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Molecular Investigation of Methicillin-Resistant *Staphylococcus aureus* Strains Recovered from the Intensive Care Unit (ICU) Based on Toxin, Adhesion Genes and *agr* Locus Type Analysis

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Abstract

Background: *Staphylococcus aureus*, as one of the most common causes of nosocomial infections, has widely spread to all parts of the world and is becoming a serious concern in public health.
Objectives: The present study aimed at evaluating the prevalence of adhesion and toxin gene profiles and their distribution among different *agr* types.
Methods: The current cross-sectional study was performed in Tehran, Iran, by analyzing 125 methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from hospitalized patients at the ICUs from March, 2016 to January, 2017. In vitro antibiotic susceptibility testing of isolates was assessed using the Kirby-Bauer disk diffusion method. The MRSA strains were genetically typed by *agr* typing and virulence and adhesion genes profile by conventional PCR.
Results: Antibiotic susceptibility testing showed that inducible macrolide-lincosamide-streptogramin B, constitutive macrolide-lincosamide-streptogramin B, and high-level mupirocin resistance phenotypes had a frequency of 18 (14.4%), 50 (56%), and 10 (31.3%), respectively. The predominant resistance profile among MDR-MRSA isolates included resistance profile to seven antibiotics (32%). A total of ten virulence genotypes were observed, from which genotype *spa*, *clfA*, *clfB*, *fnbB*, *fnbA*, *ebp*, and *can* / *tst* (36%, 45/125) comprised the majority followed by *spa*, *clfA*, *clfB*, and *fnbB* (24%, 30/125). Type I was the most prevalent *agr* type (52%), followed by type III (34.4%), type II (9.6%), I 5(5.3%), and IV (4%). All isolates carrying PVL-encoding genes and HLMUPR-MRSA strains corresponded exclusively to *agr* type I.
Conclusions: The current data demonstrated that virulence gene profiles among different *agr* types of MRSA isolates were divers. The present study suggests that molecular characterization of MRSA strains should periodically be studied.

Keywords: MRSA, MDR, ICU, *agr* Typing

1. Background

Staphylococcus aureus, as one of the most common pathogens causing community and hospital infections, is responsible for a diverse spectrum of human infections ranging from skin and soft tissue infections to food poisoning, osteomyelitis, pneumonia, endocarditis, and bacteremia (1). These bacteria are equipped with a broad range of virulence factors and it was recently shown that it is able to carry resistance to many antimicrobial agents, especially methicillin (2).

The first Methicillin-Resistant *Staphylococcus aureus* (MRSA) was reported during 1961 in the UK (3). Resistance to methicillin is mediated by the *mecA* gene, which encodes a modified penicillin-binding protein (PBP2a). Unfortunately, data obtained from recent studies showed a worldwide increase in the prevalence of this organism and high rates of mortality and morbidity in healthcare settings so that currently MRSA has become a major public health concern, especially in intensive care unit (ICU) wards (4). The epidemiological success of this pathogen, in addition to the ability to express a variety of virulence factors, is also related to its remarkable ability to acquire resistance to new antimicrobial agents (2, 5).

It is evident that MRSA infections are related to expression of a broad range of virulent factors, which are controlled by the accessory gene regulator (*agr*) locus, via encoding a specific peptide, called auto-inducing peptide

(AIP) (1, 6).

The *agr* locus consists of five genes (*agrA*, *agrC*, *agrD*, *agrB*, and *hld)* and codes for two divergent transcriptional units, RNAII and RNAIII, which are under the control of two distinct promoters, P2 and P3, respectively. The P2 operon encodes *agrB*, *agrD*, *agrC*, and *agrA* that generate the *agr*-sensing mechanism (7). The *agr* D gene encodes AIP. Furthermore, *agrB* is a transmembrane protein that appears to be involved in the secretion of an AIP signal, and *agrC* (histidine kinase) acts as a sensor of AIP concentrations and in turn modulates the activity of *agrA*. *agrA*, as a response regulator, and activates P2 or P3 promoters. *agrA* and *agrC* downregulate surface proteins and upregulate those secreted. Within the *agr* locus, there is a variable region comprised of the 39-end of the *agrB* gene, the *agrD* gene, and the 59-end of the *agrC* gene. Variation in the amino acid sequence of the last one-third of *agrB* and *agrD*, and the first half of *agrC* generates the four *agr* major groups. Associations between the *agr* genotype of isolates, specific virulence factors, and staphylococcal diseases have been reported previously (7, 8).

