RESEARCH ARTICLE

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Emergence of CC8/ST239- SCC*mec* III/t421 tigecycline resistant and CC/ST22-SCC*mec* IV/t790 vancomycin resistant *Staphylococcus aureus* strains isolated from wound: A two-year multi-center study in Tehran, Iran

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ABSTRACT

Staphylococcus aureus as an opportunistic bacterial pathogen with intrinsic and acquired resistance to many antibiotics is a worldwide problem. The current study was undertaken to evaluate the resistance pattern, and determine the genetic types of multidrug-resistant *S. aureus* isolated from wound.

This cross-sectional study was conducted over the period of two years (from December 2018 to November 2020) at the hospitals affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran. In present study, 75 multidrug-resistant *S. aureus* isolates collected from wound infections were investigated. Phenotypic resistance was assessed by Kirby–Bauer disk diffusion method. Conventional PCR was performed for the detection of virulence encoding genes. Genotyping of strains was performed based on *coa* gene polymorphism using multiplex-PCR assay. SCC*mec* typing, *spa* typing and MLST were also used to characterize the genotype of the mupirocin, tigecycline and vancomycin resistant multidrug-resistant *S. aureus* isolates.

All 75 multidrug-resistant *S. aureus* isolates in the study were confirmed as MRSA. Coagulase typing distinguished isolates into five genotypic patterns including III (40%), I (24%), IVb (16%), V (10.7%) and type X (9.3%). Resistance to tigecycline was detected in 4% of MDR-MRSA isolates and all belonged to CC8/ST239- SCC*mec* III/t421 lineage. According to our analysis, one VRSA strain was identified that belonged to *coa* type V and CC/ST22-SCC*mec* IV/t790 lineage. Resistance to mupirocin was detected in 9.3% of strains. All 7 mupirocin resistant MDR-MRSA isolates exhibited resistance to mupirocin in high level. Of these, 4 isolates belonged to CC/ST8-SCC*mec* IV/t008 (57.1%), 2 isolates belonged to CC/ST8-SCC*mec* IV/t064 (28.6%) and one isolate to CC/ST22-SCC*mec* IV/t790 (14.3%).

Altogether, current survey provides a snapshot of the characteristics of *S. aureus* strains isolated from patients. Our observations highlighted type III as predominant *coa* type among multidrug-resistant MDR strains indicating low heterogeneity of these isolates. Our study also indicates the importance of continuous monitoring of the genotypes of MDR-MRSA isolates to prevent nosocomial outbreaks and the spread of MDR isolates.

KEYWORDS

INTRODUCTION

Staphylococcus aureus as an opportunistic pathogen, is a leading cause of hospital-acquired (HA) and community-acquired (CA) infections and a significant contributor to economic and societal cost [1]. This bacterium is responsible for a number of diseases, ranging from pyogenic skin infections to life-threatening diseases [2]. According to the evidence, hospitalized patients with *S. aureus* infections had a 2-fold increase in mortality rate comparing patients with non-*S. aureus* infections [3]. The emergence of multidrug-resistant (MDR) strains especially methicillin-resistant *S. aureus* (MRSA) can lead to exacerbation of infection, treatment failure and subsequent deterioration of disease [4]. Over the last few decades, a significant rise in the prevalence of MRSA strains around the world has become a matter of concern and imposes serious economic costs on patients and health care settings [1, 2, 4]. The pathogenicity and virulence of *S. aureus* are associated with its capacity to produce several virulence factors such as toxins (exfoliative toxins (*eta*, *etb*), Toxic Shock Syndrome Toxin-1 (TSST-1), and Panton-Valentine Leukocidin (PVL), as well as adhesion factors s along with simultaneously resistance to antibacterial agents lead to successful persistence within the hospital and community and subsequently severe human and animal infections [1, 2].

