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Genetic Variability and Integron Occurrence in Methicillin Resistant *Staphylococcus aureus* Strains Recovered from Patients with Urinary Tract Infection

Mehdi Goudarzi [1,*], Anis Mohammadi [2], Hossein Goudarzi [1], Maryam Fazeli [3] and Fattaneh Sabzehali [1]

[1]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran
[3]Department of Virology, Pasteur Institute of Iran, Tehran, Iran

[*]*Corresponding author*: Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: gudarzim@yahoo.com

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Abstract

Background: Treatment for infections caused by Methicillin-Resistant *Staphylococcus aureus* (*S. aureus*) is one of the main concerns of public health.
Objectives: The aim of this study was to investigate the prevalence of toxin, enterotoxin, and resistant encoding genes and analyze the distribution of different SCC*mec* types. The prevalence of integron was also determined in *S. aureus* isolates obtained from patients with urinary tract infections (UTIs).
Methods: In the present study, 126 MRSA isolates obtained from patients with UTI were examined for susceptibility to antimicrobial agents. Genes encoding integrase, resistance, toxin, and SEs were detected by the polymerase chain reaction (PCR) screening. The SCC*mec* types were determined using the multiplex PCR. Integrase positive strains were evaluated for determination of integron classes using PCR-restriction fragment length polymorphism assay.
Results: From 126 MRSA isolates studied, 107 isolates (84.9%) were multi-drug resistant. The most prevalent genes in isolates under study was *aac* (6´)-*Ie*/*aph* (2˝) (50%) followed by *tet* (M) (47.6%), *msr* (A) (38.1%), *aph* (3´)-*IIIa* (25.4%), *erm* (A) (23%), *ant* (4´)-*Ia* (16.7%), *erm* (B) (14.3%), *msr* (B) (9.5%), and *erm* (C) (7.1%). Staphylococcal enterotoxins *sea*, *sec*, *see*, *sed*, *seg*, *seb*, and *sei* were detected in 27%, 20.6%, 16.7%, 14.3%, 11.1%, 7.1%, and 5.6% of the isolates, respectively. The results revealed that 126 MRSA isolates fell in the SCC*mec* type III (37.3%), SCC*mec* type I (23%), SCC*mec* type II (14.3%), SCC*mec* type IV (13.5%), and SCC*mec* type V (11.9%). Class 1 and 2 integrons were commonly found in 34.1% and 14.3% of the isolates, respectively. Seven isolates (5.6%) were observed to carry class 1 and 2 integrons, simultaneously.
Conclusions: The current findings showed that identification and screening of integrons and SCC*mec* elements as reservoirs that should be considered for various resistance genes to consume proper antibiotic and perform a systematic surveillance.

Keywords: Methicillin-Resistant, *Staphylococcus aureus*, Integrons, Urinary Tract Infection

1. Background

Urinary tract infections (UTIs) are among common bacterial infections, which annually influence more than 150 million people around the world. Furthermore, UTIs can infect both genders but they are more prevalent in females than their male counterparts (1, 2). They are a significant cause of death for females of all age groups, children, and older males and nearly all people experience at least one episode of UTI during their lifetime (2). Urinary Tract Infections are a severe public health problem that cause considerable economic and public health burdens and have a significant impact on the life quality of people with the disease. A wide range of pathogens, including gram-negative and gram-positive bacteria, as well as certain fungi, cause the disease (2, 3). Although the most common causative agents are *Escherichia coli*, *Klebsiella pneumonia*, and other *Enterobacteriaceae*, *Staphylococcus aureus* (*S. aureus*) as an uncommon cause of UTI, represents 0% to 6% of urinary samples and is important as a primary urinary pathogen among long-term care patients. It has been well-documented that catheterization increases the risk of *S. aureus* carriage in the urinary tract (3).

According to previously published data, although UTI causes low mortality rates, antimicrobial therapy should

not be ignored (1, 4). It has been well-documented that one-third of patients with *S. aureus* bacteriuria have a symptomatic urinary tract infection and are highly recommended for treatment with antibiotics (3). Although antimicrobial therapy has significantly reduced mortality rate from *S. aureus* infections, it can lead to the development of resistance mechanisms to antimicrobial agents (5). However, UTI treatment associated with *S. aureus*, which is now most often multi-resistant, is limited, owing to the widespread emergence of an array of antibiotic resistance mechanisms (4). The prevalence of Methicillin-Resistant *S. aureus* (MRSA) strains has significantly increased over the years, posing a major public health concern (6). The *mecA* gene has a large heterologous mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*) for carrying resistance to methicillin. Considering the genetic content and structural arrangement of SCC*mec*, eleven different types have been reported (7).