2. Objectives

The present study was conducted in order (i) to characterize the antibiotic resistance pattern, toxin, and adhesion profiles of MRSA obtained from various types of clinical samples recovered from intensive care units (ICUs) and (ii) to further investigate these isolates by *agr* typing.

3. Methods

3.1. Sampling and Methicillin Resistant Staphylococcus aureus Screening

The current cross-sectional study was conducted between March 2016 and January 2017 on 125 MRSA strains isolated from hospitalized patients at ICUs. The MRSA strains were recovered from wound (n = 53; 42.4%), blood (n = 32; 25.6%), catheter (n = 12; 9.6%), ear (n = 10; 8%), pus (n = 9; 7.2%), body fluids (n = 7; 5.6%), and urine (n = 2; 1.6%). The research was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.SM.REC.1394.156). The inclusion criterion was having MRSA isolated from hospitalized patients at ICUs. The exclusion criterion was MRSA isolated from outpatients, community acquired, and other wards of hospitals. The bacterial isolates were presumptively identified on the basis of colony morphology, gram staining, growth on mannitol salt agar, and production of catalase, coagulase, and DNase. All the isolates were confirmed making use of polymerase chain reaction (PCR) for the *nucA* gene (9). The MRSA 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 Clinical and Laboratory Standard Institute (CLSI) guidelines (10). Isolates with phenotypic resistance to oxacillin were confirmed to harbor the *mecA* gene using PCR (2). The MRSA isolates were stored in tryptic soy broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for further investigation.

3.2. Antibacterial Susceptibility Testing

The antimicrobial susceptibility test (AST) was performed using the disk-diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines for 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 ceftriaxone (CRO 30 μg). The minimum inhibitory concentration (MIC) for vancomycin and mupirocin was determined with E-test strips (bioMe'rieux), according to the manufacturer's instructions. Inducible macrolide, lincosamide, and streptogramin B ($iMLS_B$) resistance was defined for the isolates that were susceptible to clindamycin and resistant against erythromycin, detected via D-zone test and broth microdilution method, according to the CLSI procedure (10). Constitutive MLS_B ($cMLS_B$) phenotype was defined for the isolates that were resistant to both erythromycin and clindamycin. Isolates, which showed resistance to at least three or more unique antibiotic classes in addition to beta-lactam were classified as multidrug resistant (MDR). All the antibiotic disks used in the present study were supplied by Mast Co, UK. *Staphylococcus aureus* ATCC25923 and ATCC29213 were used as quality control strains.

3.3. Bacterial DNA Extraction

Total genomic DNA was extracted from each MRSA isolate using the commercial kit InstaGene Matrix (BioRad, Hercules co., CA, USA), according to the manufacture's instruction. Additional reagent was lysostaphin (Sigma-Aldrich co., USA) in a final concentration of 15 μg/mL.

3.4. Adhesion and Toxin Encoding Genes Detection

All the isolates were screened for possible presence of adhesion (*spa*, *can*, *bbp*, *ebp*, *fnbB*, *fnbA*, *clfB*, and *clfA*) and toxin (*etb*, *eta*, *pvl*, and *tst*) genes with degenerate primers as listed in Table 1.

