

Molecular Characterization, Drug Resistance and Virulence Analysis of Constitutive and Inducible Clindamycin Resistance *Staphylococcus aureus* Strains Recovered from Clinical Samples, Tehran – Iran

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Background: Macrolide-lincosamide streptogramin B family is one of the important alternative antibiotics for treating staphylococcal infections. The aim of this study was to determine the characteristics and prevalence of antibiotic resistance genes in different coagulase types of clinical *Staphylococcus aureus* strains.

Methods: In the present study, 86 isolates with different phenotypes of MLS_B resistance were investigated. In vitro susceptibility was assessed by the disk diffusion and broth microdilution methods. PCR assays were used to detect resistance-related genes. Coagulase and SCC_{mec} types were identified by multiplex PCR assay.

Results: The prevalences of constitutive MLS_B, inducible MLS_B, and MS phenotypes were found to be 23%, 14.2%, and 4.9%, respectively. The rates of resistance to mupirocin, fusidic acid, and tigecycline were found to be 9.3%, 4.6%, and 2.3%, respectively. The top three predominant resistance genes were *mecA*, *tet(M)*, *erm(C)* representing 75.6, 50, and 40.7% of isolates. *mupA* (7%), *fusB* (3.5%), and *fusC* (1.2%) genes were also detected among tested isolates. Coagulase types were mainly type II (34.9%), followed by III (32.6%), V (20.9%), and I (11.6%).

Conclusion: These findings indicated high resistance rate and low genetic variability with the prominence of *coa* type II, highlighting the particular importance of diagnosis of these strains to avoid treatment failure.

Keywords: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, polymerase chain reaction, PCR, coagulase, clindamycin

Introduction

Staphylococcus aureus is an opportunistic pathogen causing various hospital and community-acquired infections ranging from pyogenic skin infections to life-threatening diseases.¹ According to the evidence, specific virulence factors such as i) cell surface components including collagen-binding protein, clumping factor, fibronectin-binding protein, and elastin-binding protein, ii) secreted factors, e.g. staphylokinase, toxic shock syndrome toxin (TST), hemolysin, exfoliative toxins, staphylococcal enterotoxins (SEs), lipase and panton-valentine leukocidin (PVL), play a pivotal role in pathogenesis and also are related to severity of the infection.^{1,2} Recent studies have demonstrated an evolved resistance to various antibiotic types among *S. aureus*, which has raised real concerns.³⁻⁶ Due to emerging simultaneous resistance to several

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antibacterial agents, the choice of chemotherapeutic options and treatment of serious infections caused by *S. aureus* has become problematic.^{7–9} Although various antibiotics such as vancomycin, linezolid, and quinupristin-dalfopristin may be considered as drugs of choice, *S. aureus* strains with reduced susceptibility and resistance to these agents also emerged. Therefore, the use of macrolide-lincosamide-streptogramin B (MLS_B) antibiotics as an alternative approach to treating such infections was taken into consideration.^{3,4,6} However, some previous studies reported a constitutive and inducible resistance to clindamycin in *S. aureus* strains due to the indiscriminate use of MLS_B antibiotics.^{4,6,10} Therefore, particular attention should be paid to the detection of constitutive and inducible clindamycin resistance genotypes and phenotypes to prevent clinical therapeutic failure in patients with *S. aureus* infections.¹¹ With respect to the limited data in this context, the present study was designed to describe the phenotypic and genotypic resistance pattern and the presence of the virulence factors. Coagulase (*coa*) typing and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were used to characterize the genotype of the constitutive and inducible clindamycin resistance isolates.

Materials and Methods

Study Design, Isolation of Bacteria and Ethics Statement

This descriptive cross-sectional study was conducted on 204 nosocomial *S. aureus* strains recovered from clinical samples such as pus (36.3%), wound (31.9%), blood (13.7%), sputum (7.3%), cerebrospinal fluid (5.9%), and urine (4.9%) during the period of 1 year, from August 2018 to July 2019. The Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran certified the protocol of this project (IR.SBMU.MSP.REC.1396.700). At first, *S. aureus* isolates were phenotypically identified using standard microbiological and biochemical techniques and then were subjected to polymerase chain reaction (PCR) assay for the presence of *nuc* gene for definitive confirmation.¹² A total of 86 nosocomial *S. aureus* isolates were included in the study based on resistance to erythromycin and resistance and/or susceptibility to clindamycin in accordance with standard clinical and laboratory standard institute (CLSI) guidelines. Isolates with resistance to both clindamycin and erythromycin-resistant were considered to be constitutive resistance phenotype (cMLS_B). Isolates with resistance to erythromycin but susceptible

to clindamycin were tested by the D test. Inducible resistance phenotype (iMLS_B) was defined for isolates showing resistance to erythromycin and susceptible to clindamycin with a D-shaped zone around the clindamycin disk, flattened from the side of erythromycin disk. Isolates with both inhibition zones showing a circular shape (D test negative) were classified as the MS phenotype (CLSI 2019).