Mobile genetic elements, such as plasmids and transposons, can disseminate antibiotic resistance genes through horizontal gene transfer mechanisms. In the recent years, the role of integrons as a key system involved in spreading antibiotic multi-resistance has been well-established (8). Despite being motionless, integrons may be transferred by mobile genetic elements (9, 10). Based on the homology of integrase gene, several classes of integrons have been described, which are related to antibiotic multi-resistance. Class 1 and 2 integrons are commonly identified in clinical *S. aureus* isolates, while reports are limited regarding other classes (10).

Unfortunately, integron detection in clinical isolates of MRSA is not routinely performed and their presence is therefore missed. Screening for integrons in MRSA strains and its relationship with antibiotic resistance can be a useful tool for studying molecular epidemiology and also the management of infection (10).

2. Objectives

The aim of this study was: (i) to characterize the antibiotic resistance pattern, toxin, staphylococcal enterotoxins (SEs) and resistance encoding genes; (ii) to determine the prevalence of integron; and (iii) to determine molecular types of MRSA strains using *SCCmec* typing in *S. aureus* isolates, obtained from UTI.

3. Methods

3.1. Study Design and Bacterial Isolation

After screening 1425 urine samples from patients reporting UTI, 126 MRSA isolates were recovered between February, 2016 and March, 2018. Before collecting the samples, all the patients gave their verbal informed consents. The Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran) also approved this study (IR.SBMU.MSP.REC.1396.809).

Thermal boxes containing the samples were sent to the laboratory under refrigeration and immediately homogenized and inoculated on agar plates. After incubating the plates for 24 to 48 hours under aerobic conditions at 37°C, the colony count was measured. The UTI was defined as a positive urine culture for *S. aureus* with a colony count of $\geq 10^5$ CFU/mL. Standard biochemical tests, such as growth on mannitol salt agar, colony morphology on blood agar, and coagulase, DNase, and catalase production assays, were used to identify *S. aureus* isolates. A PCR assay targeting the *S. aureus*-specific *nuc* gene was performed for definitive identification of presumed identified isolates as *S. aureus* species (11). For MRSA screening, phenotypic growth was investigated around the cefoxitin disc (30 μg) and placed on plates of Mueller-Hinton agar (Merck, Germany), containing 4% NaCl. Also, the PCR method was applied for genotypic amplification of *mec*A genes (11). All the strains harbored the *mec*A gene and were confirmed as MRSA.

3.2. Antimicrobial Susceptibility Testing

Based on the Clinical and Laboratory Standards Institute (CLSI) guidelines, standard Kirby-Bauer disk diffusion method was applied for the *in vitro* assessment of antimicrobial susceptibility to antimicrobial agents (Mast Diagnostics Ltd., Merseyside, UK), including tetracycline, erythromycin, clindamycin, ampicillin, trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, and amikacin in MRSA isolates (12).

The minimum inhibitory concentration (MIC) was measured using the broth microdilution test for vancomycin and fusidic acid (12). The MIC cutoff points for vancomycin based on the CLSI criteria were as follows: resistant, $\geq$ 16 μg/mL; intermediate, 4 to 8 μg/mL; and susceptible, $\leq$ 2 μg/mL. The guidelines of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) were applied to interpret the results of resistance to fusidic acid. The resistance of MRSA strains to three antimicrobial categories (or more), besides beta-lactams, was defined as multidrug resistance (MDR) (13, 14). For quality control, *S. aureus* ATCC25923 and ATCC29213 strains were used in each run.

3.3. Genomic DNA Preparation

A QIAamp DNA mini kit (Hilden, Germany) was employed for the extraction of total genomic DNA, based on the manufacturer's protocols; nevertheless, lysostaphin (15 μg/mL) was added for cell wall lysis.

3.3.1. Polymerase Chain Reaction for Detection of Resistance and Toxin-Encoding Genes

The PCR was performed to determine the presence of resistance (*mecA*, *vanA*, *vanB*, *erm* (A), *erm* (B), *erm* (C), *msr* (A), *msr* (B), *tet* (M), *ant (4´)-Ia, aac (6´)-Ie/aph (2˝), aph (3´)-IIIa*) (15-19), toxin (*etb*, *eta*, *pvl*, *tst*) (11, 13) and SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei* and *sej*) (20) encoding genes.