Table 1. Oligonucleotide Primers Used in This Study

Target	Primer	Primer Sequence (5' → 3')	Product Size, bp	Reference
nucA	F	GCGATTGATGGTGATACGGTT	270	(2)
	R	AGCCAAGCCTTGACGAACTAAAGC		
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583	(2)
	R	ATGTATGTGCGATTGTATTGC		
luk-PV	F	TTCACTATTTGTAAAAGTGTCAGACCCACT	180	(11)
	R	TACTAATGAATTTTTTTATCGTAAGCCCTT		
tsst-1	F	TTATCGTAAGCCCTTTGTTG	398	(2)
	R	TAAAGGTAGTTCTATTGGAGTAGG		
eta	F	GCAGGTGTTGATTTAGCATT	93	(12)
	R	AGATGTCCCTATTTTTGCTG		
etb	F	ACAAGCAAAAGAATACAGCG	226	(12)
	R	GTTTTTGGCTGCTTCTCTTG		
fnbA	F	CACAACCAGC AAATATAG	1362	(13)
	R	CTGTGTGGTAATCAATGTC		
fnbB	F	GGAGAAGGAATTAAGGCG	813	(13)
	R	GCCGTCGCCTTGAGCGT		
clfA	F	GTAGGTACGTTAATCGGTT	1586	(13)
	R	CTCATCAGGTTGTTCAGG		
clfB	F	TGCAAGATCAAACTGTTCCT	596	(13)
	R	TCGGTCTGTAAATAAAGGTA		
cna	F	AGTGGTTACTAATACTG	744	(14)
	R	CAG GAT AGA TTG GTTTA		
bbp	F	CAGTAAATGTGTCAAAAGA	1055	(15)
	R	TACACCCTGTTGAACTG		
ebp	F	CAATCGATAGACACAAATTC	526	(15)
	R	CAGTTACATCATCATGTTTA		
agr	Pan F	ATGCACATGGTGCACATGC	-	(7)
	R1	GTCACAAGTACTATAAGCTGCGAT	441	
	R2	TATTACTAATTGAAAAGTGGCCATAGC	575	
	R3	GTAATGTAATAGCTTGTATAATAATACCCAG	323	
	R4	CGATAATGCCGTAATACCCG	659	

3.5. Identification of agr Alleles Using Multiplex Polymerase Chain Reaction

Multiplex PCR was performed for *agr* types detection using a primer set comprised of a common forward primer (Pan) and reverse primers (*agr*1, *agr*2, *agr*3, and *agr*4) specific to each *agr* group. These primers were designed to amplify the 441-bp fragment of the *agr* group I strains, a 575-bp fragment of the *agr* group II strains, a 323-bp fragment of the *agr* group III strains, and a 659-bp fragment of the *agr* group IV strains. The primer sequences are listed in Table 1.

4. Results

In the current study, 125 MRSA isolates from 443 various clinical specimens were evaluated. All the isolates were confirmed as MRSA following phenotypic (cefoxitin disc screening) and genotypic (amplification of the *mecA* gene) methods. A total of 93 (74.4%) MRSA isolates were recovered from male and 32 from female (25.6%) patients. The mean age of patients was 39 years ranging from 4 to 71 years. The highest and lowest prevalence rate of MRSA infection in

the present study was found to be in the 21- to 45-year-old (71.2%) and in the less than 20-year-old age groups (8%), respectively.

