Hindawi
BioMed Research International
Volume 2022, Article ID 5863310, 8 pages
https://doi.org/10.1155/2022/5863310

Research Article

Determination of Virulence Factors and Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* Strains among Different Types of *spa*, *agr*, and SCC*mec*

Mohammad Latifpour,[1] Tahmineh Narimani,[2] Amin Sadeghi,[2] and Mohammad Niakan[1]

[1]*Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran*
[2]*Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

Correspondence should be addressed to Mohammad Niakan; niakan@shahed.ac.ir

Received 18 July 2022; Accepted 3 October 2022; Published 15 October 2022

Academic Editor: Mejdi Snoussi

In order to restrict the spread of methicillin-resistant *S. aureus* (MRSA) in hospitals, it is necessary to characterize isolates rapidly and precisely. The objective of this study was to determine virulence factors and resistance profiles of MRSA strains among *spa*, *agr*, and SCC*mec* types. In total, 55 MRSA isolates were collected from clinical specimens. The MRSA isolates were characterized by antimicrobial susceptibility testing, virulence genes, *agr* typing, *spa* typing, and SCC*mec* typing. According to our findings, all MRSA strains were resistant to cefoxitin; 88% and 86.7% of which were resistant to erythromycin and clindamycin, respectively. Type II *agr* was predominant with 54.54% frequency. Among 27 different *spa* types, type t030 was most frequently (25.45%). Most MRSA isolates (63.3%) were SCC*mec* type III. The *pvl* and *tst* genes were found in 25.3% and 32.7% of MRSA isolates, respectively. Among the MRSA strains, *ermA*, *ermB*, and *ermC* were present in 50%, 33.3%, and 57.3% of cases, respectively. In addition, 43 of the 55 MRSA strains (78%) harbored aminoglycoside resistance genes. The results of our study revealed that the MRSA rate in our region is dramatically high. Better infection control guidelines in hospitals, as well as ongoing epidemiological surveillance studies, could be strongly suggested for effective prevention of the spread of MRSA to inpatients.

1. Introduction

Among human pathogens, *Staphylococcus aureus* (*S. aureus*) is one of the most common ones [1]. Methicillin-resistant *S. aureus* (MRSA) strains have emerged as a major problem in hospitals, owing to the increased mortality rate associated with some of these infections. MRSA strain outbreaks have a significant impact on morbidity, mortality, and healthcare costs [2–4]. MRSA strains express virulence factors that play a key role in infection progression. The accessory gene regulator (*agr*) system regulates the expression of numerous virulence factors in *S. aureus*, and four major *agr* types have been identified to date. Different *agr* types have different properties and distributions in various geographic regions; thus, identification of the predominant types in each location would be of benefit [5, 6].

It is clear that the spread of MRSA around the world is constantly evolving, with new strains emerging in a variety of geographical regions. The *mecA* gene, which confers beta-lactam resistance, is found on the staphylococcal cassette chromosome mec (*SCCmec*) of MRSA strains. MRSA is divided into distinct epidemiological types based on the presence of the SCC*mec* element. The SCC*mec* typing method can help distinguish community-associated MRSA (CA-MRSA) from hospital-acquired MRSA (HA-MRSA) infections [7].

Continuous MRSA surveillance in each location necessitates monitoring the epidemiology, host characteristics, and

transmission routes of emerging strains [8]. Therefore, clinicians must have a thorough understanding of MRSA's molecular epidemiology in order to assess the efficiency of preventative strategies and provide effective prophylaxis [9]. Prevention of MRSA transmission by screening patients, personnel, and the environment is a critical goal of infection control [9]. However, investigating the origins and routes of transmission of MRSA is possible only through the use of typing approaches, which are necessary for genetic characterization. There is a variety of molecular epidemiological methods used for MRSA surveillance, including multilocus sequencing typing (MLST), pulsed-field gel electrophoresis (PFGE), staphylococcal protein A (*spa*) gene sequencing, *agr*, and SCC*mec* typing [10]. Although each of these methods has a pretty good discriminating power, it has been demonstrated that combining genotyping methods is beneficial and advantageous for distinguishing distinct MRSA clones.

spa typing is a valuable typing instrument owing to its ease of use, cost-effectiveness, and uniform nomenclature, which is based solely on assessment of the repetition space in the X region of the *spa* gene [11]. The X region polymorphism, which encodes a part of the *spa* protein, is characterized by variations in tandem repeats as well as variations in base sequences within repetitions. In other words, in any strain of *S. aureus*, each motif consists of 24 base pairs, which are referred to as unique sequence motif repeats. The order of the repeats determines the *spa* type for a strain [12]. The *spa* types are important for identifying *S. aureus* outbreak isolates and infection control policies around the world. Over the last decade, studies have been conducted on the distribution of *spa*, *agr*, and SCC*mec* types in various geographic areas [13, 14]. Therefore, the current research is aimed at determining virulence and antimicrobial resistance profiles of MRSA isolates using *spa*, *agr*, and SCC*mec* typing.

2. Materials and Methods

2.1. Ethical Considerations. Ethics approval to perform this study was obtained from the institutional review board of Shahed University of Medical Sciences, Tehran, Iran (http://ethics.research.ac.ir/IR.SHAHED.REC.1398.089).