Antimicrobial Activities

The disk diffusion method using cefoxitin (30 µg) disk in Mueller-Hinton agar (Merck, Germany) according to the CLSI was applied for the screening of methicillin resistance isolates. In addition, PCR assay was used for the detection of *mecA* gene.¹²

The Kirby–Bauer disk diffusion method was used to determine the susceptibility of the isolates against penicillin, ceftriaxone, amikacin, gentamicin, tobramycin, kanamycin, tetracycline, linezolid, teicoplanin, ciprofloxacin, rifampicin, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole (Mast Co., UK) based on the CLSI recommendation (CLSI 2019). The minimum inhibitory concentration (MIC) value for vancomycin, mupirocin, tigecycline, and fusidic acid was determined using the broth microdilution method. Results for fusidic acid and tigecycline were interpreted according to the European Committee for antimicrobial susceptibility testing (EUCAST) breakpoints (<http://www.eucast.org>). Low-level and high-level mupirocin resistance (LLMUPR, HLMUPR) were defined if MIC values of 8–256 µg/mL and ≥512 µg/mL were obtained. *S. aureus* strains ATCC 25923, ATCC 43300 and ATCC 29213 were used as reference strains.

DNA Extraction

In this study, the DNA of each strain was extracted using the phenol-chloroform extraction method with the modification of adding lysostaphin (Sigma-Aldrich, United States) for bacterial lysis. DNA concentration and purity were investigated using a NanoDrop 2000 spectrophotometer (spectrophotometer, Thermo Scientific, Wilmington, DE, USA).

Amplification of Resistance-Related Genes

The resistance encoding genes *vanA*, *vanB*, *mupB*, *mupA*, *fusA*, *fusB*, *fusC*, *mecC*, *msr(A)*, *msr(B)*, *erm(A)*, *erm(B)*, *erm(C)*, *tet(M)*, *ant* (4′)-*Ia*, *aac* (6′)-*Ie/aph* (2″), *aph* (3′)-*IIIa* and virulence determinants including exfoliative toxin

(*eta*, and *etb*), Panton-Valentine leukotoxin (*pvl*), and toxic shock syndrome toxin (*tst*) genes were detected by PCR.^{12,13} In order to detect target genes, PCR amplified products were resolved using electrophoresis in 1.8% (w/v) agarose gel, stained with ethidium bromide (0.5 mg/mL) and visualized under UV light using a gel documentation system (Bio-Rad Laboratories, USA).

SCCmec Typing

For methicillin-resistant *S. aureus* (MRSA) strains, determination of SCCmec types was performed using multiplex PCR assay, as previously described by Nezhad et al *S. aureus* ATCC 10442 (type I), *S. aureus* N315 (type II), *S. aureus* 85/2082 (type III), *S. aureus* MW2 (type IV), *S. aureus* WIS 173 (type V) and *S. aureus* HDE288 (type VI) were recruited as control strains.

Staphylocoagulase (SC) Typing

Multiplex PCR assay with four-set primers (A-D), was used for determination of SC types (I–X) as previously described by Hirose et al Set A contained primers for identification of SC types I, II, III, IVa, IVb, Va, and VI while set B contained primers for identifying SC types VII, VIII, and X. Set C was used to identify SC types IX and Vb. SC types IVa and IVb were distinguished using set four primers (Set D).¹⁴