4.1. Antimicrobial Susceptibility Testing

The results of antimicrobial susceptibility testing (AST) showed the following resistance patterns among MRSA isolates: penicillin (122; 97.6%), kanamycin (105; 84%), gentamicin (95; 76%), erythromycin (88; 70.4%), tetracycline (78; 62.4%), clindamycin (70; 56%), ciprofloxacin (63; 50.4%), amikacin (60; 48%), tobramycin (58; 46.4%), ceftriaxone (49; 39.2%), mupirocin (32; 25.6%), trimethoprim-sulfamethoxazole (21; 16.8%), and quinupristin-dalfopristin (12; 9.6%). All of the isolates were susceptible to vancomycin, teicoplanin, and linezolid. Based on the results of E-test for vancomycin, 43 (34.4%) isolates had MIC of 0.5 μg/mL, 27 (21.6%) had MIC of 1 μg/mL, and 55 (44%) had MIC 2 μg/mL. Of the 32 mupirocin-resistant MRSA isolates, 10 (31.3%) showed MIC $\geq$ 512 μg/mL and were reported as high-level mupirocin resistance (HLMUPR) MRSA isolates. All the HLMUPR-MRSA strains were collected from wound samples. In the present study, $iMLS_B$ and $cMLS_B$ was detected in 18 (14.4%) and 50 (56%) MRSA isolates, respectively. Multi-drug resistance (MDR) was detected in 120 tested isolates (96%). Generally, nine different resistance profiles were identified among the investigated isolates. The predominant resistance profile among MDR isolates included a resistance profile to seven antibiotics (32%) followed by eight antibiotics (24%), five antibiotics (15.2%), nine antibiotic (14.4%), six antibiotics (8%), four antibiotics (3.2%), and three antibiotics (2.4%), simultaneously. The distribution of resistance patterns and clinical samples obtained from hospitalized patients at the ICU are presented in Table 2.

4.2. Detection of Resistance and Toxin Encoding Genes

Among adhesion genes tested, the most prevalent was *spa* gene (125; 100%) followed by *clfA* (118; 94.4%), *clfB* (115; 92%), *fnbB* (112; 89.6%), *fnbA* (104; 83.2%), *ebp* (73; 58.4%), *can* (56; 44.8%), and *bbp* (4; 3.2%) genes. Among 125 MRSA strains, the most frequent toxin genes were *tst* (84; 67.2%), *pvl* (25; 20%), eta (15; 12%), and *etb* (9; 7.2%), respectively. Different patterns of the presence of adhesion and toxin encoding genes simultaneously in MRSA strains are presented in Table 3. The *pvl* gene was detected in MRSA strains isolated from wound (72%) and blood (28%) infections. Furthermore, *pvl* positive strains were distributed among MDR-MRSA strains with resistance profile to 7 and 9 antibiotics. Isolates carrying the *tst* gene had a resistance profile to 5, 7, 8, and 9 antibiotics.

4.3. Distribution of agr Types

Multiplex-PCR analysis for *agr* typing revealed that 65 isolates (52%) belonged to *agr* group I, 43 isolates (34.4%) to *agr* group III, 12 isolates (9.6%) to *agr* group II, and 5 isolates (4%) to *agr* group IV. All the isolates carrying PVL-encoding genes belonged to *agr* type I. The remaining genes encoding toxins and adhesions were distributed among different *agr* types. All the HLMUPR-MRSA strains belonged to the *agr* group I. Of 18 isolates with $iMLS_B$ phenotype, four isolates belonged to *agr* types I (22.2%) and 14 isolates (77.8%) to *agr* type III. The distribution of different *agr* types, adhesion, and toxin encoding genes among tested isolates is summarized in Table 4.

5. Discussion

Antibiotic resistance became a great challenge in public health in the 21st century. In the recent years, the study of antibiotic resistance pattern and distribution of virulence factors among molecular types of MRSA has been an important principle for better understanding of epidemiological and clinical characterization of these bacteria (16). According to previous studies, MRSA strains have shown a wide pattern of resistance to β-lactams and other therapeutic options, such as macrolides, lincosamides, and aminoglycosides (2, 5, 16). In line with earlier reports from Iran (9, 17), Turkey (18), and Italy (19), in the present study a high level of resistance to penicillin (97.1%) was found, which can be due to the wide use of beta lactams in hospitals to treat various infections. In the current survey, a high resistance to erythromycin (70.4%) and tetracycline (62.4%) was observed. This finding is largely in accordance with that reported by Rashidi Nezhad et al. (16), Goudarzi et al. (5), and Dormanesh et al. (20). These findings reveal the fact that these antibiotics are used improperly in the treatment of common infections as well as the acquisition of resistance determinants carried by transposons, plasmids or integrons. In addition, resistance rate to aminoglycosides has been investigated by several investigators. Gentamycin is an antibiotic used to treat several types of serious infections, especially staphylococcal infections. The resistance rate to gentamicin was 76% in the present study, which is in line with Goudarzi's study (2) yet was higher than those reported by Havaei et al. (21) and lower than that those reported by Wang et al. (22). The results demonstrated relatively high resistance to kanamycin (84%), amikacin (48%), and tobramycin (46.4%), which is in agreement with earlier rates reported by Ko et al. (23), Rashidi Nezhad et al. (16) and also a study conducted by Goudarzi et al. (2). Antibiotic inactivation by plasmid or transposon-mediated aminoglycoside modifying enzymes (AMEs) is known to be the main mechanism

