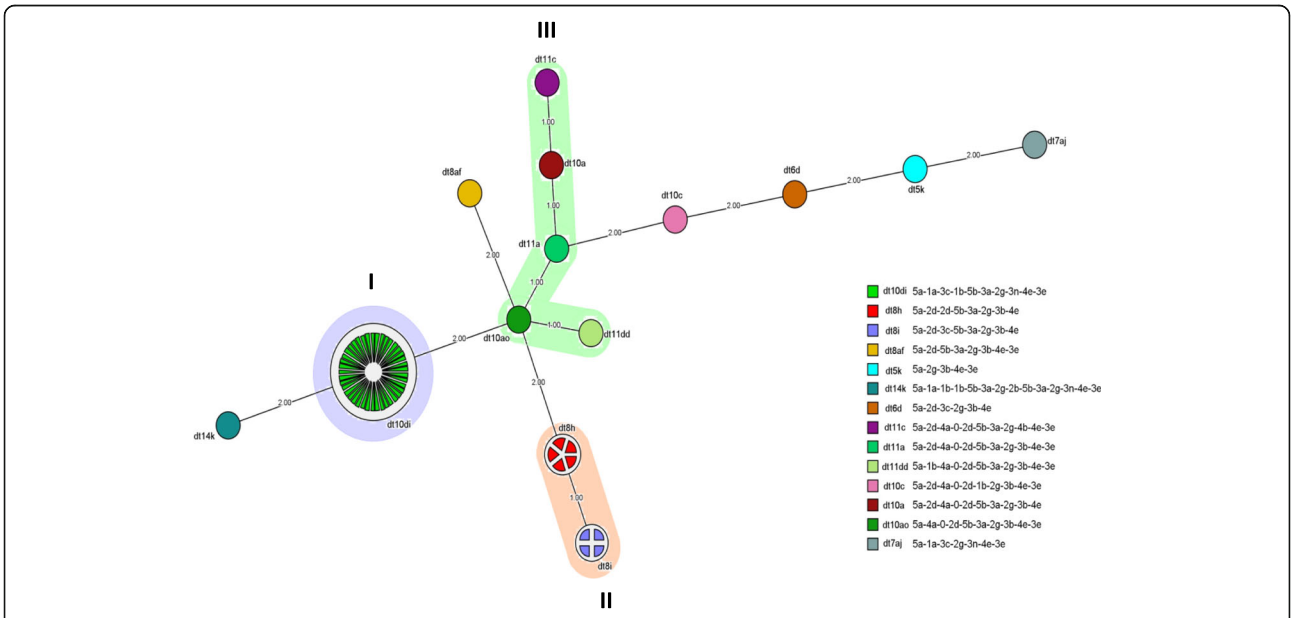

Fig. 1 Minimum Spanning Tree generated using the BioNumerics software representing the fourteen *dru* types and the fifteen *dru* repeats observed in the studied isolates. Numerical values on the branches indicate the similarities (MST distance) between different *dru* types. BioNumerics software created similarity values (termed bins) and converted these values into distance units. The bin unit distance was set at 1% (i.e., *dru* types at a distance of 1 on the MST had a similarity of more than 99%, while *dru* types at a distance of 2 had a similarity of 98–99%, and so on). *Dru* types were assigned to the same cluster (depicted in color) if they were separated by an MST distance of 1 (i.e. if they showed a similarity of at least 99%)

The prevalence of MRSA resistance to mupirocin in the present study was 68%. However, earlier studies reported lower rates of MRSA resistance to mupirocin. For instance, studies on burn patients in England, India, and Iran reported that this rate was 5.1, 22.7, and 34%, respectively [31, 38, 39]. The use of mupirocin for treating burn wound infections caused by *S. aureus* might have caused resistance to mupirocin among MRSA isolates. Previous studies in the setting of the present study showed *Pseudomonas aeruginosa* as one the main causes of burn wound infection in recent years [40, 41]. Of course, there are no detailed data about the use of mupirocin in the study setting. However, a study reported a substantially high prevalence of mupirocin-resistant MRSA among patients previously treated with mupirocin. Similarly, a high likelihood of mupirocin resistance was observed among patients with *Pseudomonas* infection treated with cefepime [42]. Mupirocin is produced by the Gram-negative bacterium *Pseudomonas fluorescens* and hence, *Pseudomonas* is inherently resistant to mupirocin [43, 44]. Furthermore, the *mupA* gene, which mediates mupirocin resistance in *Pseudomonas*, can move between bacterial isolates and thereby, cause mupirocin resistance in other isolates such as MRSA [45–47].