One of the problems in controlling *S. aureus* infections is the lack of data about molecular analysis and resistance profile [3, 4]. There are several methods with good discriminatory power and high reproducibility score such as multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) for typing of *S. aureus* clinical strains [5]. According the earlier data, these methods are time-consuming, technically complex and expensive. Regarding to high frequency of *S. aureus* related infections, it is important that a simple, accurate, rapid and inexpensive typing method for epidemiological investigations *S. aureus* isolated from clinical sources to be recruited [2, 5]. The coagulase enzyme, an extracellular protein produced by all *S. aureus* isolates, is genetically diverse and has been considered to be a hallmark for typing these strains. Based on the heterogeneity in the tandem repeat in *coa* region, ten different types of coagulase (I-X) have been described [6]. Although various data related to genetic diversity of *S. aureus* isolated from wound infections has been reported from Iran, restricted data is available on *coa* gene polymorphism. Hence, this dearth of data about *S. aureus* strains has led us to investigate the resistance pattern, and determine the genetic types of multidrug-resistant *S. aureus* (MDRSA) isolated from wound by identifying *coa* types. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing, *S. aureus* protein A (*spa*) typing and multilocus sequence typing (MLST) were also used to characterize the genotype of the mupirocin, tigecycline and vancomycin resistant MDRSA isolates.

MATERIALS AND METHODS

Sample collection and bacterial isolation

In present work, 75 MDRSA strains were isolated from 285 wound clinical samples of patients referred to the hospitals affiliated to Shahid Beheshti University of Medical Sciences over a period of 2 years (December 2018 and November 2020). All strains were isolated and identified using standard bacteriological techniques and polymerase chain reaction (PCR) of *nuc* gene [7]. The ethic Committee of Shahid Beheshti University of Medical Sciences approved present research protocol (IR.SBMU.MSP.REC.1398.492). Confirmed *S. aureus* strains were saved in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at −70 °C for detailed analysis.

Evaluation of susceptibility and resistance to antimicrobial agents

Antibiotic susceptibility test was performed according to the Kirby-Bauer disk diffusion method (15) for antibiotics penicillin (PEN), gentamicin (GEN), tetracycline (TET), erythromycin (ERY), clindamycin (CLI), ciprofloxacin (CIP), rifampin (RIF), quinupristin-dalfopristin (SYN), and trimethoprim-sulfamethoxazole (SXT) (Mast Diagnostics Ltd, Merseyside, UK). Antibiotics and their concertation were selected based on the prescription pattern of the hospital-physician/pharmacy. Results interpreted according to the clinical and the laboratory standards institute (CLSI) guideline (CLSI 2019) (http://www.clsi.org). Minimal inhibitory concentrations (MIC) for antibiotics vancomycin (VAN), tigecycline (TIG), and mupirocin (MUP) were determined by broth microdilution method. Results for tigecycline was interpreted based on the European Committee for antimicrobial susceptibility testing (EUCAST) recommendations (http://www.eucast.org).

The MIC breakpoints for vancomycin were defined as follows: susceptible, $\leq 2\,\mu g\,mL^{-1}$; intermediate, 4–$8\,\mu g\,mL^{-1}$; and resistant, $\geq 16\,\mu g\,mL^{-1}$. *S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299 were used as susceptible and resistant reference strains. According to the EUCAST guidelines, the MICs of resistance to tigecycline in *S. aureus* isolates is $>0.5\,\mu g\,mL^{-1}$. The screening of high-level mupirocin resistance (HMUPR) strains was performed by standard broth microdilution procedure in concurrence with CLSI guideline. Visible growth in $256\,\mu g\,mL^{-1}$ of mupirocin was reported as HMUPR isolates. The screening of inducible macrolide-lincosamide-streptogramin group B (iMLS_B) resistance isolates was performed on Mueller-Hinton agar (Merck, Germany) with CLI and ERY held 15 mm apart. Blunting of the circular zone of inhibition around the clindamycin disc on the side facing the erythromycin disc was reported as iMLSB phenotype. Isolates showing resistance to erythromycin while being susceptible to clindamycin with no blunting zone were classified as the MS resistance

phenotype. Resistance to both erythromycin and clindamycin was considered as constitutive (cMLS$_B$) resistance phenotype (CLSI 2019).

