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Characteristics of methicillin-resistant *Staphylococcus aureus* carrying the toxic shock syndrome toxin gene: high prevalence of clonal complex 22 strains and the emergence of new *spa* types t223 and t605 in Iran

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains that carry the *tst* gene are disseminated worldwide with varying regional incidences and different genetic backgrounds. The data on molecular characteristics of these strains is insufficient in Iran. The present study aimed to assess the characteristics and distribution of *spa* types of *tst*-positive MRSA strains. We investigated 89 MRSA isolates carrying the *tst* gene with *spa* typing, resistance gene detection and *in vitro* antimicrobial susceptibility. Of the 89 tested isolates, 61 (68.5%) were confirmed as multidrug resistant (MDR). The isolates were distributed across seven clonal complexes (CCs) including CC22 (42.7%), CC8 (28.1%), CC5 (11.2%), CC59 (5.6%), CC30 (4.5%), CC80 (4.5%) and CC15 (3.4%). *spa* typing identified 11 distinct types, with t223 (16.9%) and t790 (15.7%) being the most prevalent. All high-level mupirocin-resistant strains belonged to t002 (n = 8) and low-level mupirocin-resistant strains belonged to t790 (n = 6) *spa* types. Fusidic-acid-resistant isolates belonged to t437 (n = 3). $iMLS_B$ phenotype was observed in t005 (6.7%), t002 (5.6%), t790 (3.4%), and t030, t044 and t084 (each 2.2%). It was found that in the *tst*-carrying MRSA strains, there were genetic diversities with a majority of the t223 *spa* type. Indeed, there is a necessity for more constructive surveillance/infection control strategies to address the prevalence and prevention of the emerging *spa* types.

Keywords: Methicillin-resistant *Staphylococcus aureus*, PCR, *spa* typing, *Staphylococcus aureus*, toxic shock syndrome toxin
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Introduction

Staphylococcus aureus is one of the principal nosocomial pathogens with substantial morbidity and mortality. Unfortunately, recent reports described a continuous and heavy burden of hospital-acquired *S. aureus* infections around the world [1,2]. Currently, *S. aureus* has become an emerging problem in health-care settings and is becoming a great threat to public health. Infections caused by this bacterium are further exacerbated by the widespread circulation and emergence of drug-resistant strains; particularly, methicillin-resistant *S. aureus* (MRSA) [2]. Initial reports of MRSA strains indicated an increasing rate of their prevalence in some Asian countries [1]. Recently, a systematic review and meta-analysis reported a 43% prevalence of MRSA strains in Iran [3]. Emerging simultaneous resistance to multiple antibacterial agents among MRSA strains has significantly limited the availability of chemotherapeutic agents for the treatment of staphylococcal infections and leads to deterioration of disease [1–3].

Staphylococcus aureus produces cell-wall-associated virulence determinants and a broad spectrum of extracellular proteins, which are related to the severity of the infection [2]. A major virulence factor is toxic shock syndrome toxin-1 (TSST-1, encoded by the gene *tst*). When TSST, a protein of 29.1 kDa, enters the blood it causes the release of tumour necrosis

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factor-α, non-specific T-cell proliferation, and interleukin-1 and interleukin-2 production, which can lead to a variety of severe and life-threatening diseases [4,5]. In the absence of proper treatment, a lethal shock could occur within 24 h after the onset of symptoms [2,4,6,7]. Therefore, particular attention should be paid to MRSA strains carrying the *tst* gene. Although, recent studies have been focused on better understanding of the molecular epidemiology and detection of virulence genes of MRSA isolates carrying the *tst* gene, epidemiological data on *tst*-carrying MRSA in Iran is not sufficient. Accordingly, the present study was designed to describe the phenotypic resistance pattern, and the presence of the virulence factors. *spa* typing which was used to characterize the genotype of the MRSA carrying *tst* gene.