Our results also indicated dt10di, dt8 h, and dt8i as the most prevalent *dru* types in MRSA with SCC*mec* type IIIA (Fig. 1). The *dru* type dt10di (i.e. cluster I) was mostly prevalent in the first nine months of the study, while the *dru* types dt8 h and dt8i (i.e. cluster II) were mostly prevalent in the last trimester. These findings may be due to the fact that the *dru* types dt8 h and dt8i might have been entered to the study setting in the last trimester or might have been emerged as a result of the polymorphism of dt10ao *dru* type. Unlike our results, a study in Malaysia reported nine *dru* types in SCC*mec* type III, the most prevalent of which was the dt13d *dru* type (41%) [17]. Moreover, a study in Scotland detected 25 *dru* types and 33 *dru* repeats, with the dt10a and dt7c as the most prevalent *dru* types, respectively [16]. The greater number of *dru* types and *dru* repeats in that study compared to our study can be due to the fact that samples in the present study were selected from a single hospital, while samples in that study were selected from different hospitals.

The results of the present study also indicated the higher prevalence of cluster II in the last three months of the study. This may denote the increasing prevalence of this cluster. Moreover, compared with cluster I, cluster II had higher resistance to rifampin. Besides, the presence of the *hlb* gene was more prevalent in cluster II. The most prevalent antimicrobial resistance pattern in cluster I was erythromycin+clindamycin+cotrimoxazole+mupirocin, while the most prevalent antimicrobial resistance pattern in cluster II was erythromycin+clindamycin+cotrimoxazole+rifampin+mupirocin. The latter finding confirms the higher resistance to rifampin in cluster II. Of course, the number of samples in cluster II was small and hence, further studies with larger samples are recommended.

Virulence gene patterns in clusters and *dru* types in the present study showed that the most prevalent virulence gene patterns in cluster I were *hla* + *sea* (39%) and *sea* (12%), while the most prevalent virulence gene pattern in cluster II was *hla* + *hlb* (33.3%) (Data were not presented). Besides, *hla* + *hlb* virulence genes in the dt8i *dru* type were more prevalent than the other *dru* types. Considering the significant roles of these genes in exacerbating skin infections, the prevalence of these strains can complicate the conditions of patients with burn wound infections. Of course, because of the small sample size in cluster II, drawing definitive conclusions in this area is impossible.

Conclusion

This study shows the high prevalence of SCC*mec* IIIA among MRSA strains isolated from burn wounds in a teaching hospital in Tehran, Iran. These strains are highly resistant to multiple antibiotics. The three most common *dru* types among these strains are dt10i, dt8 h, and dt8i. Clusters with these *dru* types significantly differ from each other respecting their antimicrobial resistance patterns.

Abbreviations

ATCC: American Type Culture Collection; bp: base pair; CD: Clindamycin; CLSI: Clinical and Laboratory Standards Institute; DAD: Disk Agar Diffusion; *dru*: direct repeat unit; dt: *dru* type; E: Erythromycin; *eta*: exfoliative toxin A; LZD: Linezolid; MIC: Minimum Inhibitory Concentration; MRSA: Methicillin-Resistant *Staphylococcus aureus*; MST: Minimum Spanning Tree; MUP: Mupirocin; PCR: Polymerase Chain Reaction; *pvl*: Panton–Valentine leukocidin; RP: Rifampicin; *S. aureus*: *Staphylococcus aureus*; SCC*mec*: Staphylococcal Cassette Chromosome *mec*; SYN: quinupristin/dalfopristin; TS: trimethoprim/ Sulfamethoxazole; *tst*: toxic shock syndrome toxin; VRSA: Vancomycin-Resistant *Staphylococcus aureus*

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Availability of data and materials

Please contact author for data requests.

Authors' contributions

ME and FJ designed the study. MM and ME drafted the manuscript. RB performed data analysis. All authors provided intellectual input to the study and read and approved the final manuscript.

Ethics approval and consent to participate
This study has the formal approval of the Research Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (approval number: IR.TUMS.MEDICINE.REC.1397.235).

Consent for publication
Not Applicable.

Competing interest
The authors declare no competing interests.

Publisher's Note

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