Results

Out of 204 *S. aureus* tested isolates, the overall prevalence of cMLS_B, iMLS_B, and MS phenotypes were 47 (23%), 29 (14.2%), and 10 (4.9%), respectively. Out of 86 tested isolates, 25 isolates were obtained from hospital H1 (29.1%), 20 isolates from hospital H2 (23.3%), 18 isolates from hospital H3 (20.9%), and 23 isolates from hospital H4 (26.7%). The under-study isolates were recovered from wound (28/86, 32.6%), pus (21/86, 24.4%), blood (19/86, 22.1%), sputum (7/86, 8.1%), CSF (6/86, 7%), and urine (5/86, 5.8%). According to our analysis, the rate of invasive and non-invasive *S. aureus* was found to be 29.1% and 70.9%, respectively. All the invasive *S. aureus* isolates were methicillin-resistant with iMLS_B (40%; 10/25), MS (40%; 10/25) and cMLS_B (20%; 5/25) phenotypes. A total of 86 *S. aureus* strains included in present study, 47 were isolated from female patients (54.7%) and 39 were recovered male patients (45.3%) with a median age of 41.4 years, ranging from 15 to 59 years. Of these examined 86 *S. aureus* strains, 75.6% (65/86) and 24.4% (21/86) were MRSA and methicillin-sensitive *S. aureus* (MSSA), respectively. The

cMLS_B phenotype was observed in both MRSA (26, 30.2%) and MSSA (21, 24.4%) strains, whereas phenotypes of iMLS_B and MS were found only in MRSA strains. The most common *S. aureus* isolates with iMLS_B phenotype in the present study were isolated from wound infection (19.8%, 17/86) while cMLS_B phenotypes were from pus (22.1%, 19/86) and MS phenotypes were from blood infection (9.3%, 8/86). Distribution of different MLS_B phenotypes in *S. aureus* strains isolated from various clinical specimens is shown in Table 1. Based on data obtained from the disk diffusion test, all isolated *S. aureus* strains were found to be susceptible to linezolid, teicoplanin, and vancomycin. As summarized in Table 2, resistance to all antibacterial agents (but no amikacin and fusidic acid) was more common among MRSA isolates than among MSSA isolates. All the four of the fusidic acid-resistant isolates were MSSA isolates with cMLS_B phenotype, based on the results of the micro-broth dilution method. The rate of resistance to mupirocin was found to be 9.3%. Six isolates indicating high-level resistance to mupirocin (HLMUPR) belonged to MRSA strains with iMLS_B (5 isolates) and MS (one isolate) phenotypes and two isolates showing low-level resistance to mupirocin (LLMUPR) were identified as MRSA strains with iMLS_B phenotype. Two tigecycline resistant isolates belonged to two MRSA strains with iMLS_B and MS phenotypes.

Our findings indicated that all isolates were typed using the SC typing method. These isolates were distinguished into 4 types of SC. The predominant SC type was II (34.9%, 30/86), followed by III (32.6%, 28/86), V (20.9%, 18/86), and I (11.6%, 10/86). All the MSSA isolates belonged to SC type III. All MS phenotype MRSA isolates belonged to SC type I. Among iMLS_B phenotype MRSA isolates, the most predominant SC types were II and V representing 25.6% (22/86) and 8.1% (7/86) of isolates. cMLS_B phenotypes were distributed in

Table 1 Distribution of iMLS_B, cMLS_B and MS Phenotypes in 86 Nosocomial *S. aureus* Strains Isolated from Clinical Sources

Samples	Phenotypes			Total, n (%)
	iMLS _B	cMLS _B	MS	
Wound	17 (60.7)	11 (39.3)	–	28 (32.6)
Pus	2 (9.5)	19 (90.5)	–	21 (24.4)
Blood	10 (52.6)	1 (5.3)	8 (42.1)	19 (22.1)
Sputum	–	7 (100)	–	7 (8.1)
CSF	–	4 (66.7)	2 (33.3)	6 (7)
Urine	–	5 (100)	–	5 (5.8)
Total	29 (33.7)	47 (54.7)	10 (11.6)	86 (100)

Table 2 Antimicrobial Resistance Pattern of Nosocomial MRSA and MSSA Isolates with Inducible and Constitutive Phenotype