2.2. Detection and Isolation of MRSA. In this cross-sectional study, out of a total of 142 *S. aureus* isolates, 55 MRSA isolates were identified and included in this study, while the remaining isolates were excluded. The isolates were obtained from clinical samples including blood, urine, wounds, and cerebrospinal fluid collected from different wards (emergency, men, women, children, and intensive care unit) in Al-Zahra Hospital in Isfahan (Iran), then referred to the hospital laboratory. The *S. aureus*was identified using growth on mannitol salt agar, showing beta-hemolysis on 5% sheep blood agar, and being gram positive as well as producing catalase, coagulase, and DNase. The presence of the *nucA* gene was confirmed by PCR in all *S. aureus* isolates (Table 1).

2.3. Antimicrobial Susceptibility Testing and Detection of MRSA. The following antibiotics were tested for antibiotic susceptibility using the disk diffusion technique on Mueller-Hinton agar, and the results were recorded after incubation for 18 hours at 37°C and in accordance with the CLSI guidelines [15]: penicillin (10 μg), erythromycin (15 μg), gentamicin (10 μg), tetracycline (30 μg), clindamycin (2 μg), rifampicin (5 μg), cefoxitin (30 μg), linezolid (5 μg), and trimethoprim-sulfamethoxazole (5 μg) (Mast, Merseyside, UK). The presence of the 310-base pair (bp) PCR product of the *mecA* gene was examined in all *S. aureus* isolates (Table 1), and cefoxitin (30 μg) discs on Muller-Hinton agar plates were used to screen for MRSA isolates.

2.4. Genomic DNA Extraction. A DNA Mini Kit (Qiagen GmbH, Hilden, Germany) was used for genomic DNA extraction. Fresh colonies harvested from agar plates were washed with 500 μl TE 1x and centrifuged for 10 minutes at 5000 rpm according to manufacturer's protocol. A suspension was then prepared in 200 μl TE 1x with 20 μl lysostaphin (200 μg/ml final concentration) and incubated at 37°C for 20 minutes. Finally, the obtained DNA was dissolved 50 μl RNase-DNase-free water (Sigma). DNA concentration was measured with a spectrophotometer.

2.5. SCCmec Typing. As described previously, multiplex PCR was used to identify various MRSA isolates using genomic DNA as the template [16]. The amplification started with a 3-minute denaturation step at 94°C, then 35 cycles of 30 seconds at 94°C, 1 minute at 55°C, 1 minute at 72°C, and finally 5 minutes at 72°C for final extension.

2.6. Detection of Virulence and Resistance Genes. To detect virulence genes such as hemolysin A (hla), toxic shock syndrome toxin (tst), staphylococcal enterotoxins (sea, seb, and sec), and Panton-Valentine leukocidin, PCR was used (pvl). As previously described, PCR assays were used to investigate the common aminoglycoside resistance genes (aac (6′)-aph (2″), aph3, ant4) and macrolide resistance genes (ermA, ermB, ermC) [17, 18].

2.7. Detection of agr Types. Multiplex PCR was performed to detect *agr* types using a set of primers containing a common forward primer (Pan) and reverse primer (*agrI, agrII, agrIII*, and *agrIV*) that are unique to each *agr* group [19]. The primer sequences are shown in Table 1.

2.8. Detection of spa Types. The identified MRSA strains were subjected to PCR to detect the *spa* gene (Table 1). The amplification reaction consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 second, hybridization at 56°C for 40 second, and extension to 72°C for 50 second, followed by final extension to 72°C for 5 minutes [11]. Sequencing was then performed on the PCR products. Also, after sequencing, the spa database server (http://spaserver.ridom.de/) was used to determine different types.

2.9. Statistical Analysis. For statistical analysis, SPSS Statistics 22.0 for Windows was used. Data were presented using descriptive statistics (frequency, percentage, mean, and standard deviation).

Table 1: Oligonucleotide primers used in this study.

Primer	Sequence (5′-3′)	Amplicon size (bp)	References
nucA	CTGGCATATGTATGGCAATTGTT	613	[20]
nucA	TATTGACCTGAATCAGCGTTGTCT		
mecA	GATGAAATGACTGAACGTCCGATAA	310	[20]
mecA	CCAATTCCACATTGTTTCGGTCTAA		
agr I F	ATGCACATGGTGCACATGC	441	[19]
agr I R	GTCACAAGTACTATAAGCTGCGA		
agr II F	ATGCACATGGTGCACATGC	575	[19]
agr II R	TATTACTAATTGAAAAGTGGCCATAGC		
agr III F	ATGCACATGGTGCACATGC	323	[19]
agr III R	GTAATGTAATAGCTTGTATAATAATACCCAG		
agr IV F	ATGCACATGGTGCACATGC	659	[19]
agr IV R	CGATAATGCCGTAATACCCG		
spa F	TAAAGACGATCCTTCGGTGAGC	300-500	[11]
spa R	CAGCAGTAGTGCCGTTTGCTT		

3. Results

3.1. Detection and Isolation of MRSA. In this study, 55 clinical MRSA isolates were recovered from blood samples ($n = 12$; 21.83%), nasal ($n = 14$; 24.45%), urine ($n = 7$; 12.72%), trachea ($n = 8$; 14.54%), wound ($n = 12$; 21.83%), and synovial ($n = 2$; 3.63%). According to our data, the nasal specimen has the highest frequency of MRSA (26%). The patients were divided into 29 (52%) males and 26 (48%) females. Participants in the study ranged in age from 9 to 86. Most of the study participants were in the 21–60-year-old group (66%).