All *S. aureus* isolates were screened for methicillin resistance by the disk diffusion method using cefoxitin (30 µg) disc in Mueller-Hinton agar (Merck, Germany) and detection of the *mecA* gene as previously described. *S. aureus* isolates with zone of inhibition ≤21 mm around the cefoxitin disc were confirmed as MRSA [7]. Test performance was monitored using *S. aureus* ATCC 25923, ATCC 43300, and ATCC 29213 reference strains.

DNA isolation, screening for virulence related genes

Genomic DNA extraction was carried out using the phenol-chloroform method as described previously. The quality of DNA has adjusted approximately to 100 ng µL^{-1} which evaluated by a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). All isolates were investigated for the presence of toxin genes, namely: Panton-Valentine leukotoxin gene (*pvl*), toxic shock syndrome toxin (*tsst-1*), and exfoliative toxins A and B genes (*eta*, and *etb*) by PCR method [7] (Table 1). A previously confirmed *S. aureus* isolates harboring aforementioned toxin encoding genes were used as positive control and molecular grade nuclease-free sterile water in reaction mixture without extracted DNA template was used as a negative control in each PCR reaction.

coa typing of MDRSA isolates

Four sets of multiplex PCR reactions were used for the classification of coagulase (*coa*) types (I-X). According to the procedure of Hirose et al., set A contained primers for identifying *coa* types I, II, III, IVa, IVb, Va, and VI) while set B contained primers for identifying *coa* types VII, VIII, and X. Set C was used for identifying *coa* types IX and Vb. SC types IVa and IVb were distinguished using set D primers [6] (Table 2).

Analysis of mupirocin, tigecycline and vancomycin resistant isolates

spa *typing*. For *spa* typing, the isolates were subjected to PCR assay by amplification of the polymorphic X region of the *spa* gene with forwarding (5′-AGACGATCCTTCGGT-GAGC-3′) and reverse (5′-GCTTTTGCAATGTCATT-TACTG-3′) specific primers and protocol described by Goudarzi et al. [8]. Afterwards the PCR products were purified, sequenced for both strands and then edited. Identification of *spa* types was done in the Ridom SpaServer database (http://www.spaserver.ridom.de).

***SCC*mec *typing*.** SCC*mec* typing of MRSA isolates was done by multiplex PCR assay as stated by Boy et al. [9]. Briefly, Multiplex PCR assay mixture of 25 µl was prepared using 2 µL of template DNA (25 ng µL^{-1}), 1 µL of each primer (10 pmol), 12.5 µL of Master Mix 2x (Amplicon, Denmark), and 5.5 µL of double distilled water (Promega, USA). *S. aureus* ATCC

Table 1. The PCR conditions and oligonucleotide primers used in present research

Gene	Primer	Sequence	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Number of cycles	Amplicon size (bp)	Reference
					PCR condition (Temperature, Time)					
nuc	Forward	GCGATTGATGGTGATACGGTT	95 °C, 5 min	95 °C, 45 s	57 °C, 50 s	72 °C, 60 s	72 °C, 4 min	30	270	[7]
	Reverse	AGCCAAGCCTTGACGAACTAAAGC								
mecA	Forward	GTAGAAATGACTGAACGTCCGATAA	95 °C, 5 min	95 °C, 45 s	55 °C, 55 s	72 °C, 55 s	72 °C, 5 min	35	310	[7]
	Reverse	CCAATTCCACATTGTTTCGCTCTAA								
pvl	Forward	TTCACTATTTGTAAAAGTGTCAGACCCACT	95 °C, 5 min	94 °C, 95 s	54 °C, 50 s	72 °C, 60 s	72 °C, 3 min	35	180	[7]
	Reverse	TACTAATGAATTTTTTTATCGTAAGCCCTT								
tst	Forward	TTATCGTAAGCCCTTTGTTG	95 °C, 4 min	95 °C, 50 s	58 °C, 45 s	72 °C, 450 s	72 °C, 5 min	30	398	[7]
	Reverse	TAAAGGTAGTTCTATTGGAGTAGG								
eta	Forward	GCAGGTGTTGATTTAGCATT	95 °C, 4 min	95 °C, 60 s	55 °C, 50 s	72 °C, 45 s	72 °C, 3 min	30	93	[7]
	Reverse	AGATGTCCCTATTTTTGCTG								
etb	Forward	ACAAGCAAAAGAATACAGCG	95 °C, 5 min	95 °C, 45 s	60 °C, 45 s	72 °C, 50 s	72 °C, 5 min	35	226	[7]
	Reverse	GTTTTTGGCGTGCTTCTCTTG								

