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Molecular Characterization and Resistance Profile of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Hospitalized Patients in Intensive Care Unit, Tehran-Iran

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Abstract

Background: *Staphylococcus aureus* is an important opportunistic pathogen and can cause a wide range of infections. The ability of this pathogen to successfully persist within the hospital and the community is largely due to its remarkable ability to acquire resistance against various antimicrobial agents.

Objectives: The aim of this study was to determine the carriage of antibacterial resistance genes and virulence markers of *S. aureus* isolates from hospitalized patients in intensive care units in Tehran, the capital of Iran.

Methods: In this cross-sectional study that was conducted during an 11-month period since March 2015 to January 2016, a total of 105 *S. aureus* strains were investigated. MRSA screening was performed by phenotypic and genotypic methods. The Kirby-Bauer disk diffusion method was used to assess the sensitivity of *S. aureus* strains. The strains were typed based on the polymorphisms in SCCmec types. The presence of resistance (*ermA*, *ermB*, *ermC*, *mupA*, *msrA*, *msrB*, *tetM*, ant (4´)-*Ia*, *aac* (6´)-*Ie*/*aph* (2˝), *aph* (3´)-*IIIa*) and toxin (*etb*, *eta*, *pvl*, *tst*) encoding genes were investigated by the polymerase chain reaction (PCR) technique.

Results: In this study, 105 isolates of *S. aureus* were obtained from 299 various clinical specimens. Ninety five (90.5%) strains were confirmed as methicillin-resistant *S. aureus*. The lowest levels of resistance were related to quinupristin-dalfopristin (16.8%) while the highest levels of resistance were related to penicillin (94.7%). Multi-drug resistance was observed in 91.5% of the isolates. Type IV was the most prevalent *SCCmec* type (57.9%), followed by type III (22.1), type V (12.6%), I (5.3%), and II (2.1%). Overall, 25 isolates (26.3%) harbored PVL-encoding genes, and all of them belonged to *SCCmec* type IV. The presence of resistance genes *ant*(4´)-*Ia*, *aac*(6´)-*Ie*/*aph*(2˝), *aph*(3´)-*IIIa*, *ermA*, *ermB*, *ermC*, *msrA*, *msrB*, and *tetM* was detected in 94.7%, 81.1%, 31.6%, 31.6%, 15.9%, 18.9%, 47.3%, 21.1%, 56.8%. The frequency of the *etb*, *eta*, and *tst* genes were 1.1%, 4.2%, and 32.6%, respectively.

Conclusions: The results illustrated the diversity of antibacterial resistance and virulence gene profiles among different *SCCmec* types of *S. aureus*. The increased prevalence of methicillin-resistant *S. aureus* isolates containing different toxin and antibiotic resistance genes is a serious threat for the hospitalized patients in the intensive care units.

Keywords: MRSA, Multidrug-Resistant, Intensive Care Unit, *Staphylococcus aureus*

1. Background

Staphylococcus aureus is the major cause of infection in either hospitals or within the communities across the world (1). During the past several decades, in spite of introducing a variety of therapeutic measures including antibiotic therapy, Methicillin-resistant *S. aureus* (MRSA) strains have shown a remarkable ability in a rapid development of multi-drug resistance (MDR) (2, 3).

The ability of this pathogen to successfully persist within the hospitals and communities is largely due to its remarkable ability to acquire resistance particularly against methicillin, the further complicating treatment of infections. Methicillin resistance is mediated by *mecA* gene, that is transferred by the staphylococcal cassette chromosome mec (*SCCmec*) as a mobile genetic element with the size of 21 - 67 kbp (4). Eleven different *SCCmec* (I-XI) types have been characterized based on the nature of the *mec* and *ccr* gene complexes (4). The study of the distribution of the *SCCmec* element of *S. aureus* is essential for the molecular typing of MRSA strains (1).

Aminoglycosides are one of the most antibiotics used to treat infections caused by *S. aureus* especially

MRSA strains. The main mechanism of the resistance to aminoglycosides is the inactivation of antibiotics by aminoglycoside-modifying enzymes (AMEs) (5, 6). Methicillin-resistant *S. aureus* strains have shown a wide pattern of resistance not only to β-lactams and aminoglycosides, but also to other therapeutic options such as macrolides, lincosamides, and mupirocin (2, 3). The macrolide antibiotics as protein synthesis inhibitors are widely used in the treatment of staphylococcal infections. The different mechanisms of resistance to macrolides include: a) ribosomal binding site modification encoded by *erm* genes (*ermA*, *ermB*, and *ermC*), b) active efflux mechanism encoded by *msr* gene (7).