3.4. Multiplex Polymerase Chain Reaction Amplification for SCCmec Typing

Multiplex PCR amplification was carried out with specific primers for SCC*mec* typing, as suggested by Boye et al. (7). The controls included MRSA strains, i.e., ATCC 10442, N315, 85/2082, MW2, and WIS (SCC*mec* type I, II, III, IV, and V, respectively).

3.5. Amplification of Integrase Gene and PCR-Restriction Fragment Length Polymorphism Analysis

According to a study by Goudarzi et al. (14), integrase genes were detected, using specific primers for the conserved sites of integron-encoded integrase genes (*intI1*, *intI2*, and *intI3*) (21). To determine different classes of integrons, PCR-positive products were exposed to *RsaI* and *HinfI* restriction enzymes. Using electrophoresis on 1.5% agarose gel, the digested patterns were examined. For RFLP reactions, a final volume of 20 μL, including the integrase PCR product (10 μL), specific buffer (2 μL), double distilled water (7 μL), and 10 U *RsaI* and *HinfI* restriction enzymes (1 μL), were used (21).

3.6. Statistical Analysis

To analyze the collected data, SPSS version 18.0 (SPSS Inc., Chicago, IL) was used.

4. Results

Of 126 MRSA strains obtained from patients, 93 (73.8%) isolates belonged to female patients and 33 (26.2%) to male patients. The patients' average age was 36 years (median, 39.1, range, 4 to 65). The patients were distributed to three age group; $\leq$ 20 years old (30 patients, 23.8%), 21 to 45 years old (81 patients, 64.3%), and 46 to 65 years old (15 patients, 11.9%).

The results of antibiotic susceptibility testing revealed high levels of resistance in the isolates including to ampicillin (126/126, 100%), gentamicin (88/126, 69.8%), tetracycline (83-126, 65.9%), ciprofloxacin (76/126, 60.3%), erythromycin (71/126, 56.3%), clindamycin (52/126, 41.3%), amikacin (46/126, 36.5%), and trimethoprim-sulfamethoxazole (39/126, 31%). None of the isolates were susceptible to all of the tested antibiotics. The predominant resistance profile included resistance to four antibiotics (38.1%, 48/126), followed by five antibiotics (22.2%, 28/126), eight antibiotics (11.9%, 15/126), six antibiotics (13/126, 10.3%), and three antibiotics (13/126, 10.3%), simultaneously. All the MRSA isolates were susceptible to vancomycin and fusidic acid. The results of vancomycin MIC showed that 43 (34.1%) isolates had MIC $\geq$ 0.5 μg/mL, 39 (31%) had MIC $\geq$ 1 μg/mL, 41 (32.5%) had MIC $\geq$ 2 μg/mL, and 3 (2.4%) exhibited MIC $\geq$ 4 μg/mL. Fusidic acid could inhibit all the strains at similar MIC_{50} and MIC_{90} (0.25 μg/mL). Of the 126 MRSA isolates, 84.9% (107/126) were defined as MDR. Out of 126 MRSA clinical isolates examined, $iMLS_B$ and $cMLS_B$ were detected in 19 (15.1%) and 52 (41.3%) MRSA isolates, respectively.

Analysis of resistance encoding genes among tested MRSA strains showed that the most prevalent resistance gene was *aac* (6')-*Ie/aph* (2") (50%, 63126) followed by *tet* (M) (47.6%, 60/126), *msr* (A) (38.1%, 48/126), *aph* (3')-*IIIa* (25.4%, 32/126), *erm* (A) (23%, 29/126), *ant* (4')-*Ia* (16.7%, 21/126), *erm* (B) (14.3%, 18/126), *msr* (B) (9.5%, 12/126), and *erm* (C) (7.1%, 9/126). All isolates with inducible and constitutive resistance carried one or more macrolide resistance gene (s). Among the isolates with constitutive phenotype, the *msr* (A) gene was the most prevalent (78.8%, 41/52) followed by *erm* (A) (44.2%, 23/52), *erm* (B) (23.1%, 12/52), *msr* (B) (19.2%, 10/52) and *msr* (C) (17.3%, 9/52) while *msr* (A) (36.8%, 7/19), *erm* (A) (31.6%, 6/19), *erm* (B) (31.6%, 6/19), and *msr* (B) (10.5%, 2/19) genes were much more common in the isolates with the inducible phenotype. As shown in Table 1, 54 isolates (42.9%) harbored three resistance genes, 27 isolates (21.4%) two resistance genes, and 19 isolates (15.1%) four resistance genes, simultaneously. The findings revealed that 26 isolates did not indicate any carriage of resistance gene.

