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Phenotypic and Genotypic Study of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus* in Tabriz, Northwest Iran

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Abstract

Background: Resistance to macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics is mediated by *erm* and *msrA* genes in *Staphylococcus aureus*. The expression of these genes can lead to three phenotypes, namely constitutive resistance ($cMLS_B$), inducible resistance ($iMLS_B$), which are resistant to macrolide, lincosamide, and streptogramin B antibiotics, and MS_B phenotype, which is resistant only to macrolide and streptogramin B. Inducible clindamycin resistance is an important concern because it is not detected in routine laboratory tests.

Objectives: The aim of this study was to determine the frequency of MLS_B phenotypes and genotypes among 215 clinical isolates of *S. aureus* and then, examine their resistance to antibacterial agents, which is recommended for methicillin-resistant *S. aureus* (MRSA) isolates.

Methods: Two hundred and fifteen non-repetitive clinical isolates of *S. aureus* were collected. Resistance to antibacterial agents was determined by disk diffusion and E-test methods. Susceptibility to clindamycin and erythromycin was tested by D-test. All isolates were screened by PCR for the presence of *nucA*, *mecA*, *ermA*, *ermB*, *ermC*, and *msrA* genes.

Results: The prevalence of $iMLS_B$, $cMLS_B$ and MS_B phenotypes among all the isolates was determined as 10.69%, 34.42%, and 0%, respectively. In our study, $iMLS_B$ was prevalent more in methicillin susceptible *S. aureus* (MSSA) (11.71%) than MRSA (9.19%) isolates ($P = 0.557$). In contrast, the rate of $cMLS_B$ was significantly higher in MRSA (79.31%) than MSSA (3.90%) isolates ($P = 0.000$). No MS_B phenotype was detected in our study. The most prevalent genes were *ermC* and *ermA* with 39% and 21.5% frequencies, respectively. Six isolates showed D phenotype, while the PCR results of *erm* genes were negative. All 215 isolates of *S. aureus* were negative for the presence of *ermB* and *msrA* genes.

Conclusions: The rate of $iMLS_B$ in *S. aureus* isolates is relatively high in the Northwest Iran. Since isolates with inducible resistance may mutate and change to constitutive resistance, to prevent clinical treatment failure, D-test should be performed along with routine antibiotic susceptibility tests. In this study, *ermC* gene was the predominant genetic determinant for the expression of MLS_B resistance. This predominance is probably due to the spread of distinctive clones (which carry *ermC* gene) in our region.

Keywords: Methicillin-Resistant, Clindamycin, Iran, *Staphylococcus aureus*

1. Background

Staphylococcus aureus is one of the most common and important pathogens, accounting for diverse nosocomial and community acquired infections. The bacterium has potentiality to cause three types of disorders in human: superficial, toxin mediated, and lethal-systemic infections (1). The serious concern about this bacterium is development of antibiotic resistance, especially against methicillin, the so-called methicillin resistant *S. aureus* (MRSA) (2, 3). The emergence of MRSA has left with very few antibiotic alternatives to treat *S. aureus*-related infections, which has caused renewed interest in the usage of macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics against *S. aureus* infections (2, 4). Though these antibiotics differ structurally, they act in a similar way. They inhibit protein synthesis via binding to large subunit of ribosome (4, 5). Among these antibiotics, clindamycin is preferred because of its excellent pharmacokinetic properties (6, 7). Unfortunately, mistreatments with MLS_B antibiotics have caused an unusual increase in the rate of resistance to these antibiotics leading to clinical treatment failure (8).

Three main mechanisms have been elucidated for MLS_B antibiotics resistance in staphylococci: target site modification by a methylase enzyme encoded by *erm* genes, including *ermA*, *ermB*, *ermC*, or *ermF* (*ermA* and *ermC*

are more common); Macrolide efflux pump encoded by *msrA* or *msrB* genes; and inactivation of lincosamides due to lincosamide nucleotidyl transferase enzyme which is encoded by *inuA* gene (9, 10).