Mupirocin, also known as pseudomonic acid A, is a topical narrow spectrum antibiotic that is used for eradicating MRSA colonization and, also, the treatment of different types of staphylococcal skin infections. Mupirocin is an analogue of isoleucine that binds to isoleucyl-tRNA synthetase and blocks protein synthesis. Mupirocin resistance is classified in two categories: a) high-level mupirocin resistance that is encoded by plasmid-borne *mupA* genes, b) low level mupirocin resistance (*mupL*) that is encoded by the genes located on the bacterial chromosome (8). Nowadays, in spite of the introduction of newer antibiotics, due to the emergence and spread of various types of antibacterial resistance genes, the treatment of infections caused by MRSA strains has become problematic (3).

The most commonly detected antibiotic resistance genes in clinical *S. aureus* strains are *mecA* (methicillin), *ermA*, *ermB* and *ermC* (macrolide, lincosamide, streptogramin B), *mupA* (mupirocin), msrA and *msrB* (macrolides), *tet* (tetracycline), *ant*(4´)-*Ia*, *aac*(6´)-*Ie*/*aph*(2˝), and *aph*(3´)-*IIIa* (AMEs, aminoglycoside modifying enzymes) (9-14). MRSA strains are usually resistant to many antibiotics and may carry virulence factors that have a significant role in the pathogenicity of the diseases such as panton-valentine leukocidin (PVL), toxic shock syndrome toxin-1 (TST-1), and exfoliative toxins (*eta*, *etb*) (9, 15, 16).

2. Objectives

Given the increasing occurrence of MRSA infections in different wards of the hospitals, especially in the intensive care unit (ICU) wards, the purpose of the present research was to determine the prevalence of MRSA, antibacterial susceptibility pattern, and the carriage of resistance and virulence determinants in various types of clinical samples recovered from ICUs.

3. Methods

3.1. Study Design and Population

The 105 *S. aureus* strains analyzed in this cross-sectional study were obtained from hospitalized patients in the ICUs of seven hospitals in Tehran, Iran since March 2015 to January 2016. These isolates were recovered from different clinical samples like wound (n = 45; 42.9%), blood (n = 30; 28.6%), ear (n = 12; 11.4%), pus (n = 8; 7.6%), body fluids (n = 5; 4.8%), catheter (n = 3; 2.8%), and urine (n = 2; 1.9%). The research was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code # 143). All patients provided written informed consent for this study. Samples were transported to the laboratory within 4 hours of collection and were processed immediately. All the strains were identified by standard microbiological procedures such as colony morphology, Gram staining, growth on mannitol salt agar and production of catalase, coagulase, and DNase. All the isolates were confirmed using polymerase chain reaction (PCR) for the *femA* and *nucA* genes (10, 11). *S. aureus* isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for further investigations.

3.2. Antibacterial Susceptibility Testing

Susceptibility to kanamycin (K 30 μg), ciprofloxacin (CIP 5μg), clindamycin (CD 2 μg), tetracyclin (T 30 μg), erythromycin (E 15 μg), linezolid (LZD 30 μg), penicillin (PG 10 μg), teicoplanin (TEC 30 μg), quinupristin-dalfopristin (SYN 15 μg), amikacin (AK 30 μg), tobramycin (TN 10 μg), gentamicin (GM 10 μg), trimethoprim-sulfamethoxazole (TS 2.5 μg), and ceftriaxon (CRO 30 μg) was determined using the Kirby-Bauer disk diffusion technique in accordance with the clinical and laboratory standards institute (CLSI) (17). The minimum inhibitory concentration (MIC) for vancomycin and mupirocin was determined with E-test strips (bioMe'rieux) according to the manufacturer's instructions. According to the CLSI guidelines, the high-level mupirocin-resistant isolates showed an MIC greater than 512 μg/mL.

Inducible clindamycin resistance was detected by broth micro-dilution method according to the CLSI procedure. Briefly, the organism was regarded as positive for inducible clindamycin resistance and/or inducible macrolide, lincosamide, streptogramin ($iMLS_B$) phenotype when growth was observed in a well containing 4 μg/mL erythromycin and 0.5 μg/mL clindamycin. If growth was not observed, no inducible clindamycin resistance and/or constitutive MLSB ($cMLS_B$) phenotype was reported. Overall, inducible resistance to clindamycin was defined for the isolates that were susceptible to clindamycin and resistant against erythromycin, while constitutive resistance

was defined for the isolates that were resistant to both erythromycin and clindamycin (17). Multidrug resistance (MDR) was defined as resistance to three or more unique antibiotic classes in addition to beta-lactams (1). All the antibiotic disks used in this research were supplied by Mast, UK. *S. aureus* ATCC25923 and ATCC29213 were used as quality control strains in the antibacterial susceptibility testing.