3.2. Antibiotic Susceptibility Tests. Antibiotic susceptibility testing was performed on all MRSA isolates. All were resistant to cefoxitin and penicillin; 88% and 86.7% of them were resistant to erythromycin and clindamycin, respectively. On the other hand, all MRSA isolates were sensitive to linezolid (Table 2).

3.3. agr Typing. According to *agr* typing, 55 of the MRSA isolates belonged to one of agr types I, II, III, or IV. By using the *agr* typing method, 29.09% ($n = 16$), 54.54% ($n = 30$), 10.9% ($n = 6$), and 5.45% ($n = 3$) of isolates belonged to *agr* types I, II, III, and IV, respectively.

3.4. Prevalence of SCCmec Types, Virulence, and Resistance Genes. Most MRSA isolates (63.3%) were SCC*mec* type III. Also, the frequency of SCCmec types II and IX was 10.7% for each, 9.3% as SCC*mec* type V, 4% as SCC*mec* type I, and 2% as SCC*mec* type IV. In addition, 43 of the 55 MRSA strains (78%) harbored aminoglycoside resistance genes, with the presence of *aac(6′)-aph(2″)*, *aph3*, and *ant4* genes among MRSA isolates, were 54%, 32.7%, and 31.3%, respectively. Among the 55 MRSA strains, macrolide resistance genes *ermC*, *ermA*, and *ermB* were detected in 35 (63.6%), 11 (20%), and 9 (16.4%) isolates, respectively. In our study, genes encoding staphylococcal enterotoxins *sea*, *seb*, and *sec* were found in 48%, 25.5%, and 12% of MRSA isolates, respectively. In addition, the *pvl* and *tst* genes were found in 25.3% and 32.7% of MRSA isolates, respectively.

3.5. spa Typing. Twenty-seven *spa* types were observed in this study. There were 19 *spa* types that were found only once in all of the 55 strains analyzed. Accordingly, single types of *spa* are extremely important in MRSA strains. Table 3 shows that t030 and t037 predominate in clinical samples, especially in blood and nasal samples. As well as, phenotypic and genotypic traits of all our isolates are presented in Table 4 [21].

4. Discussion

MRSA strains are one of the leading causes of infections in hospitals, but infections from community-related MRSA have become a global public health threat over the recent decades [22]. The widespread occurrence of multidrug-resistant (MDR) MRSA augments the cost of antibiotic therapy and limits treatment options. During the last two decades, the widespread use of beta-lactam antibiotics in Iranian hospitals and medical facilities led to increased resistance to these antibiotics [23]. The results of the current study revealed that MRSA strains are resistant to erythromycin (88%), clindamycin (86.6%), tetracycline (68%), rifampicin (57%), and gentamicin (54.6%). Based on these results, linezolid was the most effective drug for MRSA in the study area. In addition, more than 93% of our MRSA isolates were MDR. We did not report the frequency of MRSA strains since the selection criteria of our study were isolation of MRSA strains, and methicillin-sensitive *S. aureus* (MSSA) strains were not included. As a protein synthesis inhibitor, erythromycin is widely utilized for the treatment of staphylococcal infections [24]. According to Mahdiyoun et al., the frequency of MRSA resistance tested for erythromycin was 84.4% [25]. A study conducted in Taiwan reported that the resistance rates for erythromycin and clindamycin were 94.9% and 86.5%, respectively [26]. High frequency of resistance to erythromycin and clindamycin antibiotics in the present study was in consistent with the findings of previous research in Iran [24] and India [27].

We also found SCCmec type III in a high percentage of MRSA isolates. Similarly, studies in Iran and other Asian

Table 2: Characteristics of antibiotic resistance pattern of MRSA and type of specimen. All results were expressed as percentages.

Type of specimen	ERY		CD		SXT		GM		TE		RA	
	S	R	S	R	S	R	S	R	S	R	S	R
Blood	1.3	20.0	0.7	20.7	9.3	12.0	10.7	10.7	7.3	14.0	8.7	12.7
Nasal	3.3	22.7	3.3	22.7	12.7	13.3	10.7	15.3	9.3	16.7	10.7	15.3
Urine	4.7	8.7	2.7	10.7	6.7	6.7	6.7	6.7	4.0	9.3	6.0	7.3
Trachea	0.7	14.0	2.0	12.7	8.0	6.7	2.0	12.7	4.0	10.7	4.0	10.7
Wound	1.3	20.0	4.7	16.7	10.7	10.7	13.3	8.0	6.0	15.3	11.3	10.0
Synovial	0.7	2.7	0.0	3.3	2.7	0.7	2.0	1.3	1.3	2.0	2.0	1.3
Total	12.0	88.0	13.3	86.7	50.0	50.0	45.3	54.7	32.0	68.0	42.7	57.3

ERY: erythromycin; CD: clindamycin; SXT: trimethoprim/sulfamethoxazole; GM: gentamicin; TE: tetracycline; RA: rifampin. All MRSA isolates were susceptible to linezolid, quinupristin/dalfopristin, and teicoplanin. S: sensitive; R: resistant. The frequency percentage was calculated according to the total number of MRSA isolates (55) not according to the number of isolates in each clinical sample. The data is presented as a percentage.