In vitro, *S. aureus* isolates with inducible resistance are resistant to erythromycin but appear susceptible to clindamycin. In this situation, therapy with clindamycin may be selective for constitutive *erm* mutants, which can lead to clinical treatment failure (6, 8); but, erythromycin-resistant staphylococci should not be assumed as clindamycin-resistant (11). Since isolates with *msrA*-mediated efflux also appear erythromycin resistant and clindamycin susceptible in in-vitro tests, such isolates do not typically become clindamycin resistant during therapy (11, 12).

Unlike constitutive (c) MLS_B ($cMLS_B$) resistance, inducible (i) MLS_B ($iMLS_B$) resistance is not recognized in conventional laboratory tests (8, 9). Inaccurate identification of $iMLS_B$ resistance may lead to clinical failure of clindamycin therapy; conversely, labeling all erythromycin-resistant staphylococci as clindamycin resistant prevents the use of clindamycin in infections caused by truly clindamycin susceptible staphylococcal isolates (6, 8, 11).

The incidence of MLS_B resistance varies significantly according to geographical region, from hospital to hospital, and age group (6, 12). On the other hand, the frequency of $iMLS_B$ in the north west of Iran has not been studied thoroughly. By using a simple disk approximation test, namely D-test, microbiology laboratories can differentiate isolates which are $iMLS_B$ resistant and harbor *erm* genes from those that are truly sensitive to clindamycin and show efflux pump mediated resistance as macrolide and streptogramin B (MS_B) or negative phenotype (due to *msrA* gene) (6, 8, 9, 11, 12).

2. Objectives

The present study investigated the frequency of MLS_B resistance among clinical isolates of *S. aureus* isolated from various teaching hospitals in the north west of Iran, including MRSA and methicillin susceptible *S. aureus* (MSSA) by phenotypic and genotypic methods and assessed resistance to therapeutic agents which are recommended for these isolates.

3. Methods

3.1. Isolation and Identification of Staphylococcus aureus

During a period of one year from February 2014 to March 2015, all clinical specimens submitted to microbiology laboratories of educational health care centers of Tabriz University of Medical Sciences in the Northwest of Iran were screened for presence of *S. aureus* isolates. Repetitive isolates from the same patient were not examined in this study. The isolates were obtained from different specimens of inpatients and outpatients such as: wound, blood, urine, abscess, fistula and catheter aspirates, sputum and other body fluids and were confirmed by standard microbiology tests (13). They were further confirmed by PCR assay for the presence of *nuc* gene in all the isolates (14). Two hundred and fifteen isolates were collected and stored in TSB broth supplemented by 30% glycerol at -70°C.

3.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility test was performed as per the clinical and laboratory standards institute (CLSI-2014) guidelines (15), with a panel of following antibiotics (MAST, Group Ltd, Merseyside, UK): oxacillin (1 μg), cefoxitin (30 μg), penicillin (10 U), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), rifampin (30 μg), ciprofloxacin (5 μg), trimetoprim-sulfametoxazol (1.25/23.75 μg), and linezolid (30 μg). The minimal inhibitory concentrations (MICs) were determined by E-test on Mueller-Hinton's agar plates (MHA, Merck, Germany) for vancomycin according to the manufacturer's recommendations (Liofilchem, Italy) and the breakpoints for resistance were those as defined by the CLSI-2014. *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 33591, *Enterococcus faecalis* ATCC 29212, and *E. faecalis* ATCC 51299 were used as the control strains.

3.3. Disk Approximation Test (D-Test)

D-zone test was performed as per the CLSI 2014 guidelines (15). Briefly, a suspension of overnight growth of isolates equivalent to 0.5 McFarland turbidity was inoculated on MHA plates. For detecting inducible clindamycin resistance, 15 μg erythromycin disks and 2 μg clindamycin disks (MAST, Group Ltd, Merseyside, UK) were placed on MHA plates (15 mm edge to edge) as part of a standard disk diffusion test. Following overnight incubation at 35°C, inducible clindamycin resistance was observed as flattening of zone towards clindamycin (D-shape), which indicates the isolate has inducible clindamycin resistance (because of *erm* gene) (9). No flattening of zone towards clindamycin indicates the isolate is erythromycin resistant only (due to *msrA* gene). As an isolate demonstrated inducible resistance, clindamycin was reported as resistant (6, 9). Based on D-test results, *S. aureus* isolates were categorized into four non-inducible and two inducible resistance phenotypes according to a previous study (9).