3.3. Extraction of Genomic DNA

The DNA of the strains was extracted using the commercial kit InstaGene Matrix (BioRad, Hercules co., CA, USA) along with the addition of lysostaphin (Sigma-Aldrich co., USA) to a final concentration of 15 μg/mL.

3.4. Methicillin-resistant S. aureus Screening

Methicillin-resistant *S. aureus* isolates were screened with cefoxitin disc (30 μg) and oxacillin disc (1 μg) on Mueller Hinton agar plates supplemented with 4% NaCl in accordance with the CLSI guidelines (17). Isolates with phenotypic resistance to oxacillin were confirmed to harbor the *mecA* gene by PCR (15).

3.5. The Detection of Resistance and Toxin Encoding Genes

PCR was performed to determine the presence of resistance (*ermA*, *ermB*, *ermC*, *mupA*, *msrA*, *msrB*, *tetM*, ant (4´)-*Ia*, *aac* (6´)-*Ie*/*aph* (2˝), *aph* (3´)-*IIIa*) and *toxin* (*etb*, *eta*, *pvl*, *tst*) encoding genes. The primer sequences are presented in Table 1.

3.6. Multiplex PCR for SCCmec Typing

Different *SCCmec* types were characterized by specific primers described by Boye et al. (4). *SCCmec* types were identified by comparing the banding patterns of MRSA to ATCC 10442 (*SCCmec* type I), N315 (*SCCmec* type II), 85/2082 (SCCmec type III), MW2 (*SCCmec* type IVa), and WIS (*SCCmec* type V) as reference strains. PCR amplification was performed in a final volume of 50 μL via EmeraldAmp MAX PCR Master Mix (Takara, Japan) for all PCR reactions. PCR conditions for the amplification of the *SCCmec* elements by thermocycler (Eppendorf co., Hamburg, Germany) were as follows: Initial denaturation for 5 minutes at 94°C, 35 cycles of denaturation at 94°C for 40 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 1 minute. The final extension was carried out at 72°C for 4 minutes. Amplified products were analyzed by electrophoresis on 1% agarose gel via ethidium bromide staining. Ultimately, the products were examined under ultraviolet illumination.

4. Results

In this study, 105 isolates of *S. aureus* were obtained from 299 various clinical specimens. Out of the 105 tested isolates, 95 (90.5%) isolates were confirmed as MRSA strains. Wound infections (47.3%) were the most prevalent infections among our samples, followed by blood infections (28.4%) and pus infections (8.4%). The results of the antibiotic susceptibility testing showed that all the strains were inhibited by vancomycin at similar MIC_{50} and MIC_{90} 1 μg/mL. The susceptibility of MRSA strains to the commonly used antibacterial agents is shown in Table 2. Out of the 29 MRSA isolates that were found mupirocin resistant, 12 (41.4%) isolates were detected as MRSA isolates with high-level mupirocin resistance and carried the *mupA* gene. All the MRSA strains carrying the *mupA* gene were isolated from wound infections.

In the present study, 67 (70.5%) MRSA isolates expressed resistance to erythromycin. Also, $iMLS_B$ was detected in 12 (12.6%), $cMLS_B$ in 50 (52.6%), and MLS_B in 5 (5.3%) isolates. The prevalence of MDR-MRSA isolates in the present study was estimated to be 91.5%. Generally, five different antibiotic resistance patterns were identified among the MRSA strains. The distribution of different resistance patterns among the MRSA isolated from the clinical samples of the hospitalized patients in ICU is presented in Table 3.

Multiplex-PCR analysis for the detection of different *SCCmec* types revealed that type IV was the most predominant (57.9%) *SCCmec* type followed by type III (22.1), type V (12.6%), I (5.3%), and II (2.1%) (Figure 1). Twenty five isolates (26.3%) were positive for PVL-encoding genes which belonged to *SCCmec* type IV. It is noteworthy that PVL genes were detected in 37% and 33% of blood and wound infections, respectively, while they were not detected in other remaining infections. Out of the 95 MRSA isolates analyzed in the current study, 31 (32.6%) isolates harbored *tst-1* encoding gene that was detected in 40%, 37.5%, 33.3% and 33.3% of wound, pus, blood, and catheter samples, respectively. The eta gene was only found in one MRSA isolated from wound infections that was assigned to *SCCmec* type IV. Two (4.2%) isolates harbored *etb* gene, which belonged to *SCCmec* type III. The distribution of different *SCCmec* types and toxin encoding genes among MRSA isolates obtained from various clinical sources is summarized in Table 4.

