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Distribution of Adhesion and Toxin Genes in *Staphylococcus aureus* Strains Recovered From Hospitalized Patients Admitted to the ICU

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a common cause of emerging nosocomial infections and are a major public health concern.
Objectives: The aim of the present study was to determine the prevalence of MRSA, its antibiotic resistance pattern, and the virulence gene profiles in hospitalized patients admitted to ICUs.
Methods: During a 6-month period, a total of 70 *S. aureus* isolates were collected from 249 patients admitted to the ICU in five hospitals. In vitro antibiotic susceptibility testing of the *S. aureus* isolates was carried out using the Kirby-Bauer disk diffusion method with 16 antibiotic disks. Molecular detection of toxin and adhesion genes was carried out using PCR.
Results: All the 70 *S. aureus* isolates were confirmed to be MRSA strains. The largest number of *S. aureus* isolates was found in the blood (42.9%) and wound (21.4%) samples. The MDR pattern was detected in 71.4% of the isolates, which were obtained from wound and blood samples. Simultaneous resistance to seven, six, five, four and three drugs was common in 35 (50%), 7 (10%), 8 (11.4%), 11 (15.7%), 2 (2.9%) and 5 (7.1%) isolates, respectively. The frequency of the spa, *fnbB*, *fnbA*, *clfB*, *clfA*, *can*, *bbp*, *ebp*, *etb*, *eta*, *pvl*, and *tst* genes was 100%, 75.7%, 74.3%, 78.6%, 71.4%24.3%, 0%, 58.6%, 2.9%, 7.1%, 21.4%, and 51.4%, respectively. In addition, among all the examined genes, the *clfB* (78.6%) and *etb* (2.9%) genes had the highest and lowest prevalence respectively.
Conclusions: In the present study, we found a high prevalence of MRSA at the hospitals studied. The findings emphasized the increased prevalence of MRSA isolates containing different toxin and adhesion genes, probably accompanied by antimicrobial resistance. Infection with such isolates worsens the clinical outcomes as well as the morbidity and mortality rates in hospitalized patients in ICUs.

Keywords: ICU, Methicillin-Resistant *Staphylococcus aureus*, Multidrug-Resistant, *Staphylococcus aureus*

1. Background

As a leading cause of infection in hospitals and within communities, *Staphylococcus aureus* is responsible for a wide range of important infections, ranging from wound infections to life-threatening diseases (1). The transmission of this bacterium easily takes place via direct contact, such as touching contaminated hands (or other body parts) and droplet transmission, and via indirect contact, such as through the breathing of contaminated air in the hospital or other environments (2). The most significant factor that contributes to the successful and extensive distribution of this pathogen is the ability of *S. aureus* strains to express a variety of virulence factors and acquire resistance to new antimicrobial agents (3, 4).

There is a broad range of virulence factors whose expression is related to the pathogenesis of *S. aureus* infections. These factors include extracellular proteins with low molecular weight and toxins such as staphylococcal enterotoxins (SEs), staphylokinase, toxic shock syndrome toxin-1 (TST-1), microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), hemolysin (alpha, beta, gamma and delta types), capsular polysaccharides, panton-valentine leukocidin (PVL), lipase, and exfoliative toxins (*eta* and *etb*). The most common cause of biofilm formation is adhesion proteins. Several types of cell wall-associated adhesion proteins have been reported, namely *fnb*, which encodes fibronectin-binding protein; *cna*, which encodes collagen-binding protein; *clf*, which encodes clumping factor; and *ebp*, elastin-binding protein (5-7). TST-1 can cause toxic shock syndrome and ETs cause peeling skin syndrome. PVL is a putative virulence factor that is hypothesized to increase the ability of the bacterium to

cause severe infections in human and animal hosts.