3.4. DNA Extraction

Briefly, DNA was extracted using dodecyl sulphate sodium salt (SDS, Merck, Germany) - proteinase K (CinnaGene, Tehran, Iran) method modified with N-cetyl-N, N, N-trimethyl ammonium bromide (CTAB, Merck, Germany) (3). The concentration of extracted DNA was confirmed by Nano drop 1000 (NanoDrop, Wilmington, USA).

3.5. Identification of nuc, mecA, erm and msrA Genes by PCR

Confirmation of all *S. aureus* isolates was carried out by PCR for amplification of *nuc* gene (279 bp) using primer pair (CinnaGene, Tehran, Iran, *nuc*/F: 5'-GCGATTGATGGTGATACGGTT-3' and *nuc*/R: 5'-AGCCAAGCCTTGACGAACTAAAGC-3') (14). PCRs were performed in a final volume of 25 μL with an automated thermal cycler (Eppendorf mastercycler gradient, Germany) with the PCR cycling conditions which were as follows: initial cycle at 94°C for 5 minute, followed by 37 cycles at 94°C for 1 minute, 55°C for 30 seconds, 72°C for 1 minute and 30 seconds, and final extension cycle at 72°C for 3 minutes and 30 seconds. PCR products were analyzed on 1.5% agarose gel, which was visualized under ultraviolet illumination (Gel documentation, UVP, UK). Negative controls for each used primer contained all the components except template DNA. *Staphylococcus aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were used as positive and negative control strains, respectively.

Staphylococcus aureus isolates were confirmed as MRSA based on the presence of *mecA* gene (310 bp) among our isolates of *S. aureus* using primer pair (CinnaGene, Tehran, Iran, *mecA*/F: 5'- GTAGAAATGACTGAACGTCCGATAA-3' and *mecA*/R: 5'-CCAATTCCACATTGTTTCGGTCTAA-3') (3). PCR cycling conditions were as follows: initial cycle at 94°C for 4 min, followed by 30 cycles at 94°C for 45 seconds, 56°C for 45 seconds, 72°C for 1 minute, and final extension cycle at 72°C for 7 minutes. *Staphylococcus aureus* ATCC 33591 and *S. aureus* ATCC 25923 were used as positive and negative control strains, respectively.

Amplification of *ermA* (139 bp), *ermB* (142 bp), *ermC* (190 bp), and *msrA* (163 bp) genes was carried out according to previous studies (16, 17). Oligonucleotide primers used for PCR were as follows: *ermA*/F: 5'-TATCTTATCGTTGAGAAGGGATT-3', *ermA*/R: 5'-CTACACTTGGCTTAGGATGAAA-3', *ermB*/F: 5'-CTATCTGATTGTTGAAGAAGGATT-3', *ermB*/R: 5'-TTTACTCTTGGTTTAGGATGAAA–3', *ermC*/F: 5'-CTTGTTGATCACGATAATTTCC-3', *ermC*/R: 5'-ATCTTTTAGCAAACCCGTATTC-3', *msrA*/F: 5'-TCCAATCATTGCACAAAATC-3', *msrA*/R: 5'-AATTCCCTCTATTTGGTGGT-3'. PCR conditions were as follows: Initial denaturation at 95°C for 3 minutes; followed by 35 cycles at 95°C for 30 seconds, various annealing temperatures (62.8°C for *ermA*, 59°C for *ermB*, 58°C for *ermC*, and 55°C for *msrA*) for 30 seconds, followed by extension at 72°C for 45 seconds and final extension at 72°C for 7 minutes. *Staphylococcus aureus* isolates with *ermA*, *ermC*, *ermB*, and *msrA* genes and *S. aureus* strains (ATCC 25923 and ATCC 29213) were used as positive and negative control strains, respectively.

3.6. Statistical Analysis

Data were analyzed by Chi-square test using SPSS 22.0 statistical software (SPSS Inc., Chicago, IL). A statistically significant difference was considered as P value < 0.05.

3.7. Ethical Approval

This work was approved by the ethics committee of Tabriz University of Medical Sciences (reference No. 5/4/3978).

4. Results

A total of 215 isolates of *S. aureus* were collected from various clinical specimens and identified by standard tests.

