Detection of Inducible Clindamycin Resistance Genes (*ermA*, *ermB*, and *ermC*) in *Staphylococcus aureus* and *Staphylococcus epidermidis*

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The aim of the present study was to survey the frequency of inducible and constitutive phenotypes and inducible cross-resistant genes by regulating the methylation of 23S rRNA (*ermA*, *ermB*, and *ermC*) and macrolide efflux-related *msrA* gene in *Staphylococcus aureus* and *S. epidermidis* strains. A total of 172 bacterial isolates (identified based on standard tests), were examined in this study. Antibiotic susceptibility was determined by the disk diffusion method, and all isolates were evaluated with respect to inducible and constitutive phenotypes. The presence of *ermA*, *ermB*, *ermC*, and *msrA* genes was investigated by a PCR assay. The constitutive resistance phenotypes showed a higher distribution among the isolates. R phenotype was detected more among *S. epidermidis* isolates (46.25%). *ermB*, *ermC*, and *msrA* genes were detected more in methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) isolates that had R and HD phenotypes (>77% strains). The *ermA* gene had the lowest frequency among MRSA, MRSE, MSSA, and MSSE strains (<14% isolates). Distribution of inducible resistance genes in MRSA and MRSE strains, and possibly other species, leads to increased constitutive resistance to erythromycin, clindamycin, and other similar antibiotics. Therefore, it can be challenging to treat infections caused by these resistant strains.

Keywords: D-phenotypes, inducible resistance, constitutive resistance, *Staphylococcus aureus*, *Staphylococcus epidermidis*

Introduction

Staphylococcus aureus is an important pathogen with a significant infectious potential due to its high pathogenicity and involvement in a broad range of infections. The advent of multidrug resistance in *S. aureus* is one of the most important challenges in health settings worldwide [1]. Macrolides, such as erythromycin, constitute an antibiotic group against infections caused by *S. aureus* and *Staphylococcus epidermidis*. As a result of their expanded use, resistance to erythromycin and other

macrolides has increased in *S. aureus* and *S. epidermidis* strains. Generally, several mechanisms are involved in macrolide resistance of *S. aureus* isolates. The most well-known mechanism in resistance to erythromycin is the reduced affinity of antibiotics for ribosomes due to ribosomal binding site modifications [2].

Clindamycin is used in the treatment of *S. aureus* infections. It is an effective drug for infections caused by *S. aureus* in case of resistance to beta-lactam antibiotics. Clindamycin and erythromycin exhibit good antibacterial activities against infections. In addition, clindamycin shows potential activity against community-acquired methicillin-resistant *S. aureus*. These strains are the most prevalent pathogens in the community [2]. Studies show that resistance to clindamycin in *S. aureus*

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and *S. epidermidis* strains is increasing worldwide.

Staphylococci acquire resistance to macrolides and also clindamycin by two primary mechanisms: macrolide efflux encoded by *msrA* gene and modification of drug binding site on ribosome controlled by erythromycin resistance methylase (*erm*) genes. Methylases reduce binding of erythromycin or in overall MLS_B¹ antibiotics to the target site in the 50S ribosomal subunit. *ermA*, *ermB* and *ermC* are three common genes responsible for the erythromycin resistance in *S. aureus*. The *msrA* gene is an ATP binding cassette transporter in the cell wall of the bacteria that induce resistance in staphylococci by extruding antibiotics to the outside of the cell [3]. The efflux mechanism yields inducible resistance to macrolides (azithromycin, erythromycin, clarithromycin, and roxithromycin) and also type B streptogramins, while it is not active against lincosamides (clindamycin and lincomycin). Inducible resistant includes two kinds of phenotypes; D and D⁺. In D phenotype, the bacteria are resistant to erythromycin and a D-shaped clear zone is formed around the clindamycin disk proximal to the erythromycin disk. In the D⁺ phenotype, in addition to the D-shaped zone around the clindamycin disk, small colonies are seen at the inhibition zone of the clindamycin disk. Ribosomal methylation causes cross-resistance to macrolides, lincosamides, and type B streptogramins. In Staphylococci, *ermA*, *ermB*, or *ermC* genes are responsible for the cross-resistant phenotype by regulation the methylation of 23S rRNA binding region. Methylation process reduces the affinity of macrolides, lincosamides, and type B streptogramins that share the common binding site. Constitutive resistant includes two kinds of phenotypes; HD (hazy D zone) and R (Resistant). In HD phenotype, in addition to the observed bacterial growth at the presence of erythromycin, two zones of growth could be observed around the clindamycin disk. In R phenotype, bacterial colonies are seen in the presence of erythromycin and clindamycin. S (Sensitive) phenotype and Neg (negative) phenotype also can be considered to erythromycin and clindamycin other than inducible and constitutive phenotypes. In S phenotype, the bacteria growth is not seen in the presence of erythromycin and clindamycin. In Neg phenotype, the bacteria is resistant to erythromycin while D shaped

clear zone is not seen around the clindamycin disk proximal to the erythromycin disk [4].