Antibiotic	65 MRSA Isolates n (%)			21 MSSA Isolates n (%)			Total n (%)
	iMLS _B	cMLS _B	MS	cMLS _B	iMLS _B	MS	
Penicillin	29 (39.7)	26 (35.6)	10 (13.7)	-	8 (11)	-	73 (84.8)
Ceftriaxone	24 (35.3)	24 (35.3)	9 (13.2)	-	11 (16.2)	-	68 (79.1)
Gentamicin	24 (43.6)	18 (32.7)	9 (16.4)	-	4 (7.3)	-	55 (64)
Kanamycin	8 (42.1)	3 (15.8)	-	-	8 (42.1)	-	19 (22.1)
Amikacin	8 (27.6)	3 (10.3)	-	-	18 (62.1)	-	29 (33.7)
Tobramycin	14 (51.9)	11 (40.7)	2 (7.4)	-	-	-	27 (31.4)
Tetracycline	19 (32.2)	18 (30.5)	4 (6.8)	-	18 (30.5)	-	59 (68.6)
Ciprofloxacin	14 (29.2)	17 (35.4)	3 (6.2)	-	14 (29.2)	-	48 (55.8)
Rifampicin	9 (69.2)	3 (23.1)	1 (7.7)	-	-	-	13 (15.1)
Mupirocin	5 (62.5)	2 (25)	1 (12.5)	-	-	-	8 (9.3)
Quinupristin-dalfopristin	5 (41.7)	2 (16.7)	1 (8.3)	-	4 (33.3)	-	12 (13.9)
Trimethoprim-sulfamethoxazole	6 (40)	-	6 (40)	-	3 (20)	-	15 (17.4)
Fusidic acid	-	-	-	-	4 (100)	-	4 (4.6)
Tigecycline	1 (50)	-	1 (50)	-	-	-	2 (2.3)

SC types III, II, and V accounting for 32.6%, 9.3%, and 12.8%, respectively.

Resistance encoding genes analysis showed that the most prevalent gene was *mecA* (75.6%, 65/86), followed by *tet(M)* (50%, 43/86), *erm(C)* (40.7%, 35/86), *ant* (4')-*Ia* (29.1%, 25/86), *aac* (6')-*Ie/aph* (2'') (20.9%, 18/86), *msr(A)* (20.9%, 18/86), *aph* (3')-*IIIa* (14%, 12/86), *erm* (B) (14%, 12/86), *msr(B)* (9.3%, 8/86), *erm(A)* (8.1%, 7/86), *mupA* (7%, 6/86), *fusB* (3.5%, 3/86), and *fusC* (1.2%, 1/86). Our findings showed that no PCR products were found for the resistance genes *vanA*, *vanB*, *mupB*, *fusA*, and *mecC*. The distribution of SCC*mec* types in the 65 MRSA isolates showed that SCC*mec* type III was the most prevalent type found in 33 isolates (50.8%), followed by type II in 20 isolates (30.8%), and type IV in 12 isolates (18.4%). SCC*mec* types I and V were not found in our isolates. The resistance profile and distribution of *coa* and SCC*mec* types in MRSA and MSSA isolates with inducible and constitutive phenotype are presented in Table 3.

Discussion

Little is known about the emergence, distribution and molecular types of constitutive and inducible clindamycin resistance *S. aureus* strains in Iran. Accordingly, our study focused on the identification of molecular characteristics and understanding of cMLS_B, iMLS_B *S. aureus* isolates epidemiology. Our research highlighted several new findings in relation to MRSA and MSSA isolates with inducible and constitutive resistance phenotype including a relatively high

prevalence of inducible and constitutive resistance and distinct molecular types with genetic diversity. According to the evidence, the prevalence rate of iMLS_B phenotype among *S. aureus* isolates was markedly varied across the geographical region and among health-care settings. The current finding showed a prevalence rate of 14.2% for iMLS_B which is higher than the reported rate in Nepal (11.48%),³ Iran (8.6%),⁵ Egypt (7.7%),¹⁵ Turkey (7.8%)⁴ and lower than those reported in India (37.5%).¹⁶ However, the results of previous studies conducted in Iran noted significant variation in iMLS_B prevalence rates in different areas^{10,17} ranging from 6% to 32.3%. This work presented a relatively high prevalence of iMLS_B phenotype, which highlighted the need to prescribe of macrolides in a logical manner, in order to change in resistance pattern. However, the true evaluation of iMLS_B *S. aureus* isolates prevalence depends on the accurate diagnosis, geographical variation, characteristic of health-care setting, and population under study.