4.1. Molecular Tests

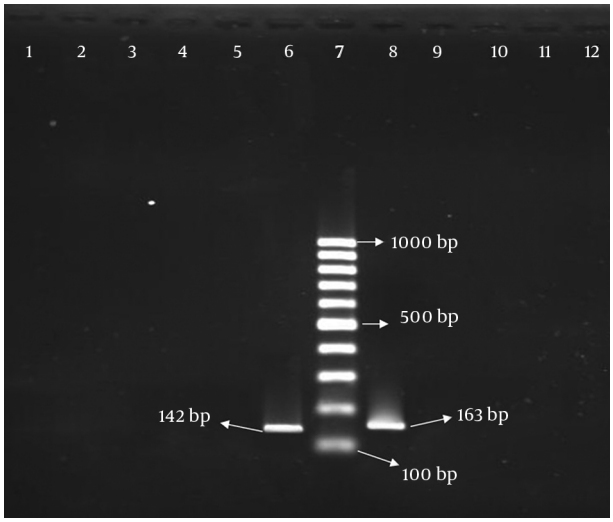
The *nuc* gene (279 bp) was amplified by all of our isolates. PCR for *mecA* gene was performed on *S. aureus* isolates that showed 87 (40.5%) as MRSA and 128 (59.5%) as MSSA. The distribution of MRSA isolates was as follows: 63 (72.41%) from in-patients and 24 (27.59%) from out-patients (P = 0.649), while for MSSA isolates it was 89 (69.53%) from in-patients and 39 (30.47%) from out-patients (P = 0.649).

According to PCR results, 46 (21.40%) and 84 (39.06%) *S. aureus* isolates were positive for presence of *ermA* and *ermC* genes, respectively. Out of 215 isolates, thirty nine (18.14%) showed both *ermA* and *ermC* genes. Although 6 (2.79%) isolates were positive on D test, they were negative for the presence of *erm* genes. All isolates were negative for the presence of *ermB* and *msrA* genes (Figure 1). The distribution of above-mentioned genes among MRSA and MSSA isolates is shown in Table 1.

4.2. Phenotypic Tests

The results of antimicrobial sensitivity test showed that all isolates (100%) were susceptible to linezolid and the majority of them (96.3%) were resistant to penicillin. The results of antibiotic susceptibility testing for other antibiotics are shown in Table 2. The rates of resistance among MRSA and MSSA isolates against studied antibiotics were as follows: penicillin, 100% and 93.75% (P = 0.017); oxacillin,

Figure 1. Separation of 142 and 163 Banding Patterns of *ermB* and *msrA* Genes on 1.5% Agarose Gel



Lanes 1 to 3, isolates without *ermB* gene; lane 4, reagent control; lane 5, *S. aureus* ATCC 25923 as negative control, lane 6, control isolate with *ermB* gene; lane 7, Size marker 1000 bp DNA Ladder; lanes 8, control isolate with *msrA* gene; lane 9, *S. aureus* ATCC 29213 as negative control; lanes 10 to 12, isolates without *msrA* gene.

Table 1. Distribution of *ermA* and *ermC* Genes among D Positive Studied Isolates[a,b]

Genes	MRSA (n = 87)	MSSA (n = 128)	Total isolates (n = 215)
***ermA* alone**	44 (50.57)	2 (1.56)	46 (21.40)
***ermC* alone**	67 (77.01)	17 (13.28)	84 (39.06)
ermA* + *ermC	38 (43.67)	1 (0.78)	39 (18.14)
Without *ermA* and *ermC*	4 (4.60)	2 (1.56)	6 (2.79)

[a] Values are expressed as No. (%).
[b] No *ermB* and *msrA* genes were detected in the present study.

89.7% and 0% (P = 0.000); cefoxitin, 96.6% and 0% (P = 0.000); clindamycin, 88.5% and 15.62% (P = 0.000); erythromycin, 86.2% and 15.62% (P = 0.000); ciprofloxacin, 83.9% and 3.12% (P = 0.000); gentamicin, 83.9% and 0.78% (P = 0.000); trimethoprim-sulfamethoxazole, 55.17% and 1.56% (P = 0.000); rifampin, 39.08% and 0% (P = 0.000), respectively. Antibiotic resistance patterns of 87 MRSA isolates are shown in Table 3. The isolates fell into 10 distinctive antibiotic resistance profiles.