Regarding the presence of SEs genes, the most prevalent gene was *sea* (n = 34; 27%), followed by *sec* (n = 26; 20.6%), *see* (n = 21; 16.7%), *sed* (n = 18; 14.3%), *seg* (n = 14; 11.1%), *seb* (n = 9; 7.1%), and *sei* (n = 7; 5.6%). None of the isolates carried *seh* and *sej* genes. In the current study, SEs genes were found in 57.1% (72/162) of MRSA isolates. Analysis of the toxin encoding genes in MRSA strains demonstrated that 34 strains (27%) harbored *tst* encoding gene, 20 strains (15.9%) *pvl* gene, three strains (2.4%) *etb* gene, and two strains *eta* gene (1.6%) (Figure 1). Distribution of enterotoxin and genetic resistance profiles are presented in Table 1.

According to the results of SCC*mec* typing, 47 isolates (37.3%) harbored SCC*mec* type III, 29 isolates (23%) SCC*mec* type I, 18 isolates (14.3%) SCC*mec* type II, 17 isolates (13.5%) SCC*mec* type IV, and 15 isolates (11.9%) SCC*mec* type V.

According to the PCR-RFLP results, out of 126 MRSA isolates tested, *int* gene was found in 68 isolates (54%). Among

Table 1. Enterotoxin and Genetic Resistance Profiles of 126 MRSA Strains Obtained from Patients with UTI

Enterotoxin Profiles	Enterotoxin Types	No. (%)
Profile I	*sea+sec+sed*	4 (3.2)
Profile II	*sea+sec+see*	5 (4)
Profile III	*sea+sec*	5 (4)
Profile IV	*sea+see*	16 (12.7)
Profile V	*sed+sec*	10 (7.9)
Profile VI	*sea+seg*	3 (2.4)
Profile VII	*seb+sei*	4 (3.2)
Profile VIII	*seg+seb*	1 (0.8)
Genetic Resistance Profiles	**Resistance Genes**	**No. (%)**
Profile A	*mecA, tet (M), erm (A), msr (C), msr (B)*	4 (3.2)
Profile B	*mecA, aac (6´)-Ie/aph (2˝), tet (M), msr (A), erm (B)*	15 (11.9)
Profile C	*mecA, aac (6´)-Ie/aph (2˝), aph (3´)-IIIa, msr (A)*	25 (19.8)
Profile D	*mecA, aac (6´)-Ie/aph (2˝), ant (4´)-Ia, ant (4´)-Ia, tet (M)*	14 (11.1)
Profile E	*mecA, aac (6´)-Ie/aph (2˝), aph (3´)-IIIa, ant (4´)-Ia*	7 (5.6)
Profile F	*MecA, tet (M), erm (A), msr (A)*	8 (6.3)
Profile G	*mecA, tet (M), erm (A)*	17 (13.5)
Profile H	*mecA, erm (C) msr (B)*	5 (4)
Profile I	*mecA, aac (6´)-Ie/aph (2˝), tet (M)*	2 (1.6)
Profile J	*mecA, erm (B) msr (B)*	3 (2.4)

these isolates, 43 (34.1%) carried class 1 integrons, 18 (14.3%) carried class 2 integrons, and seven (5.6%) carried class 1 and class 2, simultaneously (Figure 2). The characteristics related to the integron positive and negative MRSA isolates have been presented in Table 2.

5. Discussion

The increasing emergence of resistance to currently available antimicrobial agents among MRSA strains exhibiting MDR pattern has limited the choice of therapeutic options and is becoming a serious threat to public health. Therefore, novel strategies are needed for the control of infections caused by MRSA strains (13, 15, 16).

In the present study, resistance rates against ampicillin as a beta-lactam antibiotic, along with other antimicrobials, appear to be high, with the exception of amikacin (36.5%) and trimethoprim-sulfamethoxazole (31%), which is in accordance with the findings reported in Iran (13), Turkey (22), and Italy (23). High prevalence of resistance in tested MRSA isolates can be attributed to the indiscriminate use of antibiotics and poor implementation of measures, such as identification and effective control of MRSA strains and continuous and nationwide surveillance programs.