S. epidermidis is most frequently isolated from clinical cultures, especially urine and blood with the use of catheters. *S. epidermidis* accounts for approximately 13% of all cases of prosthetic valve endocarditis and 38% of all cases of cardiac abscess formation. It is also associated with a mortality rate of 24% [5]. Similar to *S. aureus*, *S. epidermidis* resistance to macrolides is mediated by methylases encoded by *erm* genes (modifying the ribosomal target of macrolides) or efflux pumps encoded by *msrA* genes [6].

D-zone test, a disk test incorporating erythromycin and clindamycin disks, is defined by the Clinical and Laboratory Standards Institute (CLSI, 2017) [7]. This test represents the inducible resistance of *Staphylococci*. With this background in mind, in the present study, we aimed to investigate the frequency of clindamycin inducible resistance genes (*ermA*, *ermB*, and *ermC*) in *S. aureus* and *S. epidermidis* strains isolated from various clinical samples.

Materials and Methods

Bacterial strains

All Gram-positive cocci, suspected to belong to the genus *Staphylococcus* were collected from Imam Hospitals of Urmia and Naqadeh in West Azerbaijan from October 2014 to October 2017. All bacterial isolates were identified by using microbiological protocols. For this purpose, samples were cultivated on nutrient agar at 37°C for 24 h. Primary single colonies were subcultured on blood agar plates to obtain pure cultures. The isolated colonies were stored in skim milk (Biomark, India), enriched with glycerol 16% at -20°C for further use. Macroscopic and microscopic characteristics were studied using mannitol salt agar, blood agar, and biochemical tests with coagulase and catalase as standard protocols. In addition, all bacterial isolates were confirmed by amplification of 16S rRNA gene using PCR method. The reference strains of *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were used as the controls [8, 9].

Antibiotic susceptibility testing

Antibiotic susceptibility of the isolates was analyzed based on the CLSI guidelines [7]. The following anti-

1. Macrolide, lincosamide and streptogramin type B

crobial disks (Mast, UK) were used in this study: oxacillin, cefoxitin, tetracycline, clindamycin, rifampicin, erythromycin, linezolid, fusidic acid, gentamicin, and mupirocin. Bacterial suspensions were adjusted at 625 nm to obtain 1.5×10^8 CFU/ml as the reference 0.5 McFarland standard. Optical density was set in the range of 0.08–0.13. Standard strains, including *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228, were used as quality control for antimicrobial susceptibility testing.

D-test

Standard CLSI double-disk diffusion test (D test) was performed using Mueller-Hinton agar (Merck, Germany) with a 15-μg erythromycin disk and a 2-μg clindamycin disk (Merck), placed at a 15-mm distance and incubated for 24 h at 35°C [8]. Standard strains including *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 were used as quality control for antimicrobial susceptibility testing.

Molecular techniques

DNA of isolated bacteria was extracted by CinnaGen kit (Iran), as recommended by the manufacturer. The presence of erythromycin-resistant methylase genes, including *ermA*, *ermB*, and *ermC*, and also *msrA* gene was confirmed in isolates by PCR method. The antibiotic resistance gene primers were selected from previous studies [10], as shown in Table 1.

To perform PCR, 2 μl of each DNA dilution was transferred directly to a 25-μl PCR mixture, containing 23 μl of Master Mix and 2 μl of each primer (Bioneer Co.,

Korea). The PCR mixtures were subjected to thermal cycling in a thermocycler (Bioer Co., China). The protocol for *ermA*, *ermB*, and *ermC* genes was as follows: denaturation for four minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 60 sec, extension at 72°C for 45 sec, and final extension for seven minutes at 72°C. This protocol was also used for the 16S rRNA gene. The annealing temperature for 16S rRNA was 54°C. The protocol for PCR of *msrA* gene was as follow: primary denaturation for five minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 52°C for 60 sec, extension at 72°C for 45 sec, and final extension for seven minutes at 72°C.