Table 2. Primers used for SC typing

Gen	Primer	*coa* type	Primer sequence	Length (bp)
Sc-R1	Coa-ant1	Common	GGGCAATTACATTTTGGAGGA	–
	coa7	Common	TGTTCCATCGTTGTATTCACG	–
	cot1	I	ATTTTTTGTATTCCTCATACTGCA	368
	cot2	II	CTTTCGCTTCTTTATAGATAGGATC	288
	cot3	III	TCAAGTCTGAATTCTTATCC	549
	cot4	Iva, IVb	AGCATTATGACCATATTGGC	665
	cot5	Va	TTACCTTGAGTCCCAATTTG	1,105
	cot6	VI	CTATAATCATGCTTATCCCA	850
Sc-R2	cot7	VII	TCAAATCAATTTTCGCCCTA	693
	cot8	VIII	GATTTTTTATTACTCCCCAGTAATA	210
	cot10	X	ACTTAATATCCTTGTCATTAGTTG	314
	cot9	IX	ATATACCGTTAGTTACACGC	591
Sc-R3	Cot1	Vb	AATCATAAAATTTCACCGGGC	411
	F4-8	IVa	TTACAGTTGGTACAACTGAAGAAGC	455
Sc-R4	F8-12	IVb	GCCAAAATACCCAACGATGGAACAG	415

10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), MW2 (SCC*mec* type IVa), and WIS (SCC*mec* type V) were recruited as reference strains.

MLST. The MDRSA strains further characterized by MLST and by amplifying and sequencing seven housekeeping genes (pta, arcC, tpi, aroE, gmk, yqiL, and glp). Sequence types (STs) were determined by the submission of the allelic profile to the online MLST database website (https://pubmlst.org/).

RESULTS

In present study, 75 MDRSA isolates were collected from wound samples using standard microbiological methods. Our analysis indicated that 31 strains were obtained from intensive care unit (ICU) (41.3%), 20 from the burn (26.7%), 14 from infectious (18.7%), and 10 from surgery (13.3%) wards. In the current work, of 75 isolates of *S. aureus*, 48 (64%) were male patients and 27 were female patients (36%) with a median age of 38.6 years, ranging from 17 to 65 years. The highest number of *S. aureus* was observed in patients of age group 21–35 years whereas the least was from patients aged <20 years.

Our analysis documented that the highest and lowest rate of resistance were related to penicillin (100%) and vancomycin (1.4%), respectively. Table 3 gives information about the resistance rate in MRSA isolates. Totally, fourteen resistance patterns were identified, wherein PEN, GEN, TET, ERY, CLI, CIP, RIF (28%, 21/75), PEN, GEN, TET, ERY, CLI, CIP, SYN, SXT (13.3%, 10/75) and PEN, GEN, ERY (9.3%, 7/75) were the top three frequently identified patterns. Based on the phenotypic method and PCR of the *mecA* gene, all 75 MDR *S. aureus* isolates under the study were confirmed as MRSA (MDR-MRSA).