Table 3: Distribution of the *spa* types among different clinical samples.

Spa type	*N* (%) of isolates						Total
	Blood	Nasal	Urine	Trachea	Wound	Synovial	*N* = 55
t275	—	1 (100)	—	—	—	—	1
t4679	—	1 (100)	—	—	—	—	1
t7685	1 (50)	—	1 (50)	—	—	—	2
t3236	—	1 (100)	—	—	—	—	1
t790	—	1 (100)	—	—	—	—	1
t030	4 (28.6)	5 (35.7)	1(7.1)	2 (14.3)	2 (14.3)	—	14
t037	1 (12.5)	2 (25)	2 (25)	1 (12.5)	1 (12.5)	1 (12.5)	8
t3769	—	—	—	—	—	1 (100)	1
t3204	—	1 (50)	—	—	1 (50)	—	2
t314	—	—	—	—	1 (100)	—	1
t5163	—	—	—	—	1 (50)	1 (50)	2
t325	1 (25)	—	—	1 (25)	2 (50)	—	4
t1587	—	1 (100)	—	—		—	1
t223	—	1 (100)	—	—		—	1
t5593	—	—	1 (100)	—		—	1
t131	—	—	—	—	1 (100)	—	1
t15871	—	—	—	1 (100)		—	1
t159	—	—	—	1 (100)		—	1
t1360	—	—	—	—	2 (100)	—	2
t692	—	1 (100)	—	—	—	—	1
t2976	—	—	1 (100)	—	—	—	1
t2104	—	—	—	—	1 (100)	—	1
t1258	—	—	—	2 (100)	—	—	2
t1403	—	1 (100)	—	—	—	—	1
t2457	1 (100)	—	—	—	—	—	1
t3182	1 (100)	—	—	—	—	—	1
t459	1 (100)	—	—	—	—	—	1
Total	10 (18.2)	16 (29)	6 (11)	8 (14.55)	12 (21.8)	3 (5.45)	55

countries have reported the high prevalence of SCC*mec* type III [28–30]. In MRSA isolates, SCC*mec* mobile genetic factor leads to an expansion of antibiotic resistance determinants as well as virulence factors, which can act as a large reservoir of resistance genes, enterotoxins, and other virulence factor genes. Our results showed that among MRSA with SCC*mec* type III, *sea*, *hla*, and *seb* were the most frequently found genes encoding virulence factors. Herein, the majority of the isolates with type III were resistant to erythromycin and clindamycin (55% and 54%) while all the isolates were

Table 4: Phenotypic and genotypic traits of all MRSA isolates in this study. Some data were collected with the study of Latifpour et al. [21].