In the current research, the prevalence of cMLS_B among *S. aureus* was found to be 23%, which was similar to reported rate, by Delialioğlu et al (24.3%),⁴ and Eksi (20.4%).¹⁸ However, a lower and higher percentage of cMLS_B were also reported in previous studies performed by Khashei et al (82.9%),⁵ Adhikari et al (29.25%),³ Mansouri et al (28.4%),¹⁹ Sedaghat et al (32.1%),¹⁷ and Sasirekha et al (13.1%).⁶ These variations in the prevalence of cMLS_B among *S. aureus* in different parts of the world could be attributed to the difference in consumption of macrolides in community and hospital settings, study design, population and geographical distribution, and the spread of specific

Table 3 Distribution of *coa*, SCCmec Types and Resistance Profiles of Nosocomial MRSA and MSSA Isolates with Inducible and Constitutive Phenotype

Phenotypes No. (%)	Virulence Genes (No.; %)	<i>coa</i> Type (No.; %)	SCCmec Type (No.; %)	Phenotypic Resistance Profile ^a (No.; %)	Genetic Resistance Profile (No.; %)	Hospitals (No.;%)	Methicillin Pattern (No.; %)
cMLS _B	<i>tst</i> (8; 30.8), <i>eta</i> (3; 11.5)	III (1; 3.8)	III (1; 3.8)	PG, CRO, GM, K, AK, TN, T, RI (1; 3.8)	<i>mecA</i> (26; 100), <i>tet(M)</i> (15; 57.7), <i>erm(C)</i> (18; 69.2), <i>erm(A)</i> (5; 19.2), <i>erm(B)</i> (2; 7.7), <i>ant (4)-Ia</i> (10; 38.5), <i>aph (3)-IIIa</i> (3; 11.5), <i>aac (6)-IeIaph (2)</i> (6; 23.1)	HI (9; 34.6), H2 (7; 26.9), H3 (6; 23.1), H4 (4; 15.4)	MRSA (26; 30.2)
		II (2; 7.7), III (6; 23.1), V (7; 26.9)	III (10; 38.5), II (5; 19.2)	PG, CRO, GM, T, CIP (15; 57.7)			
		V (4; 15.4), II (4; 15.4)	III (3; 11.6), IV (5; 19.2)	PG, CRO, TN (8; 30.8)			
		II (2; 7.7)	II (2; 7.7)	PG, GM, K, AK, TN, T, RI, CIP, SYN, MUP (2; 7.7)			
		III (7; 33.3)	-	PG, AK, T, CRO, CIP (7; 33.3)			
		III (4; 19)		CRO, GM, K, AK, T, CIP, SYN, FC (4; 19)			
		III (3; 14.3)		K, AK, T, CIP, TS (3; 14.3)			
		III (4; 19)		AK, T (4; 19)			
		III (1; 4.8)		PG, K (1; 4.8)			
		III (2; 9.6)		Without resistance (2; 9.6)			
iMLS _B	<i>pvl</i> (15; 51.7), <i>tst</i> (10; 34.5)	II (4; 13.8)	II (4; 13.8)	PG, CRO, GM, K, AK, TN, T, RI (4; 13.8)	<i>mecA</i> (29; 100), <i>mupA</i> (5; 17.2), <i>tet(M)</i> (13; 44.8), <i>mst(A)</i> (15; 51.7), <i>mst(B)</i> (6; 20.7), <i>erm(B)</i> (6; 20.7), <i>ant (4)-Ia</i> (5; 17.2), <i>aph (3)-IIIa</i> (2; 6.9), <i>aac (6)-IeIaph (2)</i> (5; 17.2)	HI (9; 31.1), H2 (5; 17.2), H3 (10; 34.5), H4 (5; 17.2)	MRSA (29; 33.8)
		II (4; 13.8), V (6; 20.7)	II (4; 13.8), IV (6; 20.7)	PG, CRO, GM, T, CIP (10; 34.5)			
		II (5; 17.2)	II (5; 17.2)	PG, CRO, GM, TS (5; 17.2)			
		II (5; 17.2)	III (5; 17.2)	PG, CRO, TN, (5; 17.2)			
		II (4; 13.8)	III (4; 13.8)	PG, GM, K, AK, TN, T, RI, CIP, SYN, MUP (4; 13.8)			
		V (1; 3.5)	IV (1; 3.5)	PG, GM, TN, T, RI, MUP, TS, SYN, TIG (1; 3.5)			
		I (3; 30)	III (10; 100)	PG, CRO, GM, T, CIP (3; 30)			
		I (5 50)		PG, CRO, GM, TS (5; 50)			
		I (1; 10)		PG, CRO, TN (1; 10)			
		I (1; 10)		PG, GM, TN, T, RI, MUP, TS, SYN, TIG (1; 10)			
MS	<i>pvl</i> (4; 40)				<i>mecA</i> (10; 100), <i>mupA</i> (1; 10), <i>tet(M)</i> (3; 30), <i>mst(A)</i> (3; 30), <i>mst(B)</i> (2; 20), <i>erm(B)</i> (1; 10), <i>ant (4)-Ia</i> (2; 20), <i>aph (3)-IIIa</i> (2; 20)	HI (2; 20), H2 (2; 20), H3 (2; 20), H4 (4; 40)	MRSA (10; 11.6)