Despite the recent data from Iran about the emergence of vancomycin-resistant *S. aureus* isolates (19), in the present study, all the MRSA isolates were susceptible to vancomycin. These findings represent the proper use and prescription guidelines of vancomycin in Iranian hospitals. Although during the two last decades, a significant trend towards increased fusidic acid resistance among MRSA isolates has been reported, the current findings indicated that none of the investigated isolates were resistant to fusidic acid. This result is in accordance with studies conducted by Aschbacher et al. (23) in Italy and Otokunefor et al. (24) in the UK. It has been well-documented that there are discrepant rates of MDR-MRSA prevalence in different geographic areas. In the present study, of the 126 MRSA isolates, 84.9% were defined as MDR, which is in line with reported results from Serbia (25) and Taiwan (26). In this study, out of 126 MRSA clinical isolates, 19 (15.1%) and 52 (41.3%) isolates showed $iMLS_B$ and $cMLS_B$ phenotype, respectively. In line with the current findings, Rashidi Nezhad et al. (13) from Iran reported that 52.6%, 12.6%, and 5.3% of their tested isolates showed cMLSB, iMLSB, and MLS_B phenotypes, respectively. Lavallee et al. (27) in Canada reported that 64.7% and 35.3% of isolates had iMLSB and cMLSB phenotypes, respectively.