The analysis of the antibiotic resistance genes by PCR revealed that *ant(4´)-Ia* gene had the highest frequency and *mupA* gene had the lowest frequency in the present study (Figure 2). The majority of the isolates carrying the *ant(4´)-Ia* gene (77.8%) were also resistant to kanamycin. The MRSA strains of the wound and blood infections had the highest prevalence of antibiotic resistance genes. The distribution of the resistance genes among 95 MRSA

Table 1. Oligonucleotide Primers Used in This Study

Target	Primer	Primer Sequence (5´ → 3´)	Product Size, bp	Reference
femA	F	CTTACTTACTGCTGTACCTG	648	(10)
	R	ATCTCGCTTGTTGTGTGC		
nucA	F	GCGATTGATGGTGATACGGTT	270	(1)
	R	AGCCAAGCCTTGACGAACTAAAGC		
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583	(15)
	R	ATGTATGTGCGATTGTATTGC		
luk-PV	F	TTCACTATTTGTAAAAGTGTCAGACCCACT	180	(9)
	R	TACTAATGAATTTTTTTATCGTAAGCCCTT		
tst-1	F	TTATCGTAAGCCCTTTGTTG	398	(15)
	R	TAAAGGTAGTTCTATTGGAGTAGG		
eta	F	GCAGGTGTTGATTTAGCATT	93	(16)
	R	AGATGTCCCTATTTTTGCTG		
etb	F	ACAAGCAAAAGAATACAGCG	226	(16)
	R	GTTTTTGGCTGCTTCTCTTG		
ant(4´)-Ia	F	AATCGGTAGAAGCCCAA	135	(10)
	R	GCACCTGCCATTGCTA		
aac(6´)-Ie/aph(2˝)	F	CCAAGAGCAATAAGGGCATACC	222	(10)
	R	CACACTATCATAACCACT		
aph(3´)-IIIa	F	CTTGATCGAAAAATACCGCTGC	269	(10)
	R	TCATACTCTTCCGAGCAAA		
ermA	F	TATCTTATCGTTGAGAAGGGATT	139	(11)
	R	CTACACTTGGCTGATGAAA		
erimB	F	CTATCTGATTGTTGAAGAAGCATT	141	(11)
	R	GTTTACTCTTGGTTTAGGATCAAA		
ermC	F	AATCGTCAATTCCTGCATGT	299	(12)
	R	TAATCGTGGAATACGGGTTTG		
msrA	F	GGCACAATAAGAGTG TTTAAAGG	940	(13)
	R	AAGTTATATCATGAATAGATTGTCCTGTT		
msrB	F	TATGATATCCATAATAATTATCCAATC	595	(13)
	R	AAGTTATATCATGAATAGATTGTCCTGTT		
mupA	F	CCCATGGCTTACCAGTTGA	1158	(14)
	R	CCATGGAGCACTATCCGA		
tetM	F	AGTGGAGCGATTACAGAA	158	(16)
	R	CATATGTCCTGGCGTGTCTA		

strains isolated from the hospitalized patients in ICUs is presented in Table 5.

All the 12 strains with the iMLS$_B$ phenotype (12.6%) carried *ermA*, *ermB*, *msrA* genes, while *ermC* and *msrB* were detected among two (16.7%) and five (41.7%) isolates. Out of 50 (52.6%) strains with the cMLS$_B$ phenotype, *ermA*, *ermC*, *msrA*, and *msrB* were found in 11 (22%), 16 (32%), 33 (66%), and 15 (30%) isolates, respectively. Out of five isolates with MLSB phenotype, two (2.1%) isolates carried *ermA* and one isolate (1.1%) carried *ermB*. Two (2.1%) isolates with MLS$_B$ phenotype were negative for these genes. None of the isolates with MLS$_B$ phenotype carried *msrA* and *msrB* genes. The most common resistance genes among iMLS$_B$ and cMLS$_B$ phenotypes were *ermA*, *ermB*, *msrA* genes (100%) and *msrA* (66%).

The results of the aminoglycoside-resistant genes screening showed *ant(4´)-Ia* as the most prevalent resistance gene found among 90 (94.7%) strains. A total of 55 (57.9%) isolates harbored the *ant(4´)-Ia* and *aac(6´)-Ie/aph(2˝)*, 20 (21.1%) isolates *ant(4´)-Ia,aph(3´)-IIIa*, and *aac(6´)-Ie/aph(2˝)*, 6 (6.3%) isolates *ant(4´)-Ia*, *aph(3´)-IIIa*, 2 (2.1%) isolates *aph(3´)-IIIa*, and *aac(6´)-Ie/aph(2˝)* simultaneously. The *ant(4´)-Ia* gene was detected alone in seven isolates (7.3%).