Micro-broth dilution test for vancomycin revealed that 33 isolates had MIC value of $0.5\,\mu g\;mL^{-1}$, 19 isolate MIC value of $1\,\mu g\;mL^{-1}$, 22 isolate MIC value of $2\,\mu g\;mL^{-1}$, and

Table 3. Resistant pattern of MDR-MRSA isolates

	Percentage	
Antibiotic	Resistant	Sensitive
Penicillin	100	0
Gentamicin	88	22
Tetracycline	90.7	9.3
Erythromycin	93.3	6.7
Clindamycin	50.7	49.3
Ciprofloxacin	54.7	45.3
Rifampin	42.7	57.3
Quinupristin- dalfopistin	24	76
Trimethoprim-sulfamethoxazole	25.3	74.7
Tigecycline	9.3	90.7
Mupirocin	4	96
Vancomycin	1.4	98.6

1 isolate MIC of $64\,\mu g\;mL^{-1}$. All 7 mupirocin resistant MDR-MRSA isolates exhibited resistance to mupirocin in high level (HLMUPR). Of the total isolates, 38 and 32 isolates exhibited cMLS$_B$ and iMLS$_B$ phenotypes accounting for 50.7% and 42.7% respectively. A total of 3 MDR-MRSA isolates (4%) were resistant to tigecycline, of which two had MIC $1\,\mu g\;mL^{-1}$ and one exhibited MIC titer of $2\,\mu g\;mL^{-1}$.

Of 75 MDR-MRSA strains, 25 (33.3%) were toxinogenic with 15 producing *pvl* (20%), and 10 *tst* (13.3%). Based on *coa* typing, predominant *coa* type was III, which included 30 isolates (40%), followed by type I in 18 isolates (24%), type IVb in 12 isolates (16%), type V in 8 isolates (10.7%), type X in 7 isolates (9.3%). Data related to the distribution of *coa* types in different wards are presented in Fig. 1. As shown in Fig. 1, the most frequency of *coa* type I was found in burn ward (72.2%), *coa* type 2 in ICU (83.4%), *coa* IVb in burn (41.7%), *coa* V in surgery (50%) and *coa* X in infectious ward (71.4%). All the *tst*-positive isolates belonged to *coa* type III (13.3%, 10/75). Among the 25-toxinogenic isolates, *pvl* was observed in isolates with *coa* type III (10.7%, 8/75), II (6.7%, 5/75) and X (2.7%, 2/75).
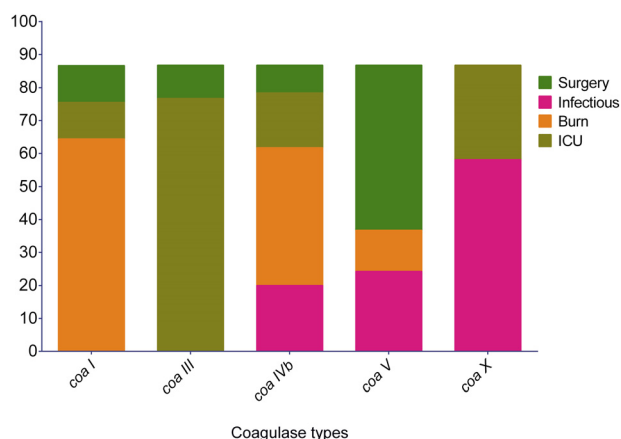
Fig. 1. Distribution of *coa* types of 75 MDR-MRSA in different wards

All tigecycline -resistant isolates belonged to *coa* type X (*n* = 3). Seven isolates with HLMUPR phenotype belonged to *coa* type I (85.7% [6/7]), and *coa* type III (14.3% [1/7]). Out of 38 strains with cMLS$_B$ phenotype, 12 isolates belonged to *coa* type I (31.6%), 12 isolates to *coa* type III (31.6%), 5 isolates to *coa* type IVb (13.1%), 3 isolate to *coa* type V (7.9%), and 6 isolates to *coa* type V (15.8%). Out of 32 isolates with iMLS$_B$ phenotype, 5 isolate belonged to *coa* type I (15.6%), 14 isolates to *coa* type III (43.8%), 7 isolates to *coa* type IVb (21.9%), 5 isolate to *coa* type V (15.6%), and one isolate to *coa* type X (3.1%). According to the micro-broth dilution test, one VRSA strain was identified that belonged to *coa* type V. Figure 2 gives information about the distribution of resistance pattern among different *coa* types of MDR-MRSA isolates.