Seventy seven out of 87 MRSA isolates (88.50%) were resistant to more than 3 antimicrobial agents, and thus they were recorded as multidrug-resistant (MDR) (Table 3). The MIC of isolates against vancomycin was in the range of 0.25 μg/mL to 6 μg/mL and the MIC_{50} and MIC_{90} for the isolates were 0.5 and 1 μg/mL, respectively. Only 3 MRSA isolates,

Table 2. Resistance Patterns of *S. aureus* Isolates Against Tested Antibiotics

Antibiotics	S	I	R
Penicillin	8 (3.7)	-	207 (96.3)
Oxacillin	137 (63.7)	-	78 (36.3)
Cefoxitin	131 (60.93)	-	84 (39.07)
Gentamicin	141 (65.6)	-	74 (34.4)
Erythromycin	120 (56)	-	95 (44)
Clindamycin	118 (55)	-	97 (45)
Trimethoprim-sulfamethoxazol	165 (76.7)	-	50 (23.3)
Ciprofloxacin	138 (64.2)	-	77 (35.8)
Rifampin	181 (84.2)	-	34 (15.8)
Linezolid	215 (100)	-	0 (0)

Abbreviations: I, intermediate; R, resistant; S, sensitive.

which showed MICs equal to 6 μg/mL, were recorded as vancomycin intermediate-resistant *S. aureus* (VISA). MRSA isolates were resistant to antibiotics significantly more than MSSA (P = 0.000) except for penicillin, linzeolide, and vancomycin. All MRSA and MSSA isolates were susceptible to linzeolide (P = 0.409) and vancomycin (P = 0.409, except 3 MRSA isolates).

Based on D test results, various phenotypes detected among 215 isolates of *S. aureus* are shown in Table 4. Table 5 depicts the rate of inducible clindamycin resistance (ICR) in MSSA and MRSA isolates. Though this rate was higher in MSSA (11.71%) than MRSA (9.19%) isolates, this difference was not meaningful (P = 0.557). In contrast, the rate of constitutive MLSB resistance was significantly higher in MRSA (79.31%) than MSSA (3.90%) isolates (P = 0.000). MS_B phenotype was not detected in this study.

Our findings revealed that 75 (86.20%) of MRSA isolates were resistant to erythromycin among whom 66 (88%) and 9 (12%) isolates were $cMLS_B$ and $iMLS_B$, respectively. On the other hand, 20 (15.62%) of MSSA isolates were resistant to erythromycin, and $cMLS_B$ and $iMLS_B$ phenotypes were seen in 5 (25%) and 15 (75%) isolates, respectively. Among 152 inpatients and 63 out-patients, ICR phenotype was observed in 14 (9.21%) and 9 (14.28%) isolates, respectively (P = 0.273).

5. Discussion

The emergence of VISA is a great concern and also an alarm for clinicians to give a second thought to the usage of this antibiotic (vancomycin) or finding an alternative treatment such as MLS_B antibiotics (2, 4-6, 8, 9, 17, 18). Unfortunately, misuses of MLS_B antibiotics have led to an unusual increase in the rate of resistance to these antibiotics

Table 3. Antibiotic Resistance Patterns of 87 Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates

Resistance Patterns	No. (Identity of Isolates)	Antibiotic Resistance Patterns	MDR[a] or Non-MDR
R_1	4 (14, 75, 111, 119)	P, OX, FOX	Non-MDR
R_2	2 (132, 135)	P, OX, FOX, GM, CC, SXT	MDR
R_3	39 (2 - 5, 8, 15, 25, 37, 41, 42, 44, 47, 61-63, 91, 98, 107, 114, 117, 118, 123, 124, 136, 140, 151, 152, 154, 156-158, 160, 162, 173, 175, 176-178, 209)	P, OX, FOX, GM, CC, E, CP, SXT	
R_4	26 (10, 11, 13, 16, 19, 22, 28, 40, 48, 57, 76, 83, 96, 97, 105, 153, 155, 159, 161, 174, 181, 182, 201-203, 206)	P, OX, FOX, GM, CC, E, CP, R	MDR
R_5	6 (60, 68, 74, 78, 125, 179)	P, OX, FOX, GM, CC, E, CP, SXT, R	MDR
R_6	1 (192)	P, OX, FOX, CC, E, CP, R	MDR
R_7	1 (7)	P, FOX, CC, E, CP, SXT	MDR
R_8	2 (20, 147)	P, FOX, CC, E	MDR
R_9	3 (109, 115, 150)	P, FOX	Non-MDR
R_{10}	3 (39, 50, 51)	P	Non-MDR