In recent decades, a wide pattern of resistance has been observed in *S. aureus* strains, not only to β-lactams but also to other therapeutic agents including macrolides, lincosides, and aminoglycosides (3, 7). An important characteristic of *S. aureus* is its ability to acquire resistance to antimicrobial agents, especially methicillin, and methicillin-resistant *S. aureus* (MRSA) has emerged as a major public health concern. Methicillin resistance develops mainly due to the presence of the *mecA* gene, which encodes a modified penicillin-binding protein (PBP2α) and has low affinity for β-lactams (4).

During the last few decades, MRSA strains have shown an extraordinary ability to rapidly develop multi-drug resistance (MDR), which has proven to be a challenge to health care providers and has taken a toll on patients too (2).

2. Objectives

The objective of the current study was to profile certain virulence genes, including those encoding adhesion proteins (*spa*, *can*, *bbp*, *ebp*, *fnbB*, *fnbA*, *clfB*, *clfA*) and toxins (*etb*, *eta*, *pvl*, *tst*), in hospitalized patients admitted to ICUs. Moreover, the pattern of antibiotic resistance was examined via antibiotic sensitivity testing.

3. Methods

3.1. Study Design and Population

The present descriptive cross-sectional study was carried out over a six-month period, between March and August 2015. A total of 70 *S. aureus* isolates were recovered from hospitalized patients admitted to the ICUs of seven hospitals. The research was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code # 6041). *S. aureus* isolates from outpatients, community-acquired infections, and other hospital wards were not included. The samples obtained from patients were transported to the laboratory within 4 h of collection and were immediately processed according to standard microbiological procedures. All the isolates were confirmed by PCR analysis of the *femA* and *nucA* genes (8, 9). The *S. aureus* isolates were stored in tryptic soy broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for further investigation.

3.2. Antimicrobial Susceptibility Testing

After the identity of the *S. aureus* strains was confirmed, their in vitro antimicrobial resistance pattern was tested using the Kirby-Bauer disk diffusion method on Mueller- Hinton agar (Mast, UK) with a panel of 16 antibiotic disks. The susceptibility interpretive criteria of the clinical and laboratory standards institute (CLSI) were used (10). After incubation for 18 hours at 37°, the results were recorded. The antimicrobial drugs tested included ampicillin (AP, 10 μg), kanamycin (K, 30 μg), ciprofloxacin (CIP, 5 μg), clindamycin (CD, 2 μg), linezolid (LZD, 30 μg), penicillin (PG, 10 μg), teicoplanin (TEC, 30 μg), amikacin (AK, 30 μg), tobramycin (TN, 10 μg), gentamicin (GM, 10 μg), trimethoprim-sulfamethoxazole (TS, 2.5 μg), and ceftriaxon (CRO, 30 μg). The minimum inhibitory concentration (MIC) for vancomycin was determined using E-test strips (AB Biodisk, Sweden) according to the manufacturer's instructions. Moreover, MDR was defined as resistance to three or more unique antibiotic classes in addition to beta-lactams. All the antibiotic disks used in the present study were purchased from Mast, UK. In every test run, *S. aureus* ATCC25923 was used as the quality control strain.

3.3. MRSA Screening

MRSA isolates were screened using cefoxitin (30 μg) and oxacillin discs (1 μg) on Mueller Hinton agar plates supplemented with 4% NaCl according to the guidelines provided by the CLSI (10). Isolates with phenotypic resistance to oxacillin were confirmed to harbor the *mecA* gene using PCR.

3.4. Genomic DNA Extraction

The QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) was used to extract genomic DNA according to the manufacturer's instructions. Lysostaphin (Sigma-Aldrich, USA), to a final concentration of 15 μg/mL, was used for cell wall lysis. The concentration of DNA was assessed using a spectrophotometer.

3.5. Detection of Adhesion and Toxin Encoding Genes

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

3.6. Statistical Analysis

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

4. Results

A total of 70 non-duplicate *S. aureus* isolates were recovered from 249 clinical specimens isolated from hospitalized patients during the 6-month period of this study.