Results

Bacterial prevalence in clinical specimens

Out of 172 isolates, 92 (53.5%) and 80 (46.5%) were identified as *S. aureus* and *S. epidermidis*, respectively. Distribution of positive clinical specimens for *S. aureus* was as follows: urine (80.4%), blood (7.6%), wound (6.5%), sputum (2.2%), catheter (0%), stretcher (0%), and thermometer (1.1%). *S. aureus* was isolated at a high rate from urine samples (80.4%), whereas it was not detected in catheter or patient's bed samples (0%). In addition, *S. epidermidis* was isolated from 77.5% of urine specimens, while it was not identified in thermometer or wound samples (0%). Distribution of bacterial strains in the clinical specimens is shown in Table 2.

Table 1. Information for primers used in this study.

Primer	Primer sequence (5'-3')	Product size (bp)	Annealing temperature	Reference
16S rRNA-F	GGA GGA AGG TGG GGA TGA CG	245	60	[11]
16S rRNA-R	ATG GTG TGA CCG GCG GTG TG			
<i>ermA</i> -F	TAT CTT ATC GTT GAG AAG GGA TT	139	54	[11]
<i>ermA</i> -R	CTA CAC TTG GCT TAG GAT GAA A			
<i>ermB</i> -F	CTA TCT GAT TGT TGA AGA AGG ATT	142	54	[11]
<i>ermB</i> -R	GTT TAC TCT TGG TTT AGG ATG AAA			
<i>ermC</i> -F	AAT CGT CAA TTC CTG CAT GT	299	54	[12]
<i>ermC</i> -R	TAA TCG TGG AAT ACG GGT TTG			
<i>msrA</i> -F	TCC AAT CAT TGC ACA AAA TC	162	54	[13]
<i>msrA</i> -R	CAA TTC CCT CTA TTT GGT GGT			

Table 2. The isolation frequency of *S. aureus* and *S. epidermidis* in different clinical specimens.

Type of specimen	<i>S. aureus</i>	<i>S. epidermidis</i>
Urine	74 (80.4%)	62 (77.5%)
Blood	7 (7.6%)	13 (16.25%)
Wound discharge	6 (6.5%)	-
Sputum	2 (2.2%)	2 (2.2%)
Pleural fluids	2 (2.2%)	1 (1.1%)
Thermometer	1 (1.1%)	-
Catheter	-	1 (1.1%)
Patient's bed	-	1 (1.1%)
Total	92 (53.3%)	80 (46.5%)

Antibiotic susceptibility testing

Resistance to ceftioxin (79.3%) was the highest in *S. aureus* strains, followed by resistance to oxacillin and erythromycin (ca. 69.6% and 67.4%, respectively). Linezolid showed potent antibacterial activity against *S. aureus* with a resistance rate of 14.1%. Following linezolid, the rate of resistance to rifampicin in *S. aureus* strains was 26.1%. Fourteen (15.2%) *S. aureus* strains were showed intermediate resistance to clindamycin. Overall, 50%, 18.5%, and 31.5% of the isolates had sensitive, intermediate, and resistant antibiogram profiles against mupirocin, respectively.

S. epidermidis strains showed high resistance to ceftioxin (83.8%). The lowest resistance of *S. epidermidis* was reported against linezolid (11.2%). The rate of resistance to oxacillin in *S. epidermidis* strains was also

found to be high. 17.5 percent of *S. epidermidis* strains showed an intermediate profile to erythromycin (the highest level), while, 1.2 percent of them had an intermediate profile to tetracycline (the lowest level). The antimicrobial resistance profile of *S. aureus* and *S. epidermidis* is shown in Table 3.

D-test

The results of D-test showed that 101 (58.7%) isolates (*S. aureus* and *S. epidermidis*) were positive for at least one out of four phenotypes (D, D⁺, HD, and R) (Fig. 1). As Fig. 2 indicates, distribution of D, D⁺, HD, and R phenotypes among *S. aureus* strains was detected respectively as 2.17%, 10.8%, 2.17%, and 35.8%. Prevalence of D, D⁺, HD, and R phenotypes among *S. epidermidis* strains was evaluated respectively as 8.75%, 8.75%, 3.75%, and 46.25%. Overall, 26 *S. aureus* and *S. epidermidis* isolates showed inducible resistance phenotypes (D = 9 and D⁺ = 17), and 75 isolates were found to have constitutive resistance phenotypes (HD = 5 and R = 70) (Table 4).