Materials and methods

Study design, isolation of bacterial isolates and ethics statement

In this cross-sectional study, 89 non-duplicated MRSA isolates carrying the *tst* gene were isolated from various clinical samples from five teaching hospitals in Tehran, Iran. The experiment was carried out from February 2018 to January 2019. Clinical specimens included wound (35.9%), blood (30.4%), pus (10.9%), urine (8.7%), sputum (5.4%), conjunctivitis (5.4%) and body fluids (3.3%) from both genders and different age groups. The Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran certified the protocol of this project (IR. SBMU. MSP.REC. 1397.711). Preliminary detection of *S. aureus* isolates used standard microbiological and biochemical techniques [8]. Positive isolates were verified by PCR targeting the *S. aureus* species-specific *nuc* gene [7]. MRSA screening was performed by the disc-diffusion method by using cefoxitin (30 μg) discs in Mueller–Hinton agar (Merck, Darmstadt, Germany) and also by detection of the *mecA* gene [8,9]. The entire strains were also analysed for the presence of the *tst* gene by PCR assay according to the previously published method [9].

Criteria for identifying hospital and community-onset

Hospital-onset (HO) *S. aureus* was established if the positive culture of *S. aureus* was obtained 96 hours or more after admission to a hospital. Community-onset (CO) infection was defined as when the culture was obtained before 4 days of hospitalization by having one or more of the following criteria: (a) a history of hospitalization, surgery, dialysis, or residence in a long-term care facility in 12 months before the culture date, or (b) the presence of a central vascular catheter within 2 days before *S. aureus* culture [8].

Antimicrobial susceptibility testing

The Kirby–Bauer disc-diffusion method on Mueller–Hinton agar was used to test the susceptibility of the isolates against amikacin, gentamicin, tobramycin, kanamycin, tetracycline, erythromycin, clindamycin, linezolid, teicoplanin, ciprofloxacin, rifampicin, quinupristin-dalfopristin and trimethoprim-sulfamethoxazole (Mast Co., Bootle, UK) based on the CLSI guideline. Susceptibility to vancomycin, mupirocin, tigecycline and fusidic acid was assessed by the broth microdilution method to determine the MIC titre. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints was used to determine MIC titres of fusidic acid and tigecycline (EUCAST 2018). The results of other antibiotics were interpreted by using the CLSI 2018 breakpoints. Low-level and high-level mupirocin resistance (LLMUPR, HLMUPR), inducible macrolide-lincosamide-streptogramin group B ($iMLS_B$) and constitutive ($cMLS_B$) macrolide-lincosamide-streptogramin group B were identified based on the CLSI guideline. Susceptibility testing was quality controlled using *S. aureus* ATCC 25923, ATCC 43300 and ATCC 29213 strains. Powders of antibiotics were all obtained from Sigma Chemical Co. (St Louis, MO, USA).

DNA extraction and amplification of resistance-related genes

Genomic DNA was extracted from pelleted bacteria using the phenol–chloroform extraction method. The genes encoding the *mecA*, *mecC*, *vanA*, *vanB*, *mupB*, *mupA*, *fusA*, *fusB*, *fusC*, *msr*(A), *msr*(B), *erm*(A), *erm*(B), *erm*(C), *tet*(M), *ant*(4′)-*Ia*, *aac*(6′)-*Ie*/*aph* (2″) and *aph*(3′)-*IIIa* were detected by PCR [10–12].

Staphylococcus aureus protein a locus (spa) typing

The *S. aureus* isolates underwent *spa* typing as recommended by Harmsen et al. [13]. It was amplified by PCR with forward (5′-AGACGATCCTTCGGTGAGC-3′) and reverse (5′-GCTTTTGCAATGTCATTTACTG-3′) primers. The PCR products were sequenced and then edited. The Ridom Spa-Server database (http://www.spaserver.ridom.de) was applied to determine the strain's *spa* type.

Results

A total of 89 *S. aureus* strains carrying the *tst*-encoding gene were recovered from 350 *S. aureus* isolates, which were all methicillin-resistant and enrolled in the current research. Of the 89 tst-positive MRSA isolates, 16 were from hospital A (18%), 18 from hospital B (20.2%), 19 from hospital C (21.3%), 21 from hospital D (23.6%) and 15 from hospital E (16.9%). The

tst-positive MRSA isolates accounted for 64% and 36% of HO (57/89), and CO (32/89) cases, respectively. Fig. 1 summarizes the distribution of HO and CO cases across hospitals.