Table 2. Distribution of Different Clinical Sample and Resistance Profile in Methicillin Resistant *Staphylococcus aureus* Isolated from Intensive Care Units

Number of Antibiotics	Resistance Profile	Number of Isolates (%)	Type of Samples (No.;%)
9	PG, K, E,T, CIP, AK, TN,CRO,MUP	18 (14.4)	W (18; 100)
8	PG, K, GM, E,CD,AK,TN, CRO	30 (24)	W (10; 33.3), B (17; 56.7), C (3; 10)
7	P,K,GM,E,T,CD,CIP	40 (32)	W (8; 20), B (7; 17.5), E (6; 15), C (9;22.5), P (5; 12.5),BF (5; 12.5)
6	P,GM,AK,TN,MUP,SYN	10 (8)	W (10; 100)
5	P,K,GM,T,TS	15 (12)	B (7; 46.7), E (3, 20), P (3; 20), U (2; 13.3)
	P,T,CIP,MUP,TS	4 (3.2)	W (4; 100)
4	K,AK,TS,SYN	2 (1.6)	W (2; 100)
3	T,CIP,CRO	1 (0.8)	BF (1; 100)
1	P	5 (4)	W (1; 20), B (1; 20), E (1; 20), P (1; 20), BF (1; 20)

Abbreviations: AK, Amikacin; B, Blood; BF, Body Fluid; C, Catheter; CD, Clindamycin; CIP, Ciprofloxacin; CRO, Ceftriaxone; E, Ear; E, Erythromycin, GM, Gentamicin; K, Kanamycin; MUP, Mupirocin; P, Pus; PG, Penicillin; SYN, Quinupristin-Dalfopristin; T, Tetracyclin; TN, Tobramycin; TS, Trimethoprim- Sulfamethoxazole; U, Urine; W, Wound.

Table 3. Virulence Patterns for Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Intensive Care Units

Adhesion/ Toxin Profile	Number of Isolates (%)
spa, clfA, clfB, fnbB, fnbA, ebp, can / tst	45 (36)
spa, clfA, clfB, fnbB	30 (24)
spa, clfA, clfB, fnbB, fnbA, ebp / pvl, tst	20 (16)
spa, clfA, clfB, fnbB / tst, eta	10 (8)
spa, clfA, clfB, fnbB, fnbA, ebp, can / pvl, tst, eta, etb	5 (4)
spa, clfA, clfB, fnbA, bbp, can / tst, etb	4 (3.2)
spa, clfA, fnbB, ebp, can	2 (1.6)
spa, clfA, clfB, ebp	1 (0.8)
spa, clfA	1 (0.8)
spa	7 (5.6)