5. Discussion

The widespread emergence of MDR *S. aureus* is becoming a great challenge in the public health (1). According to the literature, there is heterogeneity in the prevalence of MRSA in different regions and countries (1,16). In this study, methicillin resistance was detected in 90.5% *S. aureus* isolates. The high frequency of MRSA isolates in the present

Table 2. The Antibiotic Resistance Pattern of 95 MRSA Isolates Collected From Hospitalized Patients in ICU[a]

Antibiotics	Antibiotic Susceptibility (n = 95)		
	R	I	S
Penicillin	90 (94.7)	0	5 (5.3)
Linzolid	0	0	95 (100)
Teicoplanin	0	0	95 (100)
Ceftriaxone	35 (36.8)	0	60 (63.2)
Gentamicin	69 (72.6)	1 (1.1)	25 (26.3)
Kanamycin	78 (82.1)	3 (3.2)	14 (14.7)
Amikacin	56 (59)	3 (3.2)	36 (37.8)
Tobramycin	66 (69.5)	0	29 (30.5)
Mupirocin	29 (30.5)	0	66 (69.5)
Clindamycin	50 (52.6)	0	45 (47.4)
Ciprofloxacin	60 (63.2)	4 (4.2)	31 (32.6)
Erythromycin	67 (70.5)	0	28 (29.5)
Trimetoprim-sulfamethoxazole	29 (30.5)	1 (1.1)	65 (68.4)
Quinupristin-dalfopristin	16 (16.8)	0	79 (83.2)
Tetracycline	60 (63.2)	4 (4.2)	31 (32.6)

Abbreviation: ICU, intensive care unit.
[a]Values are expressed as No. (%).

Table 3. The Distribution of Different Clinical Sample and Resistance Profile in MRSA Isolated From Hospitalized Patients in ICU[a]

Number of Drugs	Resistance Profile	Number of Isolates	Type of Samples
8	PG, K, GM, TN, CIP, E, T, AK, CD	48(50.5)	W (25; 52.1), B (10; 20.8), E (7; 14.5), P (3; 6.3), C (1; 2.1), U (1; 2.1), BF (1; 2.1)
	PG, K, CIP, E, T, CRO, TS, MUP	10(10.5)	W (9; 90), P (1; 10)
7	PG, K, GM, T, CRO, TS, MUP	17(17.8)	W (4; 23.5), B (10; 58.8), C (1; 5.9), BF (1; 5.9), U (1; 5.9)
4	PG, AK, E, CRO	5(5.3)	W (2; 40), B (3; 60)
	PG, K, CD, E	2(2.1)	B (1; 50), P (1; 50)
	PG, GM, AK, CRO	3(3.1)	P (3; 100)
	E, TN, GM, K	1(1.1)	W (1; 100)
3	CIP, E, T	1(1.1)	B (1; 100)
2	TS, MUP	2(2.1)	B (1; 50), W (1, 50)
1	T	1(1.1)	B (1; 100)
	PG	5(5.3)	W (3; 60), BF (1; 20), C (1; 20),

Abbreviations: AK, amikacin; B, Blood; BF, Body fluide; C, Catheter; CD, clindamycin; CIP, ciprofloxacin; CRO, ceftriaxone; E, erythromycin; E, ear; GM, gentamicin; ICU, intensive care unit; K, kanamycin; MUP, mupirocin; P, Pus; PG, penicillin; SYN, quinupristin-dalfopristin; T, tetracyclin; TN, tobramycin; TS, trimethoprim- sulfamethoxazole; W, wound; U, urine.
[a]Values are expressed as No. (%).

study was in agreement with the findings of the studies in Iran (1), Bolivia (18), and India (19) and is higher than Hungary (20) and Croatia (21). The results of the susceptibility testing showed that all the isolates were susceptible to vancomycin, linezolid, and teicoplanin which is consistent with a previous study in Iran (1) and other studies (16-21).

Although aminoglycosides play an important role in the therapy of serious staphylococcal infections, there have, recently, been reports of increased resistance to this drug worldwide. The results of this study revealed a relatively high level resistance to aminoglycosides such as kanamycin (82.1%), gentamicin (72.6%), tobramycin (69.5%), and amikacin (59%). This finding is similar to the other observations reported from 12 Asian countries by Ko et al. (22). Also, a study conducted in Iran (1) exhibited relatively similar resistance to amikacin (64.3%), gentamicin (60%), kanamycin (57.1%), and tobramycin (57.1%) in comparison with our study. The *ant(4´)-Ia* gene was determined in 94.7% of the MRSA isolates. These results are inconsistent with a previous study by Rahimi et al. which reported that the prevalence of *aac(6′)/aph(2′′)* gene was higher than that of the two other AME genes, *ant(4′)-Ia* and *aph(3′)-IIIa* in MRSA isolates (23). Furthermore, the frequency rate of the *ant(4')-Ia* gene was higher than in the previous study performed in Iran (23) (42.2%), Japan (84.5%) (24) and Turkey (24%) (10). The rate of *aph(3´)-IIIa* gene in this study was 31.6%, which is in accordance with the results of a study in Iran by Rahimi (23), and was higher than Japan (8.9%) (24) and lower than Turkey (66%) (10). In the present study, all the MRSA isolates resistant to gentamicin were positive for the *aac(6′)/aph(2′′)* gene, which is consistent with other reports (23, 25).