Our findings revealed that linezolid, teicoplanin and vancomycin had the most desirable antimicrobial activity; whereas gentamicin (78.7%), tetracycline (77.5%) and erythromycin (62.9%) exhibited the poorest antimicrobial activity. Eight isolates indicated HLMUPR and six isolates showed LLMUPR. Of 89 tested isolates, the overall prevalence of cMLS, iMLS and macrolide-streptogramin (MS)-resistant phenotypes were 28 (28.6%), 20 (22.5%) and 8 (9%), respectively. The methicillin-resistant and $iMLS_B$ phenotypes were distributed in all the tested hospitals with the majority in hospital D (nine isolates); whereas the $cMLS_B$ isolates were recovered from hospitals A (12 isolates) and D (16 isolates). Compared with CO-MRSA, the resistance rates of HO-MRSA to tested antibiotics was higher. All the fusidic-acid-resistant isolates belonged to hospital C and the sources for the isolation of them included wound (two isolates) and pus (one isolate). In total, 61 isolates (68.5%) indicated resistance to three or more classes of antibiotics and were confirmed as MDR strains. (see Table 1).

The analysis of resistance-encoding genes among *tst*-positive MRSA strains indicated that the most prevalent gene was *tet*(M) in 56 strains (62.9%), followed by *ant*(4′)-*Ia* in 52 (58.4%), *aph*(3′)-*IIIa* in 36 (40.4%), *erm*(C) in 20 (22.5%), *erm*(A) in 19 (21.3%), *aac* (6′)-*Ie*/*aph*(2″) in 18 (20.2%), *msr*(B) in 14 (15.7%), *mupA* in 8 (9%) and *fusB* in 3 (3.4%). Our findings indicated that no PCR products for the resistance genes *vanA*, *vanB*, *mupB*, *fusA*, *fusC*, *erm*(B), *mecC* and *msr*(A) were observed (see Table 1).

The results exhibited seven clonal complexes (CCs), which were produced in the studied isolates, namely CC22 (42.7%, 38/89), CC8 (28.1%, 25/89), CC5 (11.2%, 10/89), CC59 (5.6%, 5/89), CC30 (4.5%, 4/89), CC80 (4.5%, 4/89) and CC15 (3.4%, 3/89). The *tst*-positive MRSA isolates exhibited 11 different *spa* types with t223 as the most prevalent (16.9%), followed by t790 (15.7%), t037 (14.6%), t002 (11.2%), t005 (10.1%), t030 (7.9%), t388, t437 (5.6% each), t044, t605 (4.5% each) and t084 (3.4%). All HLMUPR strains belonged to t002 (n = 8); additionally, LLMUPR strains belonged to t790 (n = 6) *spa* types. Fusidic-acid-resistant isolates belonged to t437 (n = 3). MS phenotypes were distributed in t002 (5.6%, 5/89) and t790 (3.4%, 3/89). $iMLS_B$ was observed in t005 (6.7%, 6/89), t002 (5.6%, 5/89), t790 (3.4%, 3/89), t030, t044, t084 (each 2.2%, 2/89); whereas $cMLS_B$ phenotypes were genetically diverse and distributed among almost all *spa* types. Resistance profile and the distribution of *spa* types in MRSA associated with clinical samples are presented in Table 2.

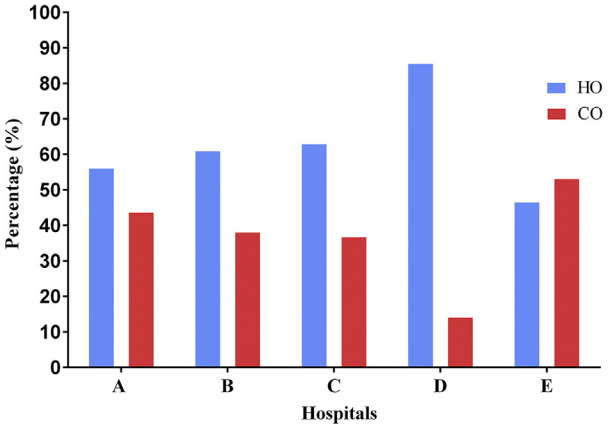

FIG. 1. Distribution of hospital-onset and community-onset cases among studied hospitals.