of aminoglycoside resistance. In the present study, 25.6% of MRSA isolates were found to be resistant to mupirocin, among which 10 (31.3%) isolates were confirmed as HLMUPR strains. Various percentages of the mupirocin resistance were reported in MRSA strains isolated from Iran (28.3%) (5, 24), India (5%) (25), Jordan (2.6%) (26), and Greek (1.6%) (27). Although the main reasons of resistance to mupirocin are not completely clear, high resistance to mupirocin among tested isolates may be due to misuse of mupirocin in the treatment of MRSA skin and soft tissue infections and also eradication of nasal carriage of *S. aureus* in health care workers. However, the study population and type of clinical samples should also be considered. In a survey performed on *S. aureus* strains isolated from burn patients conducted by Abbasi Montazeri et al. (28), it was shown that two factors affecting mupirocin resistance among *S. aureus* isolates were previous exposure to mupirocin and previous infection by *Pseudomonas aeruginosa*. Although isolates of vancomycin-resistant *S. aureus* (VRSA) and vancomycin-intermediate *S. aureus* (VISA) strains have emerged in many parts of the world, the current results showed that vancomycin, teicoplanin, and linezolid had good activity against *S. aureus* isolated from clinical samples. This finding is similar to that of previous studies conducted in Iran (6), Italy (19), and Taiwan (22). These findings highlight the high relevance of proper antibiotic prescription, good surveillance programs, and principles of infection control in health care systems. Generally, these findings suggest a gradual decrease in the vulnerability of *S. aureus* to ampicillin, erythromycin, and tetracycline whereas other antibiotics, including vancomycin, teicoplanin, and linezolid have maintained their high efficiency. In a study reported from Iran, resistance rate to trimethoprim-sulfamethoxazole was found to vary between 19.3% and 69% (2). In the present survey, it was found that 16.8% of MRSA strains were resistant to trimethoprim-sulfamethoxazole.

The results demonstrated that 18 (14.4%) isolates and 50 (56%) isolates had $iMLS_B$ and $cMLS_B$ phenotype, respectively. This finding is similar to those reported by a study conducted in Turkey, which showed that the prevalence rates of $iMLS_B$, $cMLS_B$, and MSB phenotype among MRSA strains were 18%, 23%, and 48%, respectively (29). Low resistance rate of $iMLS_B$ phenotype was detected in many countries such as Canada (35.3%) (26 az rashidi), Iran (4.18%) (24 az rashidi), and USA (7%) (30), revealing the fact that the incidence of the $iMLS_B$ resistance phenotype varies widely from one region to another. These data suggest that failure to identify $iMLS_B$ phenotype may lead to failure in treatment with clindamycin (30). In the current study, the frequency of $cMLS_B$ phenotype was found to be higher than

Table 4. Distribution of Methicillin Resistant *Staphylococcus aureus* Virulence Genes Among Different *agr* Types[a]

Toxin and Adhesion Genes	Type of *agr*				Total
	I	II	III	IV	
tst	41 (48.8)	0 (0)	43 (51.2)	0 (0)	84 (67.2)
pvl	25 (100)	0 (0)	0 (0)	0 (0)	25 (20)
eta	0 (0)	2 (13.3)	9 (60)	4 (26.7)	15 (12)
etb	0 (0)	7 (77.8)	2 (22.2)	0 (0)	9 (7.2)
clfA	65 (55.1)	8 (6.8)	43 (36.4)	2 (1.7)	118 (94.4)
clfB	63 (54.8)	10 (8.7)	40 (34.8)	2 (1.7)	115 (92)
fnbB	60 (53.6)	12 (10.7)	39 (34.8)	1 (0.9)	112 (89.6)
fnbA	63 (60.6)	9 (8.7)	31 (29.8)	1 (0.9)	104 (83.2)
ebp	27 (37)	9 (12.3)	36 (49.3)	1 (1.4)	73 (58.4)
can	26 (46.4)	2 (3.6)	23 (41.1)	5 (8.9)	56 (44.8)
bbp	1 (25)	2 (50)	1 (25)	0 (0)	4 (3.2)
Total	65 (52)	12 (9.6)	43 (34.4)	5 (4)	125 (100)

[a]Values are expressed as No. (%).

that of $iMLS_B$ phenotype; a similar finding was noted previously by Ghanbari et al. (31).

Hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) are generally distinguished from each other based on virulence and antibiotic resistance markers (2). Despite the fact that *pvl* carriage cannot be implemented as the only indicator of CA-MRSA, care should be taken to diagnose and treat infections caused by *S. aureus* strains harboring the *pvl* gene. The current study witnessed a frequency of 20% for *pvl* gene, similar to that reported by Goudarzi in Iran (24).

The most frequent toxin gene in the present study was found to be *tst* (67.2%), which is higher than that reported in Colombia (10%) (32), Malaysia (0.5%) (33), Sweden (22%) (34), and Iran (51.4%) (2).

In the present study, the frequency of eta was 12%, which was close to the rate reported in Czech (10%) (35) yet higher than the reported rate from Colombia (3%) (32) and lower than the previous rate reported from Turkey (19.2%) (36). The frequency rate of *etb* gene reported in the present study was relatively low (7.2%), which is in accordance with the results of other studies from Colombia (32) and Turkey (36).

It is well established that biofilm formation in *S. aureus* is regulated through expression of adhesion-related genes. In the current study, the most prevalent gene was the spa gene (100%) followed by *clfA* (94.4%), *clfB* (92%), *fnbB* (89.6%), *fnbA* (83.2%), *ebp* (58.4%), can (44.8%), and *bbp* (3.2%) genes. Similar findings on the frequency of *clfA* and *clfB* genes were reported by Ghasemian et al. (13), who reported high prevalence of *clfA* and *clfB* genes in comparison to other investigated adhesions. In the present study, the frequency of *fnbA* and *fnbB* genes were relatively high (13), similar to previous studies, emphasizing the important role these genes in MRSA colonization. The obtained results in present study about frequency of *ebp* (58.4%) and can (44.8%) encoding genes are, however, in contrast with those reported by Ghasemian et al. (13), who reported a frequency rate of 78% and 7%, respectively, for can and *ebps* genes in MRSA isolates. The existing difference in the frequencies of can and *ebps* genes in MRSA isolates may be justified in terms of clinical isolates and factors affecting gene regulation, which can have a role in the prevalence of these genes for colonization.

As for the frequency of *agr* specificity groups, the present study showed that the majority of tested isolates belonged to *agr* type I (52%). Indrawattana et al. (37) reported high frequency of *agr* type I (58.7%) among *S. aureus* isolated from clinical isolates. One study performed by Goudarzi et al. (5) in Iran showed *agr* type I as the dominant *agr* type among MRSA isolates. In contrast to the findings of the present study, showing that all the isolates carrying PVL-encoding genes and HLMUPR were associated with this *agr* type I, Goudarzi et al. (5) showed that PVL-positive isolates belonged to *agr* type III. The *agr* group III was the second most-common *agr* type identified in this study (34.4%). These findings are in line with those of previous reports about the predominance of *agr* III in Iran (5). In conformity with the results of the present study, low frequency of *agr* group II and *agr* group IV was reported in

studies conducted by Ben Ayed et al. (38) and Ghasemian et al. (39). The frequency of toxin and adhesive molecule-encoding genes in isolates with *agr* type I was found to be higher than that for type III in the present study, which is in line with the results reported by other studies in various areas (40). Also, distribution of *agr* types is known to vary between geographic regions. This research also found that all toxin and adhesion genes were more prevalent in isolates with *agr* type I, a finding, which was previously shown by Nowrouzian et al. (34), reporting high frequency of sea and *tst* genes in MRSA isolates harboring the *agr* type III. The body of these findings help hypothesize that *agr* type I can have an indispensible role in the regulation of staphylococcal toxins and adhesions.

To summarize, this research investigated toxin and adhesion markers in *S. aureus* isolated from hospitalized patients at ICUs. The results of the current study showed that *agr* type I was predominant among tested isolates with high frequency of toxin and adhesion genes. The high frequency of *agr* type I in this study may reflect the indispensible role of this type in regulation of staphylococcal toxins and adhesions. To appreciate the prevalence and epidemiology of adhesion and toxin genes in different molecular types of *S. aureus*, ongoing surveillance and further studies are necessary.

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