Abbreviations: CC, clindamycin; CP, ciprofloxacin; E, erythromycin; FOX, cefoxitin; GM, gentamicin; OX, oxacillin; P, penicillin; R, rifampin; SXT, trimethoprim-sulfamethoxazole.
[a]Multidrug-resistant (isolates that are resistant to more than 3 antimicrobial agents, 88.50% of the MRSA isolates are MDR).

Table 4. Inducible and Non-Inducible Phenotype of 215 *S. aureus* Isolates Based on D Test[a]

D Test Phenotypes	Resistance Phenotypes	CC Result	E Result	*S. aureus* (215 Isolates)	D Test Description
D	Inducible MLS_B	S	R	13 (6.04)	D shaped clear zone around CC disc proximal to E disc
D^+	Inducible MLS_B	S	R	10 (4.65)	
D^-	MS or Neg.	S	R	0	Clear zone only around CC disc
R	Constitutive MLS_B	R	R	71 (33.02)	Growth upto CC and E discs
HD	Constitutive MLS_B	R	R	3 (1.39)	Two zones of growth around CC disc
S	No resistance	S	S	118 (54.88)	Clear zone around both discs

Abbreviations: CC, clindamycin; D, D zone test, E, erythromycin; HD, hazy D zone; Neg, Negative; R, resistant; S, sensitive.
[a]Values are expressed as No. (%).

Table 5. MLS_B Resistance Phenotypes of *S. aureus*[a]

Isolate Name	Total Isolates	Constitutive MLS_B	Inducible MLS_B	S Phenotype
S. aureus	215	74 (34.42)	23 (10.69)	118 (54.88)
MSSA	128 (59.5)	5 (3.90)	15 (11.71)	108 (84.37)
MRSA	87 (40.5)	69 (79.31)	8 (9.19)	10 (11.49)

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

especially to clindamycin (8, 9). Therefore, it is important for microbiology laboratories to correctly recognize and report whether an *S. aureus* isolate is truly clindamycin susceptible or not. This true result can be obtained by using a simple disk agar diffusion test, described as D-zone test, because this test can exclude inducible clindamycin resistance (8, 9, 11, 12, 17).

In the present study, the prevalence of $iMLS_B$, $cMLS_B$, MS_B, and S phenotypes among all the *S. aureus* isolates was 10.69%, 34.42%, 0%, and 54.88%, respectively. The frequency of ICR was in agreement with previous findings from Iran and India (19-22). However, lower rates of ICR (5.2%, 5.3%, and 8.64%) were reported by other researchers (23-25). In contrast to our finding, the higher rates of ICR have been reported (20.3%, 20.7%, 32.3%, and 33.3%) by other investigators (17, 26-28). Such differences in the ICR pattern could be

due to differences in prescriptions of MLS_B drug groups.

In contrast to many studies, our finding in this study showed that the frequency of inducible resistance phenotype was higher in MSSA (11.71%) than MRSA (9.19%) isolates (P = 0.557) (10, 17, 23-26). Similar to our study, in a study from southeastern of Turkey it was shown that inducible clindamycin resistant strains were more prevalent in MSSA (10%) than MRSA (6.9%), nevertheless, this difference was also not significant (P = 0.434) (29).

In the present study, constitutive clindamycin resistance was seen in 74 (34.42%) *S. aureus* isolates that was comparable with two studies from Iran and India (25, 30). Other researchers have reported either much lower prevalence (12.9%, 16.6%, and 23.3%) or higher rates (36%, 37.5% and 40%) (17, 19, 23, 27, 31, 32). Our finding shows the prevalence of constitutive clindamycin resistance phenotype was 79.31% in MRSA and 3.90% in MSSA isolates (P = 0.000). This predominance has also been reported by most studies (20, 29). The reasons of above undulations among various reports are the MLS_B resistance pattern which varies widely among geographical region, age, source and type of strains, susceptibility to methicillin, and even among medical centers, and as previously mentioned, such differences could be due to differences in the form of drug usage (6, 9, 11, 12).