Further analysis on mupirocin, tigecycline and vancomycin resistant isolates showed that all tigecycline -resistant isolates belonged to CC8/ST239- SCC*mec* III/t421 clone. VRSA isolate belonged to CC/ST22-SCC*mec* IV/t790 clone. Our analysis showed a high prevalence of HLMUPR in CC8 compared to CC22 (8% vs. 1.3%). Out of 7 HLMUPR strains, 4 isolates belonged to CC/ST8-SCC*mec* IV/t008

(57.1%), 2 isolates belonged to CC/ST8-SCC*mec* IV/t064 (28.6%) and one isolate to CC/ST22-SCC*mec* IV/t790 (14.3%).

DISCUSSION

Our survey exhibited several results. i) genetic diversity of MDR-MRSA isolates with five major *coa* types (III, I, IVb, V, and X) was reported. ii) tigecycline resistant isolates belonging to the CC8/ST239-SCC*mec* III/t421 clone were found. iii) HLMUPR with a predominance of CC8 in current research was observed. iv) an isolate of CC/ST22-SCC*mec* IV/t790 clone was confirmed as MDR-MRSA with resistance to vancomycin.

The emergence of MDR MRSA is a significant challenge, and the control of these pathogenic bacteria is difficult by existing control measures. Data relating to antimicrobial activity revealed that half of MDR-MRSA isolates (50.7%) exhibited cMLS$_B$ phenotype. This finding was lower than the prevalence previously reported in Iran by Khashei et al. (82.9%) [10], while it was higher than those obtained by Adhikari et al. (29.25%) [11], and Eksi (20.4%) [12] and Sasirekha et al. (13.1%) [13].

According to the previously published data, the prevalence rate of iMLS$_B$ phenotype among MDR-MRSA isolates was found to be varied based on the geographical regions. In the study, the prevalence of iMLS$_B$ was found to be 42.7% which was higher than those obtained by Adhikari et al. from Nepal (11.48%) [11], Khashei et al. from Iran (8.6%) [10], Kilany et al. from Egypt (7.7%) [14], and Lall et al. from India (37.5%) [15]. Reasons for these variations in different geographic area could be due to study design and population, widespread use, easy access and uncontrolled policies in the prescription of macrolides, and spreading of specific clones in these area.

In this study, 9.3% of MDR-MRSA isolates exhibited resistance to mupirocin in high level. These observation was also supported by findings of Dadashi et al. that displayed
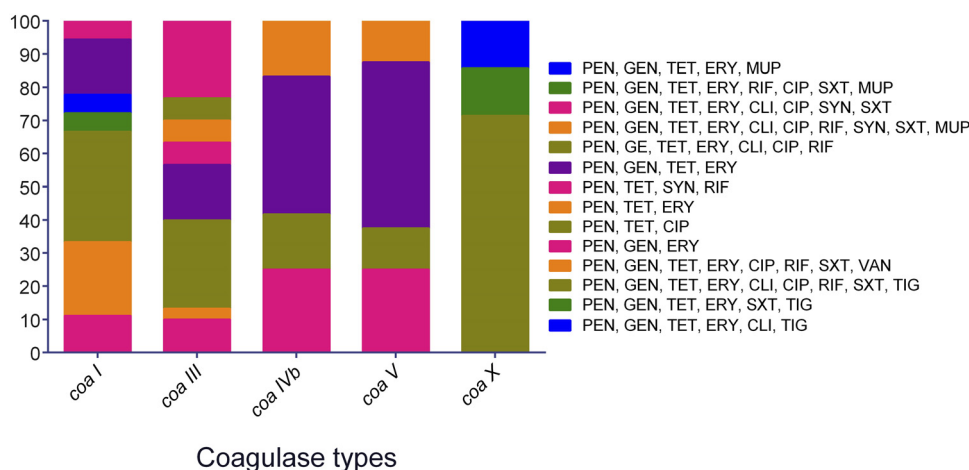