Discussion

Strains of MRSA carrying the TSST-encoding gene are particularly associated with wound and blood infections; in recent years, special attention has been given to these strains [5,9]. A recent systematic review and meta-analysis in Iran indicated that the overall prevalence of TSST-harbouring *S. aureus* clinical isolates was 21.3%, ranging from 0% to 68% in *S. aureus* clinical isolates [5]. According to the current findings, among the 350 tested *S. aureus*, 25.4% (89/350) carried the *tst*-encoding gene. Different prevalences for *tst* among *S. aureus* have been reported: from Turkey (14.2%) [14], Brazil (46.7%) [15], Korea (25.5%) [16] and China (31.4%) [17]. Contrary to Motamedifar et al., they indicated a higher frequency of *tst* in methicillin-susceptible *S. aureus* (MSSA) (18.1%) versus MRSA (11.6%) isolates [9]. The present data showed that all *tst*-harbouring isolates were methicillin resistant. Shams-Abadi et al. [5] showed that the prevalence of *tst*-harbouring MRSA strains in clinical isolates was 73.9%. The high prevalence of *tst*-carrying MRSA strains was also reported in studies from Japan (75.7%) [18] and Taiwan (75%) [19]. These differences may be a result of the type of sample and dissemination of specific clones [9,20,21].

In the present study, overall prevalence of resistance to mupirocin was found to be 15.7%. Recent studies in India [22], Jordan [23] and the USA [24] have noted mupirocin-resistance rates of 5%, 2.6% and 13.3%, respectively. Furthermore, we also observed that 9% of examined isolates were HLMUPR, which was lower than the two previous reports in Iran by Shahsavan et al. (25%) [25] and Abbasi-Montazeri et al. (17%) [26]. Differences were observed regarding the resistance to mupirocin, which may arise from the study design, dissemination of specific

TABLE 1. Antimicrobial resistance patterns of methicillin-resistant *Staphylococcus aureus* isolates carrying the *tst* genes

	89 MRSA isolates, *n* (%)		
Antibiotic	**Hospital onset**	**Community onset**	**Total, *n* (%)**
Gentamicin	49 (70)	21 (30)	70 (78.7)
Tetracycline	42 (60.9)	27 (39.1)	69 (77.5)
Erythromycin	39 (69.6)	17 (30.4)	56 (62.9)
Clindamycin	7 (20)	28 (80)	35 (39.3)
Kanamycin	28 (68.3)	13 (31.7)	41 (46.1)
Amikacin	30 (76.9)	9 (23.1)	39 (43.8)
Ciprofloxacin	25 (52.1)	23 (47.9)	48 (53.9)
Tobramycin	19 (100)	0 (0)	19 (40.5)
Rifampin	11 (52.4)	10 (47.6)	21 (23.6)
Trimethoprim-Sulfamethoxazole	12 (66.7)	6 (33.3)	18 (20.2)
Quinupristin-Dalfopristin	15 (100)	0 (0)	15 (16.9)
Mupirocin	9 (64.3)	5 (35.7)	14 (15.7)
Fusidic acid	3 (100)	0 (0)	3 (3.4)
Tigecycline	2 (100)	0 (0)	2 (2.2)
Total	57 (64)	32 (36)	89 (100)

type among patients and unrestricted policies in taking mupirocin.

In the current investigation, the frequency of iMLS phenotypes was found to be 22.5%. Reports of prevalence of inducible resistance from Iran range from 4.1% to 20.7% [27,28] Despite the discrepancies, our data was similar to previous reports by Moosavian et al. from Iran which had shown the prevalence of 32.3% [29]. Remarkably, a high prevalence of iMLS phenotypes was also reported from Jordan (76.7%) [30].