Table 1. Oligonucleotide Primers Used in This Study

Target	Primer	Primer Sequence ($5' \rightarrow 3'$)	Product Size, bp	Reference
femA	F	CTTACTTACTGCTGTACCTG	648	(8)
	R	ATCTCGCTTGTTGTGTGC		
nucA	F	GCGATTGATGGTGATACGGTT	270	(9)
	R	AGCCAAGCCTTGACGAACTAAAGC		
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583	(7)
	R	ATGTATGTGCGATTGTATTGC		
luk-PV	F	TTCACTATTTGTAAAAGTGTCAGACCCACT	180	(11)
	R	TACTAATGAATTTTTTTATCGTAAGCCCTT		
tst-1	F	TTATCGTAAGCCCTTTGTTG	398	(7)
	R	TAAAGGTAGTTCTATTGGAGTAGG		
eta	F	GCAGGTGTTGATTTAGCATT	93	(12)
	R	AGATGTCCCTATTTTTGCTG		
etb	F	ACAAGCAAAAGAATACAGCG	226	(12)
	R	GTTTTTGGCTGCTTCTCTTG		
fnbA	F	CACAACCAGC AAATATAG	1362	(3)
	R	CTGTGTGGTAATCAATGTC		
fnbB	F	GGAGAAGGAATTAAGGCG	813	(3)
	R	GCCGTCGCCTTGAGCGT		
clfA	F	GTAGGTACGTTAATCGGTT	1586	(3)
	R	CTCATCAGGTTGTTCAGG		
clfB	F	TGCAAGATCAAACTGTTCCT	596	(3)
	R	TCGGTCTGTAAATAAAGGTA		
cna	F	AGTGGTTACTAATACTG	744	(13)
	R	CAG GAT AGA TTG GTTTA		
spa	F	TAAAGACGATCCTTCGGTGAGC	Variable	(14)
	R	CAGCAGTAGTGCCGTTTGCTT		
bbp	F	CAGTAAATGTGTCAAAAGA	1055	(15)
	R	TACACCCTGTTGAACTG		
ebp	F	CAATCGATAGACACAAATTC	526	(15)
	R	CAGTTACATCATCATGTTTA		

From the 70 *S. aureus* isolates, 8 isolates (11.4%) were obtained from Mofid children's hospital, 8 isolates (11.4%) from Pars hospital, 14 isolates (20%) from Shohada Tajrish hospital, 15 isolates (21.5%) from Ayatollah Teleghani hospital, and 25 isolates (35.7%) from Imam Khomeini hospital. All the isolates were of MRSA. All the mecA gene-positive MRSA strains were found to be phenotypically methicillin resistant. The patients' mean age was 39 years (median, 40.1 years; range, 6 months to 68 years). The highest incidence of MRSA infection was observed in the 21- to 45-year-old age group (55.7%), while the lowest incidence was observed in the 6-month to 20-year age group (17.1%). Blood samples (42.9%) were the most common samples, followed by wound (21.4%), ear (11.4%), pus (10%), body fluid (7.1%), catheter (4.3%), and urine samples (2.9%).

All the isolates were susceptible to linezolid, teicoplanin, and vancomycin. According to the results of microbroth dilution, all the strains were inhibited by vancomycin at a similar MIC_{50} and MIC_{90} of 1 μg/mL. The highest rate of resistance was observed against ampicillin and penicillin (97.1%), followed by ciprofloxacin (71.4%), amikacin (64.3%), gentamicin (60%), and clindamycin (60%). The rate of drug resistance against the other tested antibiotics was between 8.6% (for ceftriaxon) and 57.1% (for kanamycin). From among the 70 MRSA isolates, 50 (71.4%) were MDR. MDR strains were most commonly identified among MRSA strains isolated from blood and wound samples. Table 2 presents the antimicrobial susceptibility patterns of the isolates. The major resistance profile among isolates in the present study included resistance to seven antimicrobial drugs and four antimicrobial drugs, which was found in 35 (50%) and 11 (15.7%) of the isolates respectively. The MRSA isolates that were resistant to seven antibiotics were isolated mostly from blood and wound samples. Table 3 presents the distribution of the resistance profiles among different types of clinical samples.