At least one type of *erm* genes (*ermA*, *ermB*, or *ermC*) was detected in all *S. aureus* isolates and 93.75% of *S. epidermidis* isolates. The *ermB* and *ermC* genes were the most frequent genes among *S. aureus* (44.6% and 43.5%, respectively) and *S. epidermidis* (38.8% and 43.8%, respectively) strains, while *ermA* was detected in a few *S. aureus* (13%) and *S. epidermidis* (11.3%) strains. In addition, *ermB* and *ermC* genes were found to have a high frequency in MRSA (57.1% and 53.1%, respectively) and methicillin-resistant *S. epidermidis*

Table 3. Antibiogram profile of *S. aureus* and *S. epidermidis*.

Antibiotic	Concentration µg/disc	<i>S. aureus</i>			<i>S. epidermidis</i>		
		Resistant n (%)	Intermediate n (%)	Sensitive n (%)	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Oxacillin	1	64 (69.6)	6 (6.5)	22 (23.9)	59 (73.8)	5 (6.2)	16 (20)
Ceftioxin	30	73 (79.3)	-	19 (20.7)	67 (83.8)	-	13 (16.2)
Clindamycin	2	42 (45.7)	14 (15.2)	36 (39.1)	37 (46.2)	6 (7.5)	37 (46.2)
Erythromycin	15	62 (67.4)	20 (21.7)	10 (10.9)	58 (72.5)	14 (17.5)	8 (10)
Linezolid	30	13 (14.1)	-	79 (85.9)	9 (11.2)	-	71 (88.8)
Fusidic acid	10	34 (37)	8 (8.7)	50 (54.3)	36 (45)	4 (5)	40 (50)
Tetracyclin	30	58 (63)	2 (2.2)	32 (34.8)	53 (66.2)	1 (1.2)	26 (32.5)
Gentamicin	10	47 (51.1)	4 (4.3)	41 (44.6)	43 (53.8)	-	37 (46.2)
Rifampicin	5	24 (26.1)	1 (1.1)	67 (72.8)	19 (23.8)	5 (6.2)	56 (70)
Mupirocin	20	29 (31.5)	17 (18.5)	46 (50)	30 (37.5)	6 (7.5)	44 (55)

Fig. 1. Different inducible and constitutive phenotypes on Muller Hinton agar plate. CLI: clindamycin, E: erythromycin. 1; In D phenotype, the bacteria is resistant to erythromycin and a D shaped clear zone was seen around the clindamycin disk proximal to the erythromycin disk. 2; In D+ phenotype, in addition to the observed D shaped zone around the clindamycin disk, small colonies were grown at the inhibition zone of the clindamycin disk. 3; In HD (hazy D zone) phenotype, in addition to the observed bacterial growth at the presence of erythromycin, two zones of growth could be observed around the clindamycin disk. 4; In R (Resistant) phenotype, bacteria growth was seen in the presence of erythromycin and clindamycin. 5; In S (Sensitive) phenotype, bacteria growth was not seen in the presence of erythromycin and clindamycin. 6; In Neg (negative) phenotype, the bacteria is resistant to erythromycin while D shaped clear zone was not seen around the clindamycin disk proximal to the erythromycin disk.

Fig. 2. Frequency of inducible and constitutive phenotypes among *S. aureus* and *S. epidermidis* strains. R: resistant, HD: Hazy D zone. A high percent of *S. epidermidis* and *S. aureus* isolates showed R phenotype as a constitutive resistance.

(MRSE) (50% and 53.8%, respectively) strains (Table 5).

The frequency of *ermA*, *ermB*, and *ermC* genes among *S. aureus* and *S. epidermidis* strains which had at least one out of four D-phenotypes was 22.2%, 55.6%, and 33.3%, respectively. The corresponding frequencies of these genes in D⁺-positive strains were 5.9%, 41.2%, and 76.5%, respectively. All HD-positive strains (100%) concurrently possessed both *ermB* and *ermC* genes.

Overall, 20% of HD-positive strains contained *ermA* genes. All HD-positive strains (100%) were also positive for *msrA* gene. The frequency of *ermA*, *ermB* and *ermC* genes in R-positive strains was 22.9%, 78.6%, and 77.1%, respectively (Figs. 3 and 4). Among D-negative strains, only one strain was positive for *ermA* gene. Also, 7% of these strains were positive for *msrA* gene.

Table 4. Distribution of inducible and constitutive resistance among *S. aureus* and *S. epidermidis* strains.