Recently published data from Asian countries indicated a low prevalence of resistance to fusidic acid (<10%) [29,31]. We noted a low prevalence (3.4%) of resistance to fusidic acid among our isolates. Rahimi et al. found similar rates of fusidic acid resistance among MRSA (3%) [32]. Different resistance rates to fusidic acid have been described in many countries: Greece (62.4%), Ireland (19.9%), Australia (7.0%), Canada (7.0%) and the USA (0.3%) [33].

As illustrated in Table 2, 11 different *spa* types were detected in this work, which were distributed in seven CCs. In agreement with data that indicated CC22 as the most common type in in Gaza, Palestine [6], the present research reported a prevalence of this CC in 42.7% of isolates. In this study, CC22 had three important *spa* types (t223 in 16.9% of isolates, t790 in 15.7% of isolates and t005 in 10.1% of isolates). This finding supports previous results from Gaza in which t223 was the predominant *spa* type, accounting for 16.7% of isolates [6]. A recent report from Kuwait also indicated that t223 was one of the common *spa* types detected in MRSA strains investigated from 1992 to 2010 (4.7%) [34]. t790 was the other *spa* type identified in CC22 (15.7%). This *spa* type is one of the most successful and persistent types reported from Saudi Arabia [35] and Jordan [36]. A contradictory result was reported by Boswihi et al. in Kuwait [34]. A study by Japoni-Nejad et al. in Iran [37] also indicated the low frequency of t790 among their tested isolates. Although virulence determinants and antibiotic resistance profiles in t790 isolates were found to be varied, *tst* carriage and resistance to mupirocin in t790 strains have been described by several researchers [8,38]. The attained data demonstrated that 10.1% of isolates were related to *spa* type t005. These findings are similar to those reported in Iran by Khademi et al. [39], that indicated a 7.1% prevalence rate of *spa* type among *S. aureus* isolated from clinical samples. Contrastingly, other studies have demonstrated *spa* type t005 as the most frequent *spa* type (47.4%) detected among Panton–Valentine leucocidin-positive MSSA strains [40].

Isolates of CC8 corresponded to t037 (14.6%), t030 (7.9%) and t388 (5.6%). According to the evidence, *spa* type t388 and t037 are associated with health-care-associated MRSA, which was found in Europe, Asia and America [34,38]. Observed frequencies of these *spa* types were similar to a previous report by Ohadian Moghadam et al. from Iran on 66 *S. aureus* strains, which recognized 11 different *spa* types with the most prominent *spa* types being t037 and t030, and low frequency of t388 [41]. The *spa* type t030 was previously reported as one of the most common types from different countries [34,38]. Notably, most t030 MRSA isolates were resistant to fluoroquinolones and tetracycline; a finding that was in accordance with Li's study; furthermore, they indicated that all of the tested isolates were resistant to tetracycline, rifampicin and fluoroquinolones [42].

The present work confirmed that CC30 (to which t605 belongs) was detected in four isolates (4.5%). *tst*-positive CC30 strains have been described in Iran [8,41], Kuwait [34] and Palestine [6]. In a 700-bed tertiary teaching hospital in Greece, it was documented that among 18 tst-positive MSSA strains obtained, CC30 was the predominant clone in the intensive care unit accounting for 55.5% (10/18) and the remaining eight strains were classified into three additional sequence types including ST2123 (33.3%), ST27 (5.6%) and ST45 (5.6%) [7].

In this study, CC80 and *spa* type t044 ranked sixth among *tst*-positive MRSA isolates, accounting for 4.5% of isolates. This observation is supported by a study conducted in Gaza, Palestine during 2008 and 2012, which documented the presence of CC80 and *spa* type t044 in *tst*-positive MRSA isolates. This would suggest the potential for greater numbers of isolates harbouring both toxin-encoding genes [6]. Also, several studies have reported the presence of this *spa* type in *S. aureus* isolates from Iran. Mirzaii et al. analysed 37 *S. aureus*

TABLE 2. Distribution of *spa* types and resistance profiles of MRSA carrying *tst* gene