Isolate number	*spa* type	*agr* type	Sample type	SCC*mec*	Virulence genes	Resistance profile	Resistance genes
1.	t030	II	Blood	III	*hla*, *sea*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermC*
2.	t030	II	Blood	III	*tst*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermB*
3.	t030	IV	Wound	III	*pvl*, *hla*, *sea*, *seb*, *sec*	FOX, ERY, CD, SXT, GM, TE	*aac* (6′)-*aph* (2″), *ant4*, *ermC*
4.	t030	II	Trachea	III	*hly*, *sea*	FOX, ERY, CD, SXT, GM, RA	*aac* (6′)-*aph* (2″), *aph3*
5.	t030	I	Nasal	III	*hly*, *sea*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*
6.	t030	II	Nasal	III	*pvl*, *tst*, *hly*, *sea*	FOX, ERY, CD, TE	*ermC*
7.	t030	II	Blood	III	*tst*	FOX, ERY, CD, SXT, GM, RA	*aph3*, *ermA*
8.	t030	I	Nasal	III	*hla*, *sea*	FOX, SXT, GM, TE, RA	*aph3*, *ant4*, *aac* (6′)-*aph* (2″)
9.	t030	II	Nasal	IX	*hla*	FOX, ERY, CD, SXT, GM, TE, RA	*ermC*
10.	t030	I	Nasal	V	—	FOX, GM	*ant4*, *aph3*, *ermA*
11.	t030	I	Blood	V	*pvl*, *hla*, *sea*	FOX, ERY, TE	*ermC*, *ermA*
12.	t030	IV	Wound	II	*hla*, *sea*, *seb*	FOX, ERY, CD, SXT, TE, RA	*ermC*, *ermB*
13.	t030	I	Trachea	II	*hla*, *tst*	FOX, ERY, TE	*ermC*, *ermB*
14.	t030	I	Urine	IX	*hla*, *tst*	FOX, ERY, CD	*ermC*, *ermA*
15.	t037	III	Urine	II	*hla*, *sec*	FOX, TE	—
16.	t037	I	Urine	III	*pvl*, *tst*, *hla*, *sea*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *ant4*, *aph3*
17.	t037	I	Nasal	III	*hla*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*
18.	t037	III	Synovial	III	*hla*	FOX	—
19.	t037	II	Wound	III	*hla*, *sea*	FOX, TE	—
20.	t037	II	Nasal	IX	*pvl*, *tst*, *hla*, *sea*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*
21.	t037	I	Trachea	IX	*hla*, *sea*	FOX	—
22.	t037	I, II	Blood	IX	*hla*, *sea*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *ermC*
23.	t1258	II	Trachea	III	*tst*, *hla*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermA*
24.	t1258	I	Trachea	I	*pvl*, *hla*, *sea*	FOX, ERY, CD, TE, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermC*
25.	t1360	I, II	Wound	III	*hla*, *sea*	FOX, ERY, CD, SXT, RA	*aac* (6′)-*aph* (2″), *ermC*
26.	t1360	III	Wound	IX	*hla*, *sea*, *sec*	FOX, ERY, CD, SXT, GM, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermC*
27.	t131	II	Wound	III	*pvl*, *hla*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *ermC*
28.	t5163	II	Trachea	III	*pvl*, *hla*, *seb*	FOX, ERY, CD, GM, TE	*aac* (6′)-*aph* (2″), *ermC*
29.	t5163	I	Wound	III	*hla*	FOX, ERY, CD, TE	*ant4*, *ermC*
30.	t7685	II	Urine	III	*tst*, *hla*	FOX, ERY, CD, SXT, GM, TE	*aac* (6′)-*aph* (2″), *aph3*, *ermC*
31.	t7685	III	Blood	III	*tst*, *hla*, *sea*	FOX, ERY, CD, SXT, GM	*aac* (6′)-*aph* (2″), *ant4*, *ermC*
32.	t1587	II	Nasal	III	*hla*, *sea*	FOX, ERY, CD, GM, TE	*aac* (6′)-*aph* (2″), *aph3*, *ermB*
33.	t1403	II	Nasal	V	*tst*, *hla*, *sea*, *seb*	FOX, ERY, SXT, GM	*aac* (6′)-*aph* (2″), *ant4*
34.	t15871	II	Trachea	III	*pvl*, *hla*	FOX, ERY, CD, GM, TE, RA	*ant4*, *ermC*
35.	t159	I	Trachea	III	*hla*, *sea*, *seb*, *sec*	FOX, ERY, CD, GM, TE, RA	*ant4*, *ermC*
36.	t2104	II	Wound	III	*hla*, *sea*	FOX, ERY, CD, TE, RA	*aac* (6′)-*aph* (2″), *ermC*
37.	t275	II	Nasal	III	*pvl*, *hla*	FOX	—
38.	t223	II	Nasal	V	*tst*, *seb*	FOX, ERY, CD, TE	*ant4*, *ermA*
39.	t2457	II	Blood	III	*pvl*, *hly*, *seb*	FOX, ERY, CD, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*
40.	t314	II	Wound	III	*hla*	FOX, ERY, CD, GM, TE, RA	*aac* (6′)-*aph* (2″), *ermC*
41.	t2976	II	Urine	III	*pvl*, *tst*, *hla*, *sea*	FOX, ERY, CD, SXT, GM, TE, RA	*aac* (6′)-*aph* (2″), *aph3*, *ermA*
42.	t3182	I	Blood	III	*pvl*, *tst*, *hla*, *sea*	FOX, ERY, CD, GM, TE, RA	*ant4*, *ermC*
43.	t3236	III	Nasal	III	*pvl*, *hla*	FOX, ERY, CD, GM, TE	*aac* (6′)-*aph* (2″), *ermC*

Table 4: Continued.

Isolate number	*spa* type	*agr* type	Sample type	SCC*mec*	Virulence genes	Resistance profile	Resistance genes
44.	t3204	II	Wound	II	*hla*, *seb*	FOX, ERY, CD, SXT, GM, TE, RA	*aac (6′)-aph (2″)*, *ant4*
45.	t3204	I	Nasal	III	*hla*, *sea*	FOX, ERY, CD, GM	*ermC*, *ermB*
46.	t325	I	Trachea	III	*hla*	FOX, ERY, CD, GM, TE, RA	*aac (6′)-aph (2″)*, *ant4*, *ermC*
47.	t325	II	Wound	III	*pvl*, *tst*	FOX, ERY, GM	*ant4*, *ermA*
48.	t325	IV	Wound	III	*hla*, *sea*	FOX, ERY, CD, GM, TE	*aph3*, *ant4*, *ermA*
49.	t4679	II	Nasal	III	*pvl*, *tst*, *hla*, *sea*, *seb*	FOX, ERY, CD, GM, RA	*aac (6′)-aph (2″)*, *ermB*
50.	t3769	II	Synovial	I	*pvl*, *hla*, *sea*	FOX, ERY, CD, RA	*ermA*, *ermB*
51.	t459	III	Blood	V	*hla*, *sea*	FOX, ERY, CD, TE, RA	*ermA*, *ermB*
52.	t5593	II	Urine	IX	*hla*	FOX, ERY, CD, SXT, GM, TE	*ant4*, *ermC*
53.	t692	III	Nasal	III	*hla*	FOX, ERY, CD, SXT, GM, TE, RA	*ant4*, *ermA*
54.	t790	II	Nasal		*hla*, *sea*	FOX, ERY, CD, SXT, GM, TE	*aac (6′)-aph (2″)*, *ermC*
55.	t459	II	Nasal	III	*hla*, *sea*	FOX, ERY, CD, GM	*ant4*, *ermB*