Regarding the presence of aminoglycoside-modifying

Table 2. Distribution of MRSA Molecular Types Isolated from Patients with UTI

Integron Types[a]	SCC*mec* Type	Toxin Profile, No. (%)	Enterotoxin Encoding Genes	Antibiotic Resistance Gene Profiles [a], No. (%)	Antibiotic Resistance Profile, No. (%)	No. (%)
I	I	*tst* 3 (33.3)	*sea* 3 (33.3), *sec* 4 (44.4)	Profiles C 4 (44.4) and B 3 (33.3)	AP, CIP, T, E, CD, AK, GM, TS 4 (44.4)	9 (7.1)
					AP, CIP, T, GM 2 (22.2)	
					AP, CIP, T, E, CD, TS 3 (33.3)	
	II	*etb* 1 (16.7)	*sea* 2 (33.3), *see* 1 (16.7), *sei* 2 (33.3)	Profiles A 4 (66.7) and C 2 (33.3)	AP, CIP, T, E 2 (33.3)	6 (4.8)
					AP, E, AK, GM, T 3 (50)	
					AP, AK, E, CD, GM 1 (16.7)	
	III	*tst* 10 (41.7)	*sea* 8 (33.3), *sec* 11 (45.8), *see* 5 (20.8), *sed* 4 (16.7)	Profiles C 12 (50), G 8 (33.3) and D 4 (16.7)	AP, CIP, T, E, CD, AK, GM, TS 4 (16.7)	24 (19)
					AP, CIP, T, GM 8 (33.3)	
					AP, AK, E, CD, GM 7 (29.2)	
					AP, CIP, T, E 5 (20.8)	
	IV	*pvl* 4 (100)	*sea* 2 (50), *see* 2 (50)	Profile E 3 (75)	AP, CIP, T, GM 3 (75)	4 (3.2)
					AP, AK, E, CD, GM 1 (25)	
II	II	*etb* 2 (66.7)	*sea* 1 (33.3), *seg* 1 (33.3), *seb* 2 (66.7)	Profiles C 1 (33.3), D 1 (33.3) and F 1 (33.3)	AP, E, AK, GM, T 2 (66.7)	3 (2.4)
					AP, E, CD, AK, TS 1 (33.3)	
	III	*tst* 2 (50)	*seb* 2 (50), *sei* 3 (75)	Profiles C 2 (50), B 1 (25) and D 1 (25)	AP, CIP, T, E, CD, AK, GM, TS 2 (50)	4 (3.2)
					AP, GM, TS 2 (50)	
	IV	*pvl* 5 (83.3)	*sea* 4 (66.7), *see* 3 (50), *seg* 1 (16.7)	Profiles E 3 (50) and B 3 (50)	AP, CIP, T, GM 2 (33.3)	6 (4.8)
					AP, CIP, T, E, CD, TS 2 (33.3)	
					AP, GM, TS 2 (33.3)	
	V	*pvl* 2 (40)	*sea* 2 (40), *seg* 2 (40)	Profiles F 4 (80) and C 1 (20)	AP, E, CD, AK 1 (20)	5 (3.9)
					AP, CIP, T, E, CD, AK, GM, TS 3 (60)	
					AP, CIP, T, E, CD, TS 1 (20)	
I and II	I	*tst* 1 (25)	*seg* 1 (25), *seb* 2 (50)	Profiles B 3 (75) and D 1 (25)	AP, CIP, T, E, CD, AK, GM, TS 2 (50)	4 (3.2)
					AP, CIP, T, E, CD, TS 1 (25)	
					AP, E, CD, AK, GM 1 (25)	
	III	*eta* 2 (66.7)	*sed* 3 (100), *sei* 2 (66.7)	Profiles F 2 (66.7) and H 1 (33.3)	AP, CIP, T, E 2 (66.7)	3 (2.4)
					AP, E, AK, GM, T 1 (33.3)	
Without integron	I	*tst* 7 (43.8)	*sed* 1 (6.3), *seg* 4 (25), *seb* 3 (18.7), *see* 4 (25)	Profiles B 4 (25), D 5 (31.3) and G 3 (18.7)	AP, CIP, T, GM 5 (31.3)	16 (12.7)
					AP, E, CD, AK, GM 4 (25)	
					AP, CIP, T, E, CD, TS 4 (25)	
					AP, GM, TS 3 (18.7)	
	II	-	*sed* 2 (22.2), *seg* 5 (55.6)	Profiles C 33.3), and H 2 (22.2)	AP, CIP, T, E 2 (22.2)	9 (7.1)
					AP, CIP, T, GM 7 (77.8)	
	III	*tst* 11 (68.8)	*sec* 8 (50), *see* 4 (25), *sed* 8 (50)	Profiles D 2 (12.5), G 3 (18.7), E 1 (6.3) and H 2 (12.5)	AP, CIP, T, GM 9 (56.3)	16 (12.7)
					AP, E, CD, AK, GM 3 (18.7)	
					AP, CIP, T, E, CD, TS 1 (6.3)	
					AP, GM, TS 3 (18.7)	
	IV	*pvl* 7 (100)	*sea* 5 (71.4), *see* 6 (85.7)	Profiles B 1 (14.3) and G 3 (42.9)	AP, E, CD, AK, GM 3 (42.9)	7 (5.6)
					AP 4 (57.1)	
	V	*pvl* 2 (20)	*sea* 7 (70), *sec* 3 (30)	Profiles F 1 (10), I 2 (20), and J 3 (30)	AP, CIP, T, E 1 (10)	10 (7.9)
					AP, E, AK, GM, T 1 (10)	
					AP, CIP, T, E, CD, TS 1 10)	
					E, CD, AK 2 (20)	
					AP 5 (50)	

Abbreviations: AK, amikacin; AP, ampicillin; CD, clindamycin; CIP, ciprofloxacin; E, erythromycin; GM, gentamicin; T, tetracycline; TS, trimethoprim-sulfamethoxazole.
[a] Genetic resistance profiles are presented in Table 1.

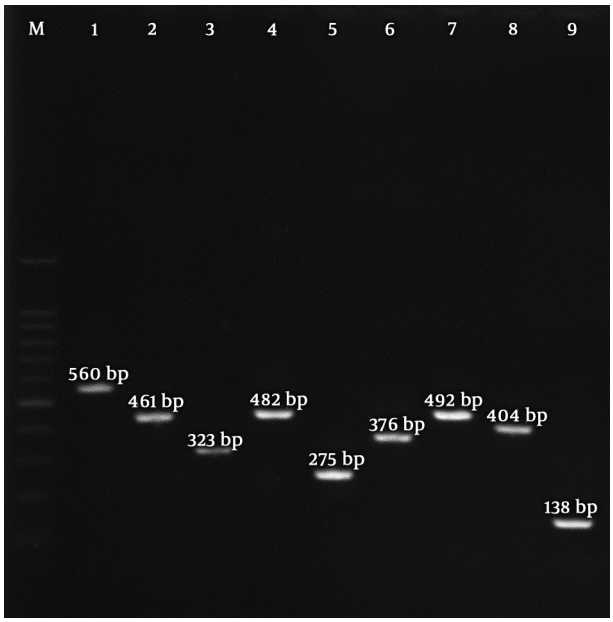

Figure 1. Lane M, 100-bp DNA ladder (Fermentas, UK); lane 1 *sea* gene, lane 2 *sei* gene, lane 3 *seg* gene, lane 4 *see* gene, lane 5 *sec*, lane 6 *seh* gene, lane 7 *sed* gene, lane 8 *seb* gene, and lane 9 *shj* gene.