Abbreviations: PG, penicillin; CRO, ceftriaxone; GM, gentamicin; K, kanamycin; AK, amikacin; TN, tobramycin; T, tetracycline; TS, trimethoprim-sulfamethoxazole; FC, fusidic acid; CIP, ciprofloxacin; SYN, quinupristin-dalfopristin; TIG, tigecycline; RI, rifampicin; MUP, mupirocin.

molecular types. As presented in Table 2, the cMLS_B phenotype was higher in MRSA (30.2%) as compared to MSSA (24.4%) and iMLS_B and MS phenotypes were found only in MRSA strains, which is consistent with previous reports from Nepal,³ Iran,¹⁹ Egypt,¹⁵ and Turkey.⁴

In our study, low resistance rate to mupirocin (9.3%) was noted which is in agreement with other studies conducted in Iran (6%),⁷ India (5%),⁸ and Jordan (2.6%).²⁰ Furthermore, the findings of the present study demonstrate that 7% of tested isolates were found to have resistance to mupirocin at a high level which are quite similar to the results of a study conducted by Liu and colleagues in China. They reported a prevalence rate of 6.6% for isolates with high-level mupirocin-resistant.²¹ However, there has been a higher prevalence of HLMUPR strains in Iran (25%)²² and Egypt (61.5%).²³ Different results of these studies may be due to study design (patient characteristics and specimen types), specific type dissemination among patients and unrestricted policies in taking mupirocin. This study showed that all HLMUPR isolates were *mupA*-positive (7%). González-Domínguez et al (27.2%)²⁴ and Abbasi-Montazeri et al (34%)⁷ reported a higher percentage of *mupA*. Shahsavan et al reported that *mupA* was responsible for the resistance to mupirocin only in MRSA strains with cMLS_B phenotype. Conversely, in the present research, this gene was present in MRSA strains with iMLS_B and MS resistance phenotype.

Tigecycline is a reliable treatment option against many infections caused by MDR isolates especially MRSA. So far, there have been few reports published on the emergence of *S. aureus* strains with reduced susceptibility and resistance to tigecycline. We found the low numbers of tigecycline resistant isolates (2.3%). However, different resistance rates to tigecycline among *S. aureus* isolates are reported in Libya (3.6%),²⁵ Turkey (2%),²⁶ and Iran (6.6%).⁹

Recently published data from Asian countries indicated a low prevalence of resistance to fusidic acid (<10%).^{27,28} We found a low prevalence (4.7%) of resistance to fusidic acid among our isolates carrying *fusB* (3 isolates), and *fusC* (1 isolate) genes. This observation is consistent with data from a recent multicenter study in Iran that showed a low prevalence of MRSA fusidic acid-resistant (3%) among 726 studied *S. aureus* isolates.²⁸ Various resistance rates to fusidic acid have been described in many countries such as Greece (62.4%), Ireland (19.9%), Australia (7.0%), Canada (7.0%), and the United States (0.3%).²⁹ Notably, this study showed that fusidic acid resistance was

only seen among MSSA isolates which was in contrast to a report from China that indicated the prevalence of fusidic acid resistance among MRSA isolates was higher significantly than that among MSSA isolates.³⁰ Yu and colleagues also reported a 10.5% incidence of *fusB* genes while *fusC* and *fusA* genes were not detected in any of the isolates examined.³⁰ This indicates that *fusB* is the predominant determinant responsible for resistance to fusidic acid among MSSA isolates with cMLS_B resistance phenotype in Iran.