resistant to gentamicin. The SCCmec typing has also been done in other parts of Iran in accordance with our study. A study by Ebrahim-Saraie et al., Moshtagheian et al., and Parhizgari et al. reported that SCCmec type IV dominated MRSA isolates; however, in line with our findings, most studies conducted in Iran described SCCmec type III as the predominant SCCmec type [31–33]. In the present study, among MRSA strains, *ermC* (63.6%) was the most commonly detected macrolide resistance gene, followed by *ermA* (20%) and *ermB* (16.4%). Also, the most frequently identified aminoglycoside resistance gene was *aac (6′)-aph (2″)* (54%). These findings are in contrary with the study by Hau et al. [34] conducted with clinical MRSA in the United States in which an incidence of 91.5% for ermA and 12.7% for *aac (6′)-aph (2″)* was reported. A similar finding was also reported by Yılmaz and Aslantaş [35] that the *ermC* and *aac (6′)-aph (2″)* genes were detected in 91.5% and 50% of *S. aureus* isolates, respectively.

In view of the widespread of MRSA isolates, it is imperative that the treating physician encourages the preservation of glycopeptides and linezolid only in the case of MRSA. In Iran, a major MRSA-associated problem is the result of increased incidence and hospitalization rates. Therefore, for screening, epidemiology, surveillance, and infection control, rapid and accurate typing of MRSA isolates is crucial [23]. Several genotyping techniques are available for identifying *S. aureus* strains in epidemiological studies. Sequence-based typing methods, such as MLST and *spa* typing, have several obvious advantages; for example, they are easily used, portable, reproducible, and able to provide comparable results compared to tape-based methods, such as small macrorestricted analysis [14]. One of the key regulators of *S. aureus*, which is involved in the regulation of bacterial virulence factors, is the *agr* system. There are currently four *agr* types identified in *S. aureus* strains (I, II, III, and IV). According to our study, the predominant agr type among the 55 MRSA isolates was type II, with a frequency of 54.54 percent. The majority (69.5%) of the isolates studied by Ghasemian et al., showed *agr* I, followed by *agr* III (30.5%) [36]. The *agr* type III is the most prevalent type of MRSA isolate, according to a study by Goudarzi et al., in Iran [37]. There is a significant relationship between *agr* types and specific pathogens [38], and the distribution of *agr* types varies by geographic region. The selected regions of the *spa* gene are usually short repeats of sequences with enough polymorphisms to allow isolated typing [20]. In the current work, 27 different *spa* types were found. *spa* typing analysis indicated that *spa* type t030 was the most common *spa* type found in 25.45% of isolates. The second most frequently identified *spa* type in our study was t037. These results are consistent with those of other studies in Iran and other Asian countries [10]. The *spa* type t037 was previously reported by Alreshidi et al. in Saudi Arabia [39], Chen et al. in China [40], and Goudarzi et al. in Iran [37]. In agreement with our study, in China, t030 was found to be one of the most common *spa* types (52.0%) of MRSA isolates [40]. We believed that t030 would result in longer bacterial survival and easier transmission. In this study, we reported that 7.27% of our isolates had *spa* type t325 and 19 *spa* types, which were detected, each, in one isolate.

5. Conclusion

According to recent studies, presence of these common SCC*mec* (III), *spa* (t030), and *agr* (II) types indicates that these MRSA strains are actively circulating in the healthcare setting of Isfahan Province. Despite the high diversity of our *spa* types (27/55) in this study, most of them are classified as t030 and t037 and are probably phylogenetically related. There is a possibility that these strains may spread to other parts of Iran or the world in relation to the tourism industry in Isfahan Province. Therefore, the current study emphasizes the importance of molecular typing in tracking global trends in the emergence, spread, and persistence of epidemic MRSA strains. Better infection control guidelines in hospitals, as well as ongoing epidemiological surveillance studies,

could be strongly suggested for effective prevention of bacteria spread to inpatients and control nosocomial infections.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Consent

Consent is not necessary.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

We would like to thank the Department of Microbiology and the Al-Zahra laboratory at Isfahan University of Medical Sciences for supporting the practical work. This study was funded by the Shahed University of Medical Sciences' Vice Chancellor for Research.

References

[1] Y. Guo, G. Song, M. Sun, J. Wang, and Y. Wang, "Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus," *Frontiers in Cellular and Infection Microbiology*, vol. 10, no. 107, p. 263, 2020.

[2] A. S. Lee, H. de Lencastre, J. Garau et al., "Methicillin-resistant Staphylococcus aureus," *Nature Reviews. Disease Primers*, vol. 4, no. 1, p. 18033, 2018.

[3] A. Hassoun, P. K. Linden, and B. Friedman, "Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment," *Critical Care*, vol. 21, no. 1, pp. 211–211, 2017.