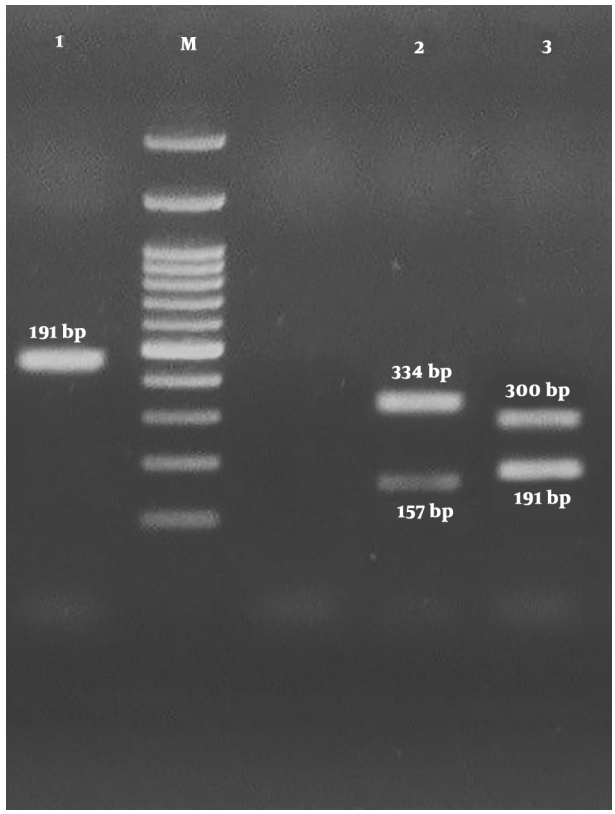

Figure 2. The PCR-RFLP results for integrase gene products. Lane M, 100-pb DNA ladder; lane 1, *HinfI* and *RsaI*-treated products indicating class 1 integrons; lane 2, *RsaI*-treated products indicating class 2 integrons; lane 3, *HinfI*-treated products indicating class 2 integrons.

enzyme genes, as noted above, *aac* (6´)-*Ie*/*aph* (2˝) (50%) was the most frequent gene followed by *aph* (3´)-*IIIa* (25.4%) and *ant* (4´)-*Ia* (16.7%), which is in contrast to the findings of Rashidi Nezhad et al.'s study (13) that reported *ant (4')-Ia* gene as the most prevalent gene (94.7%), followed by the *aac (6′)/aph (2′′)* (81.1%) and *aph (3')-IIIa* (31.6%) genes, respectively. In contrary, Ida et al. (28) and Yadegar et al. (29) reported the ant *(4)-Ia gene* as the most prevalent gene in 84.5% and 58% of *S. aureus* strains in comparison with other modifying enzyme genes. Analysis of macrolide resistance encoding genes indicated that resistance to erythromycin was due to *msr* (A) (38.1%) among MRSA isolates from Iran. The different rate of macrolide resistance genes was reported by several investigators (13). The observed differences reflected differences in policies related to antibiotic prescription, treatment protocols, and also dissemination of resistance gene in geographic regions, where the study was carried out.

Regarding the findings of multiplex PCR, it was found that 57.1 % of the isolates contained at least one SE gene. Similarly, in a study on *S. aureus* producing enterotoxin isolated from skin infections, a relatively high prevalence of SEs (45%) was described (30). A study conducted in Turkey reported that 62.6% of *S. aureus* isolates were enterotoxigenic (31). A remarkable finding regarding the presence of staphylococcal enterotoxin genes was the high prevalence of *sea* (n = 34; 27%). Similar results were reported by Mashouf et al. indicating a prevalence of 25.5% of *sea* gene in *S. aureus* strains isolated from animal-originated foods (32). In a study conducted by Aydin et al. (31) in 2007 to 2008, the prevalence of *sea* genes was 8.6%. In contrast to the results of several studies, which showed that enterotoxin-like (SEl) toxin genes, mainly *seg* and *sei*, were found in isolates from patients rather than strains of food samples and food poisoning (31), the present study indicated classical enterotoxin genes (*sea* to *see*) were more common SEl genes.