CC	*spa* types	Phenotypic resistance profile [a] (*n*, %)	Genetic resistance profile (*n*, %)	Hospitals (*n*, %)	*n* (%)
22	223	T, GM, E, CD, K, AK, CIP (5, 33.3) T, GM, E, CD, TN, SYN, RI (4, 26.7) T, E, TIG CD (2, 13.3) CIP, GM (4, 26.7)	*MecA* (15,100), *tet*(M) (12, 80), *ant (4′)-Ia* (6, 40), *aph (3′)-IIIa* (2, 13.3), *aac (6′)-Ie/aph* (2) (7, 46.7), *erm*(C) (5, 33.3), *erm*(A) (3, 20), *msr*(B) (8, 53.3)	A (5, 33.3), B (4, 26.7), D (6, 40)	15 (16.9)
	790	GM, T, E, AK, MUP, RI (5, 35.7) T, E, TS, SYN, MUP (1, 7.1) T, GM, E, CD, TN, SYN, RI (4, 28.6) GM, T, TN, CIP, CD (3, 21.5) T (1, 7.1)	*MecA* (14,100), *tet*(M) (10, 71.4), *ant (4′)-Ia* (10, 71.4), *aph (3′)-IIIa* (4, 28.6), *aac (6′)-Ie/aph* (2) (5, 35.7), *erm*(C) (6, 42.9), *erm*(A) (3, 21.4), *msr*(B) (5, 35.7)	A (2, 14.3), B (1, 7.1), C (5, 35.7), D (3, 21.4), E (3, 21.4)	14 (15.7)
	005	T, GM, AK, CIP, TS, E (6, 66.7) T, GM, E, CD, TN, SYN, RI (3, 33.3)	*MecA* (9,100), *tet*(M) (7, 77.8), *ant (4′)-Ia* (8, 88.9), *aph (3′)-IIIa* (5, 55.6), *erm*(C) (2, 22.2), *erm*(A) (2, 22.2)	A (3, 33.3), B (3, 33.3), C (2, 22.2), D (1, 11.1)	9 (10.1)
8	037	T, GM, E, CD, K, AK, CIP (4, 30.8) T, GM, TN, CIP, CD (4, 30.8) GM, CIP (2, 15.3) No resistance (3, 23.1)	*MecA* (13,100), *tet*(M) (9, 69.2), *ant (4′)-Ia* (7, 53.8), *aph (3′)-IIIa* (6, 46.1), *aac (6′)-Ie/aph* (2) (5, 38.5), *erm*(C) (5, 38.5), *erm*(A) (4, 30.8), *msr*(B) (1, 7.7)	A (2, 15.4), B (4, 30.8), C (3, 23.1), D (4, 30.8)	13 (14.6)
	030	T, GM, AK, CIP, TS, E (2, 28.6) GM, CIP (4, 57.1) No resistance (1, 14.3)	*MecA* (7,100), *tet*(M) (4, 57.1), *ant (4′)-Ia* (6, 85.7), *aph (3′)-IIIa* (5, 71.4), *aac (6′)-Ie/aph* (2) (1, 14.3)	B (2, 28.6), D (2, 28.6), E (3, 42.9)	7 (7.9)
	388	T, GM, E, CD, K, AK, CIP (3, 60) T (2, 40)	*MecA* (5,100), *tet*(M) (2, 40) *ant (4′)-Ia* (3, 60), *aph (3′)-IIIa* (2, 40), *erm*(A) (3, 60)	C (2, 40), E (3, 60)	5 (5.6)
30	605	T, GM, E, CD, K, AK, CIP (2, 50) No resistance (2, 50)	*MecA* (4,100), *tet*(M) (1, 25) *ant (4′)-Ia* (2, 50), *erm*(A) (1, 25)	C (2, 50), E (2, 50)	4 (4.5)
80	044	T, GM, AK, CIP, TS, E (2, 50) T, GM, E, CD, K, AK, CIP (1, 25) CIP, GM (1, 25)	*MecA* (4,100), *tet*(M) (2, 50) *ant (4′)-Ia* (4, 100), *aph (3′)-IIIa* (4, 100), *erm*(A) (3, 75)	B (1, 25), E (2, 50), D (1, 25)	4 (4.5)
15	084	T, GM, AK, CIP, TS, E (2, 66.7) No resistance (1, 33.3)	*MecA* (3,100), *tet*(M) (1, 33.3), *aph (3′)-IIIa* (2, 66.7)	C (2, 66.7), D (1, 33.3)	3 (3.4)
59	437	T, GM, K, TN, FC (1, 20) T, FC (2, 40) CIP, GM (1, 20) No resistance (1, 20)	*MecA* (5,100), *fusB* (3, 60), *aph (3′)-IIIa* (2, 40)	B (1, 20), C (3, 60), E (1, 20)	5 (5.6)
5	002	GM, T, E, AK, MUP, RI (5, 50) T, E, TS, SYN, MUP (3, 30) T, GM, AK, CIP, TS, E (2, 20)	*MecA* (10,100), *mupA* (8, 80), *tet*(M) (8, 80), *ant (4′)-Ia* (6, 60), *aph (3′)-IIIa* (4, 40), *erm*(C) (2, 20),	A (4, 40), B (2, 20), D (3, 30), E (1, 10)	10 (11.2)