The macrolide antibiotics as protein synthesis inhibitors are widely used in the treatment of staphylococcal infections (7). In the current survey, the *ermA* gene predominated in the strains with the inducible phenotype, while *ermC* was more common in the isolates with the constitutive phenotype, which is similar to the data reported by Ghanbari et al. (26). In this study, the frequency of the $cMLS_B$ phenotype was 50 (52.6%), $iMLS_B$ was 12 (12.6%), and MLS_B was 5 (5.3%). In Turkey, the prevalence of $iMLS_B$, $cMLS_B$, and MSB phenotypes among MRSA strains was 18%, 23%, and 48%, respectively (27). In another study conducted by Lavallee et al. (28) in Canada, inducible clindamycin resistance was detected in 64.7% MRSA isolates and 35.3% of

Table 4. The Distribution of Antibiotic Resistance Genes in the MRSA Strains of Various Clinical Infections Isolated from Hospitalized Patients in ICU

Type of Samples (No)	Distribution of Antibiotic Resistance Genes										
	mecA	*ant(4´)-Ia*	*aac(6´)-Ie/aph(2˝)*	*apih(3´)-IIIa*	*ermA*	*ermB*	*ermC*	*msrA*	*msrB*	*tetM*	*mupA*
Wound	45 (47.3)	45 (47.3)	45 (47.3)	15 (15.8)	15 (15.8)	8 (8.4)	5 (5.3)	20 (21.1)	11 (11.6)	25 (26.3)	12 (12.6)
Blood	27 (28.4)	27 (28.4)	20 (21.1)	10 (10.4)	8 (8.4)	3 (3.2)	10 (10.4)	15 (15.8)	2 (2.1)	19 (20)	0
Ear	7 (7.4)	7 (7.4)	2 (2.1)	0	0	0	2 (2.1)	5 (5.3)	4 (4.2)	6 (6.3)	0
Pus	8 (8.4)	5 (5.3)	5 (5.3)	1 (1.1)	5 (5.3)	3 (3.2)	0	0	3 (3.2)	4 (4.2)	0
Body fluids	3 (3.2)	2 (2.1)	0	0	0	1 (1.1)	0	0	0	0	0
Catheter	3 (3.2)	2 (2.1)	3 (3.2)	3 (3.2)	2 (2.1)	0	1(1.1)	3 (3.2)	0	0	0
Urine	2 (2.1)	2 (2.1)	2 (2.1)	1 (1.1)	0	0		2 (2.1)	0	0	0
Total	95 (100)	90 (94.7)	77 (81.1)	30 (31.6)	30 (31.6)	15 (15.9)	18 (18.9)	45 (47.3)	20 (21.1)	54 (56.8)	12 (12.6)

Abbreviation: ICU, intensive care unit.

Table 5. The Distribution of Different *SCCmec* Types and Toxin Encoding Genes Among MRSA Isolates Obtained From Various Clinical Sources

Type of Samples (No)	Distribution of *SCCmec* Types					Toxin Encoding Genes			
	I	II	III	IV	V	*pvl*	*tst*	*eta*	*etb*
Wound (45)	2 (4.4)	0	9 (20)	25 (55.6)	9 (20)	15 (33.3)	18 (40)	1 (2.2)	2 (4.4)
Blood (27)	2 (7.4)	1 (3.7)	7 (25.9)	15 (55.6)	2 (7.4)	10 (37)	9 (33.3)	0	0
Ear (7)	0	0	5 (71.4)	2 (28.6)	0	0	0	0	0
Pus (8)	1 (12.5)	0	0	6 (75)	1 (12.5)	0	3 (37.5)	0	0
Body fluids (3)	0	1 (33.3)	0	2 (66.7)	0	0	0	0	0
Catheter (3)	0	0	0	3 (100)	0	0	1 (33.3)	0	0
Urine (2)	0	0	0	2 (100)	0	0		0	0
Total (95)	5 (5.3)	2 (2.1)	21 (22.1)	55 (57.9)	12 (12.6)	25 (26.3)	31 (32.6)	1 (1.1)	4 (4.2)

these isolates showed constitutive MLSB phenotype. The rate of the $iMLS_B$ phenotype in the present study is higher than those reported by Ghanbari et al. (26) (4.18%), and Schreckenberger et al. (7%) (29). In a study conducted by Fiebelkorn et al. (30) 34% of 114 erytromycin resistant *S. aureus* isolates demonstrated constitutive resistance to clindamycin and 29% showed inducible resistance. Overall, the occurrence of the $iMLS_B$ resistance phenotype varies widely in geographic regions (29). In this survey, the frequency of $cMLS_B$ phenotype was higher than $iMLS_B$ phenotype, which is in accordance with the results obtained by Ghanbari et al. (26).