Fig. 2. Distribution of resistance pattern among different *coa* types of MDR-MRSA isolates

the pooled prevalence of HLMUPR MRSA clinical isolates was found to be 8.1%. Meanwhile, they also indicated a different rate of HLMUPR MRSA isolates in Asia (12.1%), Europe (8.0%), and the USA (5.9%) [16]. Different results were achieved by Shittu et al. They reported the prevalence of HLMUPR MRSA isolates in Africa ranged between 0.5 and 38% [17]. However, much higher rates were also reported by findings from India (26.1%) [18], the USA (19.3%) [19], and Korea (5.7%) [20]. The probable cause of this can be injudicious and widespread use, uncontrolled policies in the prescription of these antibiotics, easy access to antibiotics without prescription, inexpensive drugs, and spreading of specific lineage in these areas.

Tigecycline as an alternative option for the treatment of MDR-MRSA is recommended for treating skin and soft tissue infections. Despite of low resistance rate to tigecycline among MRSA strains, the prescription and consumption of this antibiotic still has limitations in treating staphylococcal infections [21]. Although reported rate of resistance to tigecycline was found to be rare, evidence markedly indicated an increase in the prevalence of tigecycline resistant MRSA isolates in recent years [21, 22]. Although, tigecycline is not on the list of drugs used for routine treatment of *S. aureus* related infections in Iran, in the present study, three tigecycline-resistant MRSA isolates (4%) were found. A study conducted by Mardziah et al. in Malaysia noted the occurrence of five tigecycline resistant isolates among 90 MRSA isolates [22]. In other Malaysian study conducted by Atshan and colleagues a high frequency of resistance to tigecycline (26.7%) among MRSA strains between 2009–2010 was noted [23]. Similarly, a previous study conducted by Zorgani et al. in Libya indicated resistance to tigecycline in 3.6% of their *S. aureus* isolates tested [24]. In an experiment performed by Yousefi et al. on 54 *S. aureus* isolated from urinary tract infections, 6.6% isolates were found to be resistant to tigecycline [25]. These observations were also supported by findings from Canada, USA, Honduras, El Salvador, France, Germany, Italy, Poland, Nigeria, China, and Taiwan [21]. Although, the underlying reason for the increased resistance to tigecycline in MRSA is not fully explored, it may be due to prior exposure to tigecycline, use of this antibiotic in combination with other related antibiotics, such as minocycline, genetic alteration of efflux pumps and spreading of specific clones in these regions.

Decreased susceptibility and subsequent resistance to vancomycin, as an active drug for treatment of MDR *S. aureus* infections, have been reported in many regions around the world [4]. Nowadays, the VRSA is being an emerging public health issue in throughout the world. Our finding indicated the prevalence of VRSA in 1.3% of tested isolates. In a 2020 systematic review and meta-analysis, Shariati et al. reported a trends towards an increasing prevalence of VRSA in different areas. They noted an increasing trend 2-fold of VRSA after 2010 comparing to before 2010. The results of aforementioned study also showed an overall prevalence of 1.5% for VRSA strains [26]. These observations were also supported by findings from Japan, USA, India, and Iran. Reasons for considerable

increasing trend of VRSA could be inappropriate and over use, poor hygiene standards, defect in implementation of antibiotic stewardship programs, and different attitudes towards antibacterial treatments.