It was found that among the adhesion protein- and toxin-coding genes, the most commonly found and the least commonly found genes were *spa* (100%) and *etb* (2.9%), respectively. It was also observed that all the MRSA isolates carried a minimum of two virulence genes. The *spa* genes were present in all the isolates, too, but they were most

Table 2. The Susceptibly Patterns of the 70 MRSA Isolates Obtained From ICU Hospitalized Patients Against 13 Antimicrobial Agents

Antibiotics	Antibiotic Susceptibility (n = 70), No. (%)		
	R	I	S
Penicillin	68 (97.1)	0	2 (2.9)
Ampicillin	68 (97.1)	0	2 (2.9)
Vancomycin	0	2 (2.9)	68 (97.1)
Teicoplanin	0	0	70 (100)
Ceftriaxon	6 (8.6)	0	64 (91.4)
Gentamicin	42 (60)	5 (7.1)	23 (32.9)
Kanamycin	40 (57.1)	3 (4.3)	27 (38.6)
Amikacin	45 (64.3)	5 (7.1)	20 (28.6)
Tobramycin	40 (57.1)	6 (8.6)	24 (34.3)
Linzolid	0	0	70 (100)
Clindamycin	42 (60)	0	28 (40)
Ciprofloxacin	50 (71.4)	4 (5.7)	16 (22.9)
Trimetoprim-sulfamethoxazole	21 (30)	9 (12.9)	40 (57.1)

Table 3. Distribution of Different Clinical Samples and the Resistance Profile of MRSA Isolates Obtained From ICUs

Resistance Profile	Number of Isolates (%)	Type of Samples (No., %)
PG, AP, GM, AK, TN, CD, CIP	35 (50)	B (28, 80), W (7, 20)
PG, AP, AK, CIP, TS	8 (11.4)	W (3, 37.5), E (3, 37.5), P (2, 25)
PG, AP, GM, CD, CIP, TS	7 (10)	E (3, 43), P (2, 28.5), BF (2, 28.5)
PG, AP, CRO, TS	6 (8.6)	E (2, 33.3), P (2, 33.3), BF (1, 16.7), U (1, 16.7)
PG, AP, K, TN	5 (7.1)	B (2, 40), P (1, 20), BF (2, 40)
PG, AP, AK	2 (2.9)	W (2, 100)
PG, AP,	5 (7.1)	W (3, 60), C (2, 40)

Abbreviations: AP, ampicillin; K, kanamycin; CIP, ciprofloxacin; CD, clindamycin; PG, penicillin; AK, amikacin; TN, tobramycin; GM, gentamicin; TS, trimethoprim-sulfamethoxazole; CRO, ceftriaxone; B, Blood; W, Wound; E, Ear; P, Pus; BF, Body fluid; C, Catheter; U, Urine.

commonly isolated from blood and wound samples.

Fifteen isolates (21.4%) were found to be positive for *pvl*-encoding genes. Isolates carrying the *pvl* gene were obtained from blood (66.7%), wound (20%), and body fluids (13.3%). Most strains carried the spa, *fnbB*, *fnbA*, *clfB*, and *clfA* genes, whereas the *bbp* gene was not detected. Table 4 provides a summary of the distribution of virulence determinants among the isolates obtained from different clinical sources. As can be seen, a total of seven different patterns were identified based on the presence of different virulence genes. Table 5 illustrates the coexistence of various genes. Co-existence of the *spa*, *clfA*, *clfB*, *fnbA*, *fnbB*, and *ebp* genes was most commonly observed (57.1%), while co-existence of the spa, *ebp*, and *tst* genes showed the lowest prevalence (1.4%). Moreover, the majority (96%) of the MDR-MRSA variants that harbored these virulence-related genes were identified in MRSA strains isolated from blood and wound samples.