[4] M. Damavandi, M. Safarpour Dehkordi, A. Dehghan, F. Heibati, R. Taghaddosi, and A. Gholipour, "Detection of antiseptic resistance genes among Staphylococcus aureus colonising nurses and coagulase-negative staphylococci isolated from clinical specimens at teaching hospitals in southwest of Iran," *Jundishapur Journal of Microbiology*, vol. 10, no. 1, article e39285, 2016.

[5] L. Tan, S. R. Li, B. Jiang, X. M. Hu, and S. Li, "Therapeutic targeting of the Staphylococcus aureus accessory gene regulator (agr) system," *Frontiers in Microbiology*, vol. 9, 2018.

[6] C. Jenul and A. R. Horswill, "Regulation of Staphylococcus aureus virulence," *Microbiology spectrum*, vol. 7, no. 2, 2019.

[7] M. Miragaia, "Factors contributing to the evolution of mecA-mediated β-lactam resistance in staphylococci: update and new insights from whole genome sequencing (WGS)," *Frontiers in Microbiology*, vol. 9, 2018.

[8] N. A. Turner, B. K. Sharma-Kuinkel, S. A. Maskarinec et al., "Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research," *Nature Reviews. Microbiology*, vol. 17, no. 4, pp. 203–218, 2019.

[9] K. T. Kavanagh, "Control of MSSA and MRSA in the United States: protocols, policies, risk adjustment and excuses," *Antimicrobial Resistance and Infection Control*, vol. 8, no. 1, p. 103, 2019.

[10] P. Asadollahi, N. N. Farahani, M. Mirzaii et al., "Distribution of the most prevalent spa types among clinical isolates of methicillin-resistant and -susceptible Staphylococcus aureus around the world: a review," *Frontiers in Microbiology*, vol. 9, 2018.

[11] B. Strommenger, C. Braulke, D. Heuck et al., "spaTyping ofStaphylococcus aureusas a frontline tool in epidemiological typing," *Journal of clinical microbiology*, vol. 46, no. 2, pp. 574–581, 2008.

[12] M. Hallin, A. W. Friedrich, and M. J. Struelens, "spa typing for epidemiological surveillance of Staphylococcus aureus," *Methods in molecular biology*, vol. 551, pp. 189–202, 2009.

[13] S. Ravaioli, D. Campoccia, W. Ruppitsch et al., "Comparison of automated ribotyping, spa typing, and MLST in 108 clinical isolates of Staphylococcus aureus from orthopedic infections," *International Journal of Molecular Sciences*, vol. 23, no. 3, p. 1660, 2022.

[14] S. Abbasian, N. N. Farahani, Z. Mir et al., "Genotypic characterization of Staphylococcus aureus isolated from a burn centre by using agr, spa and SCCmec typing methods," *New microbes and new infections*, vol. 26, pp. 15–19, 2018.

[15] Wayne. P, *Performance standards for antimicrobial susceptibility testing; twenty-eight informational*, CLSI, 2018.

[16] T. Ito, K. Kuwahara-Arai, Y. Katayama et al., "Staphylococcal cassette chromosome mec (SCCmec) analysis of MRSA," *Methods in molecular biology*, vol. 1085, pp. 131–148, 2014.

[17] M. Mehrotra, G. Wang, and W. M. Johnson, "Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance," *Journal of Clinical Microbiology*, vol. 38, no. 3, pp. 1032–1035, 2000.

[18] J. Nishi, H. Miyanohara, T. Nakajima et al., "Molecular typing of the methicillin resistance determinant (mec) of clinical strains of Staphylococcus based on mec hypervariable region length polymorphisms," *The Journal of Laboratory and Clinical Medicine*, vol. 126, no. 1, pp. 29–35, 1995.

[19] V. Cázares-Domínguez, S. A. Ochoa, A. Cruz-Córdova et al., "Vancomycin modifies the expression of the agr system in multidrug-resistant Staphylococcus aureus clinical isolates," *Frontiers in Microbiology*, vol. 6, pp. 369–369, 2015.

[20] S. Hoseini, M. Niakan, H. Saderi, and M. Emaneini, "Comparison of cefoxitin disk diffusion and PCR for mecA gene methods for detection of methicillin resistant Staphylococcus aureus," *Daneshvar Medicine*, vol. 22, no. 114, pp. 41–46, 2015.

[21] M. Latifpour, R. V. Goering, S. A. Havaei et al., "Identification of two major direct repeat unit clusters, 8i and 11ce, among methicillin resistant Staphylococcus aureus strains: the emergence of novel dru types and repeats," *Molecular biology reports*, vol. 49, no. 9, pp. 8229–8239, 2022.

[22] S.-M. Li, Y.-F. Zhou, L. Li et al., "Characterization of the multidrug resistance gene cfr in methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from animals and humans in China," *Frontiers in microbiology*, vol. 9, pp. 2925–2925, 2018.

[23] S. M. Kareem, S. S. Aljubori, and M. R. Ali, "Novel determination of _spa_ gene diversity and its molecular typing among _Staphylococcus aureus_ Iraqi isolates obtained from different clinical samples," *New Microbes and New Infections*, vol. 34, article 100653, 2020.