Our study indicated that MDR- MRSA strains were assigned to five *coa* gene. Similarly, Afrogh et al. [27] reported six different patterns of *coa* gene among *S. aureus* strains isolated from staff nose and patients in Iran. However, other similar results were achieved by Younis Omar et al. [28] and Ibrahim et al. [29]. In contrast, a study performed by Abdulghany et al. [30] distinguished 15 different *coa* types among 58 MRSA isolates. In our study, *coa* type III was the most predominant *coa* type among tested isolates (40%). In a research performed in 2010 in Japan, Hirose et al. indicated a different result. The study showed *coa* type II, VII and I accounted for 91.9%, 3.9%, and 1.7% of isolates as top three *coa* type identified among *S. aureus* clinical isolates [6]. In other research conducted by Mohajeri et al. [31], five *coa* PCR types were obtained. They revealed that out of 96 *coa*-positive MRSA isolates, 29 (30.2%) belonged to genotype pattern III, 27 (28.2%) to IV, 15 (15.6%) to I, 13 (13.5%) to II and 12 to (12.5%) to IV. Consistent with other findings [23, 27, 31], we found that MDR- MRSA strains are differed considerably among the countries.

In our study, VRSA isolate belonged to CC/ST22-SCC*mec* IV/t790 lineage. In contrast to our findings, earlier studies from Iran displayed that VRSA strains belonged to ST239-SCC*mec* III/t037 and ST1283-SCC*mec* III/t037 clones [32, 33]. A systematic review and meta-analysis conducted by Shariati et al. indicated that despite of VRSA and VISA has been associated with many clones such as CC5, CC8, CC30, and CC45, however, the majority of VRSA strains belonged to CC5 in the USA [26]. In other research conducted by Tiwari et al. from India [34], it was observed that VRSA isolate belonged to CC/ST8-SCC*mec*IV/t008 clone.

In the current study, we found that all mupirocin resistant MDR-MRSA isolates exhibited HLMUPR phenotype and the majority of them belonged to CC/ST8. In accordance with our finding, mupirocin resistance in CC/ST8 MRSA clone has been reported earlier. Similarly, study performed by Udo et al. in Kuwait during a period of eighteen years in 13 health care settings indicated high prevalence of HLMUPR strains which belonged to CC/ST8-SCC*mec* IV/t064 clone [35].

In consistent with our results, studies from Ireland [36], and Nigeria [37] have shown resistance to mupirocin among CC/ST8 MRSA isolates. We also noted a CC/ST22-SCC*mec* IV/t790 isolate with HLMUPR phenotype in this study. This observation was also supported by Goudarzi et al.'s findings from Iran that confirmed the presence of HLMUPR-MRSA strains related to CC/ST15 (40%), CC/ST22 (23.3%), and CC/ST8 (36.7%) clones among examined MRSA strains [38]. These results support this assumption that CC/ST8 and ST22 strains are actively circulating in our hospitals.

Our data demonstrated that all tigecycline -resistant MRSA isolates belonged to CC8/ST239- SCC*mec* III/t421 lineage. In this regard, Dabul and colleagues analyzed 36 *S.*

aureus strains isolated from infection and nasal sites and found 10 tigecycline-resistant *S. aureus* strains which all belonged to ST105-SCC*mec*II lineage [39]. This lineage has been identified in both community and health care settings in many countries including Kuwait, China, the United Arab Emirates, Japan, Switzerland, the UK, Australia, and Spain [16].

This study confirms high rate of MDR among MRSA strains isolated from wound. Since there is a considerable increasing trend for simultaneous resistance to antibiotics among MRSA strains in Iran, the emergence of tigecycline, mupirocin and vancomycin-resistant MRSA strains in our hospitals must not be neglected. Our findings indicate the need for efficient control protocols and stricter precautions to stop the dissemination of these isolates in both communities to hospitals. Present study also suggests continuous monitoring of the genotypes of MDR-MRSA isolates to prevent nosocomial outbreaks and development of resistance to these antibiotics.

Competing interests: All authors declare that they have no competing interests.

Author contribution: MG, BH, and MJ conceived and designed the study. BH, MJN, HG, MD, MH, AH, and MMM contributed in comprehensive research. MG, BH and MJ wrote the paper. MG, MH, HG and MD participated in manuscript editing.

Ethics approval and consent to participate: This study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran (IR.SB-MU.MSP.REC.1398.492).

Consent for publication: Not applicable.

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