[a] E, erythromycin; T, tetracycline; CD, clindamycin; GM, gentamicin; TS, trimethoprim-sulfamethoxazole; FC, fusidic cid; CIP, ciprofloxacin; SYN, quinupristin-dalfopristin; TIG, tigecycline; TN, tobramycin; AK, amikacin; RI, rifampicin; K, kanamycin; MUP, mupirocin.

strains isolated from different sources and found that only four isolates (10.8%) isolated from the hands and nose of personnel and also from the environment of the intensive care unit belonged to *spa* type t044 [43]. Asadollahi et al. also demonstrated that the t044 *spa* type was one of the most common *spa* types in 11 countries [44].

The other CC detected among *tst*-positive MRSA strains was CC5 (11.2%) assigned to single *spa* type t002. As stated in previous documents, CC5 is distributed in both community and hospital environments. Recent studies have shown the presence of CC5/t002 clones in Asian and European countries, such as Iran, Japan, Korea, the United Arab Emirates, Kuwait, Ireland and Australia [34,38]. Based on our analysis, all HLMUPR strains belonged to *spa* type t002 (n = 8). Similarly, in an experiment conducted in 2016 in Spain, María González-Domínguez et al. revealed resistance to mupirocin in CC55-*spa* t002 strains. The present survey indicated that all HLMUPR CC5/t002 isolates were positive for the *mupA* gene. This finding contradicted previous studies stating that CC5/t002 isolates could not carry the *mupA* gene [45].

Based on the evidence, CC59 (to which t437 belongs) has limited geographical spread. The present study showed that CC59 was present in five isolates, accounting for 5.6%. These findings correspond to previous reports from other countries including Australia, Ireland, the UK, Korea, Kuwait and Taiwan. Notably, resistance to fusidic acid encoded by *fusB* was also detected in three isolates. This finding is consistent with Shore et al. in Ireland [46].

As mentioned, the frequency of CC15/t084 was found to be 3.4%; in fact, two isolates were confirmed as $iMLS_B$. Sangvik et al. [47] reported that t012 (8.8%), t084 (5.6%) and t065 (5.2%) were the most common *spa* types in north Norway. It was previously thought that t084 could be detected only in MRSA; nonetheless, there are reports indicating high distribution of this type among MSSA strains [34,38]. To the best of our knowledge, this is the first report regarding the emergence of *spa* types t223 and 605 in Iran.

In conclusion, this study is the first report regarding the molecular characteristics of *tst*-positive MRSA strains in Iran. These strains belonged to diverse genetic backgrounds with a predominance of CC22. Our investigation revealed the high prevalence of MDR patterns that highlighted the rational usage of antibiotics to minimize the spread of *S. aureus* with MDR in Iran; however, some resistance patterns were related to certain

spa types. Our results ought to be further investigated in other health-care settings in Iran to keep track of the emerging *spa* types.

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Conflict of interest

All authors declare that they have no conflict of interest.

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