5. Discussion

The high incidence of infections caused by *S. aureus*, particularly MRSA strains, in ICUs has become a serious threat to world health in recent decades. In such a scenario, ICUs, as high-risk environments for MRSA infections, must be investigated in more detail (16). As there is only limited data available on the distribution of virulence genes in MRSA samples isolated from hospitalized patients in ICUs, the present study was conducted in an attempt to characterize MRSA strains isolated from ICUs at seven hospitals in Tehran, Iran. The main sources of *S. aureus* isolates, according to the findings of the present study, were blood and wound specimens, which is in keeping with previously reported data (7).

A significant element in the good prognosis of infections caused by *S. aureus* isolates is the accurate and early determination of MRSA infections. In the present study, MRSA screening showed that all the tested isolates were MRSA; this has been previously reported in other studies from Iran by Vahdani et al. (16) and Mirzaii et al. (17). However, this incidence is higher than that found in other studies in Taiwan (18), Nigeria (19), and Hungary (20). The studies from Iran indicate that the incidence of MRSA in clinical specimens has increased over time. Yet, the prevalence rates for MRSA infections in these studies are varied. This could be attributed to the differences in the standard infection control programs at ICUs, study design, antibiotic

Table 4. Incidence of Virulence Determinants in MRSA Strains Isolated From ICU Patients

Sample	Adhesions, No. (%)						Toxins, No. (%)					Total, No. (%)
	clfA 50 (71.4)	*clfB* 55 (78.6)	*fnbA* 52 (74.3)	*fnbB* 53 (75.7)	*Ebp* 1 (58.6)	*Bbp* 0 (0)	*Can* 17 (24.3)	*spa* 70 (100)	*Tst* 30 (51.4)	*Pvl* 15 (21.4)	*Eta* 5(7.1)	*Etb* 2(2.9)	
Blood	29 (58)	30 (54.5)	30 (57.7)	30 (56.6)	18 (43.9)	0	12 (70.6)	30 (42.9)	24 (80)	10 (66.7)	4 (80)	2 (100)	30 (42.9)
Wound	15 (30)	14 (25.5)	15 (28.8)	12 (22.6)	14 (34.1)	0	2 (11.8)	15 (21.4)	6 (20)	3 (20)	1 (20)	0	15 (21.4)
Ear	0	0	2 (3.8)	0	2 (4.9)	0	0	8 (11.4)	0	0	0	0	8 (11.4)
Pus	0	4 (7.3)	5 (9.7)	1 (1.9)	4 (9.8)	0	3 (17.6)	7 (10)	0	0	0	0	7 (10)
Body fluids	3 (6)	4 (7.3)	0	5 (9.4)	2 (4.9)	0	0	5 (7.1)	0	2 (13.3)	0	0	5 (7.1)
Catheter	3 (6)	3 (5.4)	0	3 (5.7)	0	0	0	3 (4.3)	0	0	0	0	3 (4.3)
Urine	0	0	0	2 (3.8)	1 (2.4)	0	0	2 (2.9)	0	0	0	0	2 (2.9)

Table 5. Virulence Patterns of the MRSA Strains Isolated From ICU Patients

Virulence Genes	Number of Isolates (%)
Spa, clfA, clfB, fnbA, fnbB, ebp	40 (57.1)
Spa, clfA, clfB, fnbA, fnbB, ebp, can, tst, pvl	10 (14.3)
Spa, clfB, can, tst, pvl	5 (7.1)
Spa, fnbB, eta, tst	3 (4.3)
Spa, fnbA, can, eta, etb, tst	2 (2.9)
Spa, ebp, tst	1 (1.4)
Spa, tst	9 (12.9)

prescriptions, sample types, the investigated population, and laboratory testing to determine methicillin resistance. Phenotypic testing for MRSA screening, as performed in the present study, showed that its sensitivity was similar to that of PCR. These results are in agreement with previous reports (21).