Based on previous reports, the most frequent toxin-encoding gene in MRSA isolated from clinical samples is *tst* (13, 14). As stated in Table 2, in 126 MRSA isolates, the presence of *tst* was detected in 34 strains (27%). The rate reported in the current study is approximately similar to the reported rate from Sweden (22%) (33) and lower than another study from Iran (32.6%) (13). In the current work, the *pvl* gene was detected in 15.9% of tested isolates. Previous published data by the current authors from Iran revealed PVL-MRSA carriage rate of 15.1% in 2017 (14). How-

ever, a high prevalence of PVL was reported by several investigators (4, 13, 14). In this study, the presence of *eta* gene was confirmed in two strains (1.6%), which was close to the study conducted in Colombia (3%) (34) and the reported rate from Iran (1.1%) (13). Consistent with the current results, several studies have indicated the low frequency of *etb* genes (13, 14).

SCC*mec* type III is a common type reported in Iran (14), China (35), and Brazil (36), emphasizing the nosocomial origin of the strains. Similar to other studies that showed a higher percentage of *tst* genes among SCC*mec* type III (14), the present study showed that 48.9% of strains harbored SCC*mec* type III containing the *tst* gene (23/47). The other frequent SCC*mec* type identified in this study was type I (23%) followed by II (14.3). Conversely, in a study from Iran (published in 2017) conducted on 95 MRSA isolates collected from ICU patients, a prevalence of 5.3%. 2.1%, 22.1%, 57.9%, and 12.6% for types I - V, was documented, respectively (13). However, several reports have shown the various rates of SCC*mec* types I and II in examined isolates, albeit at a low level (13, 37). In this survey, SCC*mec* type IV and V were found in 13.5% and 11.5% of MRSA isolates. Inconsistent with the present results, Perez-Vazquez et al. (83.3%) reported high frequency of SCC*mec* type IV (37). Based on the present findings, the low frequency of SCC*mec* IV shows its lower mobility, compared to other SCC*mec* types.

The present study indicated that all PVL-positive strains were from SCC*mec* IV and V classes. This finding is in line with previous results showing that nearly all PVL-positive strains belong to SCC*mec* IV (13, 14).

As is generally accepted, integrons are key systems involved in spreading antibiotic multi-resistance among pathogenic bacteria. The present findings indicated class 1 integron as the most common type of integron (34.1%). On the other hand, class 2 integron was found in 14.3% of the isolates, while seven isolates (5.6%) carried both class 1 and 2 integrons. This, in agreement with other studies, shows that the percentages of integron class 1 is higher than integron class 2 (38).

This finding is similar to a study by Xu et al. from China, which identified class 1 integrons in 53% of *S. aureus* isolates (39). The current results are inconsistent with a study by Guney from Turkey, which showed that the tested isolates did not harbor class 1 integrons (22). Recent evidence supports the hypothesis that class 1 integrons can act as antimicrobial resistance reservoirs in MRSA strains. Discrepancies in the prevalence of integron classes can be attributed to the different geographic regions, the bacteria strains, or indiscriminate and overuse of antibiotics.

To summarize, the current findings indicated that SCC*mec* type III and class 1 integron were predominant among tested isolates with a high frequency of toxin, SEs, and resistance encoding genes. It can be deduced that simultaneous existence of these factors could confer to improve survival in various environments and lead to treatment failure. Therefore, identification and screening of integrons and SCC*mec* elements as reservoirs for various resistance genes could aim to select proper treatment protocols and also successful implementation of antibiotic stewardship and existing antibiotic resistance surveillance programs.

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Footnotes

Authors' Contribution: Study concept and design, development of the study, data interpretation, and manuscript revision: Mehdi Goudarzi, Maryam Fazeli, and Hossein Goudarzi; phenotypic and molecular studies and manuscript drafting: Hossein Goudarzi, Anis Mohammadi, Mehdi Goudarzi and Fattaneh Sabzehali; performing experimental procedures: Anis Mohammadi, Mehdi Goudarzi, Fattaneh Sabzehali and Maryam Fazeli; participation in the acquisition of data and statistical analysis: Mehdi Goudarzi, Hossein Goudarzi and Maryam Fazeli; study supervision: Mehdi Goudarzi and Hossein Goudarzi.

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