In the present study, of 29 isolates (30.5%) that presented mupirocin resistance phenotype, of which 41.4% harbored the *mupA* gene as confirmed as high-level mupirocin resistant MRSA that is lower than the previous rate reported by Abbasi-Montazeri et al. (31) from the south-west of Iran (34%) and is higher than India (5%) (32), and Greece (1.6%) (8). In this survey, the resistance of the isolates was confirmed by the amplification of *mupA*. In a study conducted by Shahsavan et al. (33), it was shown that 25% of mupirucin resistant MRSA isolates carried the *mupA* gene. Unrestricted policies that allow the improper and widespread use of mupirocin for long periods in hospitals and health care settings and the origin of the isolates and clinical samples are the most important causes of variation in the incidence rate of resistance to mupirocin in MRSA isolates (8, 32, 33).

The *tetM* was the second most detected antibiotic resistance gene in the MRSA strains among the clinical isolates in this study. This observation is in agreement with Dormanesh et al.'s (34) study. They showed that the most commonly detected antibiotic resistance genes in the MRSA strains were *tetK* (89.18%), *mecA* (71.62%), *msrA* (56.75%), and *tetM* (54.05%). In accordance with the results of the studies in Italy (35), Croatia (21), and Hungary (20), a high resistance to ciprofloxacin was seen in the current study (63.2%), which could be due to the widespread use of ciprofloxacin in the treatment of MRSA infections, mutation in gyrase or topoisomerase IV enzymes, and efflux pumps (36).

The most frequent toxin gene in this survey was *tst* (32.6%) that was recovered from blood (33.3%), pus (37.5%), and wound (40%) samples. The results obtained in the current study are higher than those reported in Sweden 22% (37) and Colombia (10%) (38) and lower than a study conducted in Iran 51.4% (1). In line with a study which reported the prevalence of 2% -35% of PVL genes among MRSA strains (39), 25 isolates (26.3%) obtained from blood (37%)

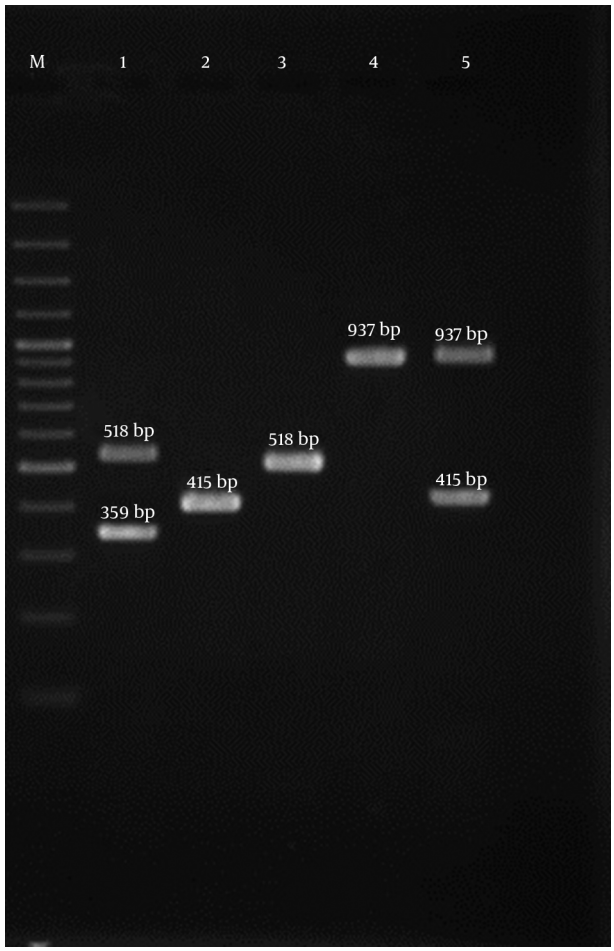

Figure 1. Lane M, 100-bp DNA Ladder (Fermentas, UK); Lane 1 *SCCmec* Type V; Lane 2 *SCCmec* Type I; Lane 3 *SCCmec* Type III; Lane 4 *SCCmec* Type II; Lane 5 *SCCmec* Type IV.

and wound (33.3%) which belonged to *SCCmec* type IV were positive for the PVL-encoding genes. The frequency of the eta gene in this study was 1.1%. The frequency rates of eta gene reported from Colombia (38) and Malaysia (40) were 3% and 0%, respectively. The range from 0% in Colombia (38) and Malaysia (40) to 9.2% in Turkey (41) for the *etb* gene frequency was reported. The frequency of the *etb* in this study was 4.2%, which is in accordance with Eftekhar et al. study from Iran (42).