Hazy D (HD) phenotype was detected in 3 (3.45%) MRSA isolates. This type of resistance must be considered as R phenotype and its rate has been reported rarely in different countries (10). MS_B or negative phenotype was not found in our study and all the erythromycin resistant and clindamycin susceptible isolates showed inducible resistance phenotype. The rates of MS_B phenotype among *S. aureus* isolates have been reported to vary from 5.7% to 44.8% in other countries (19-21, 25-28, 31). These differences in the rates of MS_B phenotype which is related to *msrA* genes emphasize the importance of performing D-test for differentiation of truly clindamycin susceptibility from $iMLS_B$ phenotype and selecting proper therapeutic agent.

In this present study, *ermA* and *ermC* genes were observed in 46 (21.40%) and 84 (39.06%) isolates, respectively, while much higher frequencies have been reported in other Iranian studies (60.3% - 54.8% and 41.1% - 17.7%) (17, 33). Other studies conducted in various parts of the world have shown that *ermA* and *ermC* were responsible for the majority of resistance to erythromycin among *S. aureus* isolates (4, 34, 35). In some studies, *ermA* is predominant, while in the others *ermC* is more prevalent than *ermA* genes (36, 37). In contrast to our finding, in all the above-mentioned studies, the rate of *ermA* gene was more than the rate of *ermC* gene. However, in agreement with our finding, a study carried out in Denmark showed 16% and 84% of *S. aureus* isolates were harboring *ermA* and *ermC*, respectively (37). Spiliopoulou et al. (37) have reported in Greece that *ermC* gene with 70% prevalence is the predominant genetic determinant compared to *ermA* gene with 22%. This predominance is probably due to the spread of distinctive clones (which carry *ermC* gene) in the mentioned countries and our region.

Based on the findings of our study and some other studies, no *ermB* gene has been found in studied isolates of *S. aureus* (17, 33, 38). But in 3 studies conducted in France, Brazil, and Turkey, the frequency of *ermB* gene was 0.7%, 2.2%, and 8.3%, respectively (16, 39, 40). Our finding did not show any *msrA* gene that is similar to the two available studies from Iran (17, 33). However, different low rates of *msrA* gene have been reported by other researchers (16, 38, 40).

A notable finding of the present study was the co-presence of *ermA* and *ermC* in a significant number (39, 18.14%) of our isolates. In other studies, the presence of both genes has been reported in their studied isolates with different rates (16, 17, 33, 38). Six *S. aureus* isolates which had $iMLS_B$ phenotype did not carry any of *ermA* and *ermC* genes, therefore, other genes or factors may have a significant role in resistance to erythromycin. Similar finding has been reported in other studies from Iran and Turkey (16, 17, 33).

5.1. Conclusions

The rate of inducible resistance to clindamycin in *S. aureus* isolates is relatively high in the north west of Iran. Since isolates with inducible resistance may mutate and change to constitutive resistance, for excluding inducible clindamycin resistance, microbiology laboratories must correctly recognize clindamycin susceptibility in *S. aureus* isolates by using D-test. ICR frequency was higher in MSSA than MRSA isolates. Our finding showed *ermC* as the predominant genetic determinant. This predominance is probably due to the spread of distinctive clones (which carry *ermC* gene) in our region.

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Footnotes

Authors' Contribution: Mojtaba Nikbakht and Mohammad Ahangarzadeh Rezaee participated in study concept, design, and administrative, technical and material

support. Mojtaba Nikbakht, Mohammad Ahangarzadeh Rezaee and Javid Sadegi participated in acquisition of data and statistical analysis. Mojtaba Nikbakht, Mohammad Ahangarzadeh Rezaee, Alka Hasani and Mohammad Reza Nahaei participated in drafting and critical revision of the manuscript for important intellectual content. Mojtaba Nikbakht, Mohammad Ahangarzadeh Rezaee, Alka Hasani, Mohammad Reza Nahaei and Javid Sadegi participated in analysis and interpretation of data. Mojtaba Nikbakht carried out all experiments including phenotypic and molecular experiments. All authors read and approved the final manuscript.

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