As stated in a literature review, MRSA strains are usually resistant to macrolides, lincosides, and aminoglycosides, as well as all currently available beta-lactam antimicrobial agents such as penicillin and cephalosporin (16). In the current study, a high level of resistance to ampicillin and penicillin (97.1%) was observed; this was followed by ciprofloxacin (71.4%), amikacin (64.3%), gentamicin (60%), and clindamycin (60%) resistance. Previous studies in Iran (16, 17), Italy (22), and Turkey (23) also reported the rate of resistance against beta-lactam antibiotics, as demonstrated in the presented study.

Similar to the studies carried out in Italy (22) and Hungary (20), high resistance to ciprofloxacin was observed in our study (71.4%). The high resistance against ciprofloxacin observed in the present study may be related to the use of erythromycin in treating diseases caused by *S. aureus* and its variants. Other possible reasons include the permeability effect, efflux pumps, and decreased availability of quinolones at the target site (24).

The current study revealed relatively high resistance to amikacin (64.3%), gentamicin (60%), kanamycin (57.1%), and tobramycin (57.1%). High resistance to aminoglycosides was previously reported by Ko et al. (25) in a study of 74 MRSA strains isolated from 12 Asian countries. The main mechanism of aminoglycoside resistance is drug inactivation by plasmid or transposon-mediated aminoglycoside-modifying enzymes (AMEs) (7).

According to the data collected in the present study, none of the isolates inhibited by concentration of vancomycin did exceed 1 μg/mL. These findings are in line with those reported in other studies carried out in Italy (22) and Taiwan (18). Although some studies have shown that there has been a gradual increase in the resistance of MRSA variants against vancomycin in Iran (7), the results obtained in the current study indicate that limited and appropriate use of vancomycin, establishment of appropriate prescription protocols, and standard infection control measures in health care systems can be effective in decreasing the resistance of MRSA to vancomycin.

As shown in Table 2, co-trimoxazol and ceftriaxone demonstrated the lowest resistance rate among the MRSA isolates. The resistance rate to co-trimoxazole varies between 19.3% and 69% in Iran (7). Similarly, varying levels of resistance against co-trimoxazole have been reported worldwide (26).

The increase in the prevalence of MDR *S. aureus* strains has restricted the number of therapeutic options available and resulted in severe morbidity and mortality in hospitalized patients. This problem is currently a challenge and is viewed as a public health concern, especially in Iran. In the present study, we reported a substantial increase in the prevalence of MDR MRSA strains, as we found its incidence to be 71.4%. However, the prevalence of MDR ranges widely among different nations, from 83.9% in Serbia (27) to 75.8% in Taiwan (18).

The most prevalent toxin gene was found to be *tst*, with a prevalence rate of 51.4%, which is higher than that reported in previous studies conducted in Iran (11.6%) (12), Sweden (22%) (28), Malaysia (0.5%) (6) and Colombia (10%) (29). An interesting finding in the present study is that

all the MRSA strains that harbored the *tst* gene were recovered from blood (80%) and wound (20%) samples. These results are in line with those of other studies which reported high frequency of the *tst* gene in MRSA strains isolated from blood and wound samples (12). However, a study in Malaysia (6) found that the *tst* gene was only isolated from respiratory samples.

Community acquired-MRSA (CA-MRSA) may be more virulent than hospital acquired-MRSA (HA-MRSA) (7). These two types can be distinguished from each other based on their virulence factors. Although *pvl* cannot be used as a sole marker of CA-MRSA, it is important to promptly diagnose and treat infections caused by *S. aureus* strains harboring the *pvl* gene. In the current study, the incidence rate of MRSA strains harboring the *pvl* gene was observed to be 21.4%, which is similar to the results of other studies that reported the prevalence to be 2% - 35% among MRSA strains (30, 31). Most of the isolates carrying the *pvl* gene were obtained from blood samples (66.7%), which is in contrast to the findings reported in other studies, where skin and soft tissue infections were the main presentations (30).