[24] M. Goudarzi, S. S. Seyedjavadi, M. J. Nasiri, H. Goudarzi, R. Sajadi Nia, and H. Dabiri, "Molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis," *Microbial Pathogenesis*, vol. 104, pp. 328–335, 2017.

[25] S. M. Mahdiyoun, H. Kazemian, M. Ahanjan, H. Houri, and M. Goudarzi, "Frequency of aminoglycoside-resistance genes in methicillin-resistant Staphylococcus aureus (MRSA) isolates from hospitalized patients," *Jundishapur journal of microbiology*, vol. 9, no. 8, p. e35052, 2016.

[26] W.-Y. Wang, T.-S. Chiueh, J.-R. Sun, S.-M. Tsao, and J.-J. Lu, "Molecular typing and phenotype characterization of methicillin-resistant Staphylococcus aureus isolates from blood in Taiwan," *PLoS One*, vol. 7, no. 1, article e30394, 2012.

[27] Indian Network for Surveillance of Antimicrobial Resistance group I, "Methicillin resistant Staphylococcus aureus (MRSA) in India: prevalence & susceptibility pattern," *Indian Journal of Medical Research*, vol. 137, no. 2, pp. 363–369, 2013.

[28] A. E. Namvar, M. Afshar, B. Asghari, and A. Rastegar Lari, "Characterisation of SCCmec elements in methicillin-resistant Staphylococcus aureus isolated from burn patients," *Burns*, vol. 40, no. 4, pp. 708–712, 2014.

[29] M. Aires de Sousa, M. I. Crisostomo, I. Santos Sanches et al., "Frequent recovery of a single clonal type of multidrug-resistant Staphylococcus aureus from patients in two hospitals in Taiwan and China," *Journal of Clinical Microbiology*, vol. 41, no. 1, pp. 159–163, 2003.

[30] G. Arakere, S. Nadig, G. Swedberg, R. Macaden, S. K. Amarnath, and D. Raghunath, "Genotyping of methicillin-resistant Staphylococcus aureus strains from two hospitals in Bangalore, South India," *Journal of Clinical Microbiology*, vol. 43, no. 7, pp. 3198–3202, 2005.

[31] H. S. Ebrahim-Saraie, M. Motamedifar, J. Sarvari, and S. M. Hoseini Alfatemi, "Emergence of SCCmec type I obtained from clinical samples in Shiraz teaching hospitals, South-West of Iran," *Jundishapur Journal of Microbiology*, vol. 8, no. 6, article e16998, 2015.

[32] S. Moshtagheian, M. Halaji, H. Sedaghat et al., "Molecular characteristics of methicillin-resistant Staphylococcus aureus nasal carriage from hospitalized patients and medical staff in Isfahan, Iran," *Annali di Igiene*, vol. 30, no. 3, pp. 237–244, 2018.

[33] N. Parhizgari, S. S. Khoramrooz, S. A. Malek Hosseini et al., "High frequency of multidrug-resistant Staphylococcus aureus with SCCmec type III and Spa types t037 and t631 isolated from burn patients in southwest of Iran," *APMIS*, vol. 124, no. 3, pp. 221–228, 2016.

[34] S. J. Hau, J. S. Haan, P. R. Davies, T. Frana, and T. L. Nicholson, "Antimicrobial resistance distribution differs among methicillin resistant Staphylococcus aureus sequence type (ST) 5 isolates from health care and agricultural sources," *Frontiers in Microbiology*, vol. 9, 2018.

[35] E. Ş. Yılmaz and Ö. Aslantaş, "Antimicrobial resistance and underlying mechanisms in Staphylococcus aureus isolates," *Asian Pacific Journal of Tropical Medicine*, vol. 10, no. 11, pp. 1059–1064, 2017.

[36] A. Ghasemian, S. Peerayeh, and M. Mirzaee, "Detection of accessory gene regulator groups genes and cassette chromosome mec types among Staphylococcus aureus isolated from intensive care unit patients," *Asian Pacific Journal of Tropical Disease*, vol. 5, no. 2, pp. 153–157, 2015.

[37] M. Goudarzi, H. Goudarzi, A. M. Sá Figueiredo et al., "Molecular characterization of methicillin resistant Staphylococcus aureus strains isolated from intensive care units in Iran: ST22-SCCmec IV/t790 emerges as the major clone," *PLoS One*, vol. 11, no. 5, article e0155529, 2016.

[38] F. L. Nowrouzian, O. Dauwalder, H. Meugnier et al., "Adhesin and superantigen genes and the capacity of Staphylococcus aureus to colonize the infantile gut," *The Journal of Infectious Diseases*, vol. 204, no. 5, pp. 714–721, 2011.

[39] M. A. Alreshidi, A. A. Alsalamah, R. A. Hamat et al., "Genetic variation among methicillin-resistant Staphylococcus aureus isolates from cancer patients in Saudi Arabia," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 32, no. 6, pp. 755–761, 2013.

[40] H. Chen, Y. Liu, X. Jiang, M. Chen, and H. Wang, "Rapid change of methicillin-resistant Staphylococcus aureus clones in a Chinese tertiary care hospital over a 15-year period," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 5, pp. 1842–1847, 2010.