ant (4')-Ia as the most prevalent aminoglycoside resistance gene was present in 25 strains (29.1%) which was higher than those reported in Turkey (24%)³¹ and lower than that reported rate in India (9%).³² However, much higher rates have also been reported by Nezhad et al (94.7%)¹² and Ida et al (84.5%).³³ In our research, the prevalence of *aac* (6')-Ie/*aph* (2'') as the second commonly detected aminoglycoside resistance gene was found to be 20.9% which is lower than that reported by Ardic et al (60.5%).³¹ Akpaka et al reported that in MRSA strains, 20% harbored *ant* (4')-Ia gene.³⁴ Many reports from different parts of Asia such as Turkey (8%),³¹ and India (9%)³⁴ have shown a low prevalence of the *ant* (4')-Ia gene. Likewise, in the current work, 14% of isolates were found to carry *aph* (3')-IIIa. This variation in aminoglycoside resistance determinant frequency displayed that factors such as study design, specimen types, different policies in aminoglycosides consumption, and horizontal gene transfer among the strains may be involved.

Based on the literature, inducible and constitutive resistance in *S. aureus* strains is mediated by both *erm* and *msr* genes. Our analysis recorded *erm*(C) (40.7%) and *erm*(A) (8.1%) as the highest and lowest erythromycin resistance gene. Although there is a discrepancy in the prevalence rate of *erm*(C) gene in different parts of the world, a similar finding from Iran has also been reported.¹⁷ These findings are in concordance with those described by Fasihi et al, which reported prevalence of *erm*(C) and *erm*(A) genes to be 20.5% and 11% for MRSA strains.³⁵ In accordance with our results, Schmitz and colleagues reported that the *erm*(A) gene was more common in MRSA isolates compared to MSSA isolates (88% vs 38%) and occurred mainly in cMLS_B expression strains.³⁶ A high percentage of *msr*(A) (20.9%) was obtained in the current research, similar to a study conducted by Sedaghat et al (43.6%),¹⁷ Nezhad et al (47.3%).¹² In accordance with several investigators who reported the prevalence of *erm*(B) gene at a

low level, we detected *erm(B)* gene in 5.8% and 8.1% inducible and constitutive resistant strains, respectively. A study from Texas also showed a high frequency of *erm(B)* gene in inducible resistant strains (46.3%).³⁷ This discrepancy could be because of the distribution of some specific clonal lineages in communities and hospitals and might be more closely related to the usage of particular macrolides and ketolides in our health-care settings.

Our data related to SCC*mec* types are in concordance with many studies that demonstrated SCC*mec* types I, II, III are related to hospital-acquired *S. aureus* infections while IV and V are prominent types in community-acquired *S. aureus* infections.^{12,21,24} We confirmed SCC*mec* type IV in MRSA isolates with cMLS_B (5.8%) and iMLS_B (8.1%) phenotype. This finding supports a shift in these isolates from our community to hospital.

According to the *coa* typing results, predominant SC type was II (34.9%), followed by III (32.6%), V (20.9%), and I (11.6%). This was in comparison to the previous report by Hirose et al in Japan which indicated *coa* type II, VII and I accounted for 91.9%, 3.9% and 1.7% of isolates.¹⁴ We detected 2 SC types (II–V) among iMLS_B *S. aureus* strains suggesting the clonal distribution of tested isolates in this region of Iran. In research involving 157 *S. aureus* strains from clinical specimens, nine different patterns of *coa* gene have been detected.³⁸ In another study from Thailand on 129 MRSA isolates from 17 hospitals, Janwithayanuchit et al determined four different genotypes from coagulase gene typing of tested strains.³⁹ They showed that the most prevalent *coa* type was III (82.2%) followed by IV (14%), II (2.3%), and I (1.5%). The analysis was carried out by Younis Omar et al on 75 MRSA isolated from different ICUs grouped into three different types based on the polymorphism of *coa* gene products by PCR.⁴⁰ The similarity between *coa* genes of examined strains highlighted that it may be as a predictor for specific inducible resistant *S. aureus* strains.

Conclusion

Given the presence of various types of MLS_B resistance in our survey, special attention should be given to diagnosing these resistance types in order to judicious use of clindamycin. However, some resistance patterns were related to certain SCC*mec* and *coa* types. These strains have low genetic variability with a predominance of *coa* type II and SCC*mec* type III. Further researches should be performed in other regions of Iran to keep track of the emerging *coa* types.

Funding

This work was supported by the fund in the Research Deputy of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant No. 12688).

Disclosure

The authors declare that they have no conflict interest.

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