With regard to the other toxin genes, the eta gene was present in five isolates (7.1%) and was observed to be associated with etb in two isolates (2.9%). In the present study, the incidence rate of the *eta* gene was higher than that reported in other studies from Iran 0.68% (12), Colombia (3%) (29), and Malaysia (0%) (6). Unlike the results of the present study, high prevalence of the *eta* gene was reported in studies conducted in Czech (10%) (5) and Turkey 19.2% (32). It was observed that the incidence of the etb gene differs across studies, ranging from 0% in Colombia (29) and Malaysia (6) to 9.2% in Turkey (32).

According to the literature, *S. aureus* biofilm formation is regulated through expression of adhesion-related genes and mediates the spreading of antibiotic resistance. In the current study, the prevalence rates of the *clfA*, *clfB*, *fnbA*, *fnbB*, *can*, *ebp* and *bbp* genes were found to be 71.4%, 78.6%, 74.3%, 75.7%, 24.3%, 58.6%, and 0%, respectively. The results for the *clfA* and clfB genes obtained in the present study were in contrast to those reported by Ghasemian et al. (3), who reported high prevalence of the *clfA* and *clfB* genes (100%). In the present study, similar to the studies previously reported, the incidence of the *fnbA* and *fnbB* genes was relatively high (3), which shows the important role of these genes in the colonization of MRSA strains. The present study showed that the can, *ebp*, and *bbp* genes accounted for 24.3%, 58.6%, and 0% of *S. aureus* infections, respectively. These results are in agreement with those of other studies which found that the incidence of the bbp genes in MRSA clinical isolates was lower. These results, however, are in contrast with those reported by Ghasemian et al. (3), who found that the can and *ebps* genes were detected in 78% and 7% of MRSA isolates, respectively. This discrepancy in the incidence of the can and *ebp* genes in MRSA isolates can be explained by the type of clinical isolate and the factors which affect gene regulation that may be important in the prevalence of these genes for colonization. It is worth noting that in wound and burn infections, due to the high incidence of laminin and collagen in tissues, *S. aureus* isolates with overexpression of several virulence factors, such as can and ebp, can easily and rapidly bind to the specific receptors.

The majority of the genes examined in this study were found to be significantly more prevalent among MDR-MRSA strains isolated from blood and wound infections than from other infection sites. As previously confirmed, the clinical origins of the isolates and infection sites may be significant factors related to the ability of the isolates to form biofilms.

5.1. Conclusion

In the present study, data on the antibiotic susceptibility and the distribution of the virulence genes of *S. aureus* were reviewed in hospitalized patients in ICUs. The results demonstrated high prevalence of MDR MRSA strains among the isolates. The high prevalence of antibiotic resistance in the MRSA strains indicated that the antimicrobial agents currently used for treating MRSA infections may be inadequate. Therefore, it is essential for clinicians to consider the treatment guidelines for MRSA infections. It is also worth mentioning that the incidence of toxin- and adhesion-related genes in MRSA strains isolated from wound and blood samples was higher than that in strains isolated from other clinical samples. Future studies should aim at understanding the different virulence and resistance profiles, which can be vital for risk prediction and the treatment of MRSA infections.

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Footnotes

Authors' Contribution: Fereshteh Eftekhar, Mehdi Goudarzi, Hossein Goudarzi, Mehdi Azad and Hadi Azimi: study concept and design, development of the study, data interpretation, and manuscript revision; Razieh Rezaee,

Fereshteh Eftekhar and Mehdi Goudarzi: phenotypic and molecular studies and manuscript drafting; Razieh Rezaee, Hossein Goudarzi and Mehdi Azad: experimental procedures; Hadi Azimi: acquisition of data and statistical analysis; Fereshteh Eftekhar, Mehdi Goudarzi: study supervision. All the authors have read and approved of the final manuscript.

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