SCCmec types I, II, III are related to hospital-associated MRSA (HA-MRSA) while the *SCCmec* types IV and V are prominent types of community-associated MRSA (CA-MRSA) (1). The distribution of *SCCmec* types among the MRSA strains indicated the high frequency of *SCCmec* type IV (57.9%) in the present study, all of which were PVL positive. This finding was inconsistent with the previous reports about the predominance of *SCCmec* III in most Asian countries (22). In this study, the resistance to antibacterial agents and MDR pattern among isolates with *SCCmec* type III was more prevalent than that of *SCCmec* type IV, while the distribution of the virulence factors were vice versa, which is in line with the findings of other studies (16, 21).

In conclusion, the results of this study illustrated that the *SCCmec* type IV was predominant among ICU patients. The higher frequency of some antibiotic resistance genes in this study which could be a challenge for the public health, emphasizes that infection control measures should be prioritized in the ICUs of our hospitals.

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Footnotes

Authors' Contribution: Mehdi Goudarzi and Seyed Mansour Meybodi: study concept and design, the development of the study, data interpretation, and manuscript revision; Ramin Rashidi, Mehdi Goudarzi and Seyed Mansour Meybodi: phenotypic and molecular studies and manuscript drafting; Ramin Rashidi, Mehdi Goudarzi and Maryam Fazeli: performing experimental procedures; Ramin Rashidi and Maryam Fazeli: participation in the acquisition of data and statistical analysis; Mehdi Goudarzi and Seyed Mansour Meybodi: Study supervision. All the authors read and approved the final manuscript.

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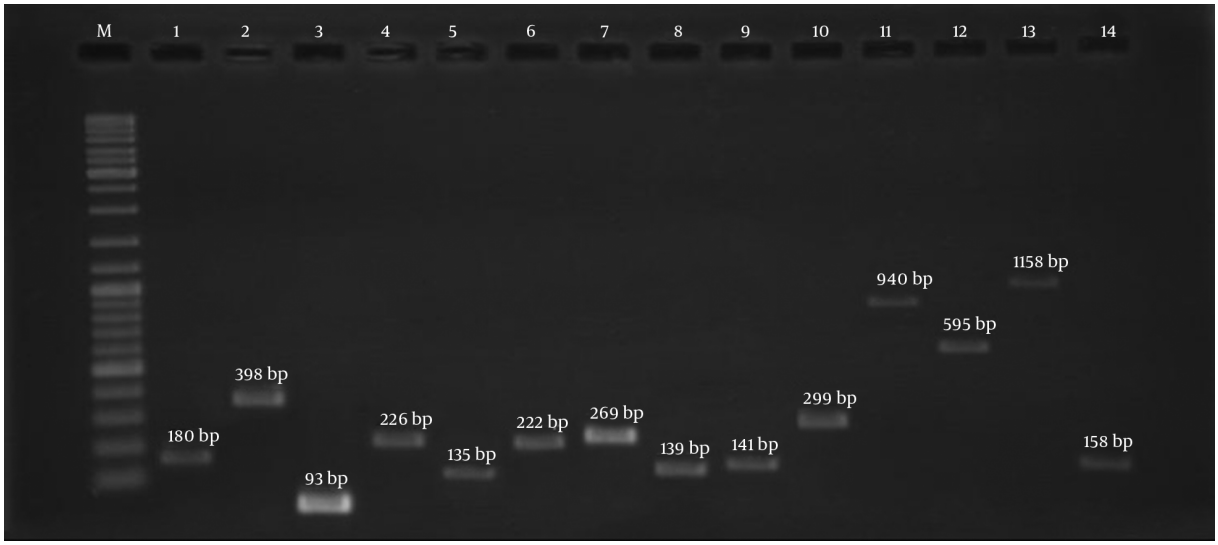

Figure 2. Lane M, 100-bp DNA Ladder (Fermentas, UK); Lane 1 *luk-PV* Gene, Lane 2 *tst* Gene, Lane 3 eta Gene, Lane 4 *etb* Gene, Lane 5 *ant(4´)-Ia* Gene, Lane 6 *aac(6´)-Ie/aph(2´)* Gene, Lane 7 *aph(3´)-IIIa* Gene, Lane 8 *ermA* Gene, Lane 9 *ermB* Gene, Lane 10 *ermC*, Lane 11 *msrA* Gene, Lane 12 *msrB* Gene, Lane 13 *mupA* Gene, and Lane 14 *tetM* Gene.

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