D-phenotypes	Resistance phenotype	ERY Result	CLI Result	<i>S. aureus</i> 92 (53.49%)		<i>S. epidermidis</i> 80 (46.5%)	
				MRSA 49 (53.3)	MSSA 43 (46.7)	MRSE 52 (65)	MSSE 28 (35)
D zone ^a	Inducible Resistance	R	S	1 (8.3)	1 (2.9)	3 (14.3)	4 (12.1)
D ⁺ zone ^b	Inducible Resistance	R	S	5 (41.7)	5 (14.3)	5 (23.8)	2 (6.1)
HD zone ^c	Constitutive Resistance	R	R	0	2 (5.7)	0	3 (9.1)
R zone ^d	Constitutive Resistance	R	R	6 (50)	27 (77.1)	13 (61.9)	24 (72.7)
Total				12 (42.9)	35 (67.3)	21 (48.8)	33 (67.3)

ERY; Erythromycin, CLI; Clindamycin; a; in D zone phenotype, the isolates are resistant to Erythromycin while sensitive to Clindamycin. b; in D⁺ zone phenotype, the isolates also are resistant to Erythromycin while sensitive to Clindamycin. c; in HD zone phenotype, the isolates are resistant to both Erythromycin and Clindamycin. d; in R zone phenotype, the isolates also are resistant to both Erythromycin and Clindamycin.

Table 5. Frequency of *ermA*, *ermB*, *ermC* and *msrA* genes among *S. aureus* and *S. epidermidis* isolates.

	<i>S. aureus</i> N (%)	<i>S. epidermidis</i> N (%)	MRSA N (%)	MSSA N (%)	MRSE N (%)	MSSE N (%)
<i>ermA</i>	12 (13)	9 (11.3)	7 (14.3)	5 (11.6)	7 (13.5)	2 (7.1)
<i>ermB</i>	41 (44.6)	31 (38.8)	28 (57.1)	13 (30.2)	26 (50)	5 (17.9)
<i>ermC</i>	40 (43.5)	35 (43.8)	26 (53.1)	14 (32.6)	28 (53.8)	7 (25)
<i>msrA</i>	43 (46.7)	40 (50)	29 (59.2)	14 (32.6)	31 (59.6)	9 (32.1)

MRSA; Methicillin-resistant *Staphylococcus aureus*, MSSA; Methicillin-Sensitive *Staphylococcus aureus*, MRSE; Methicillin-Resistant *Staphylococcus epidermidis*, MSSE; Methicillin-Sensitive *Staphylococcus epidermidis*.

Genes sequence analysis

One example of all genes includes 16S rRNA, *msrA*,

ermA, *ermB*, and *ermC* were sequenced. Some sequence information of these genes was shown as Table 6.

Fig. 3. Distribution of *erm* and *msrA* genes among the isolates of *S. aureus* and *S. epidermidis* with inducible and constitutive resistant. As the figure shows, the frequency of *ermB*, *ermC*, and *msrA* genes in HD and R phenotypes in *S. aureus* and *S. epidermidis* strains showed a high percentage than the prevalence of these genes in D and D⁺ phenotypes. The percentage of the *ermA* gene is approximately similar in D, HD, and R phenotypes.

Fig. 4. Agarose gel electrophoresis the PCR product of investigated genes in clinical strains of *S. aureus* and *S. epidermidis*. A; Lane 1: *ermA* positive isolate, Lane 2: *ermC* positive isolate, Lane 3: *ermB* positive isolate, Lane 4: *msrA* positive isolate, Lane 5: 16S rRNA gene of *S. aureus*, Lane 6: 16S rRNA gene of *S. epidermidis*, Lane 7: *erm* negative isolate, Lane 8: PCR negative control. M: Size marker 100 bp. B to E; distinctive *erm* genes in clinical strains of *S. aureus* and *S. epidermidis*. F; *msrA* gene in clinical strains of *S. aureus* and *S. epidermidis*. S.a; *Staphylococcus aureus*, S.e; *Staphylococcus epidermidis*.

Table 6. Genes sequence analysis.

Gene name	Direction	Size (bp)	GC%	ORF Number
<i>ermA</i>	F	115	34.8	2
	R	115	37.4	2
<i>ermB</i>	F	118	44.1	2
	R	121	44.6	1
<i>ermC</i>	F	261	26.1	1
	R	278	25.2	1
<i>msrA</i>	F	128	36.7	2
	R	140	35.7	3
16S rRNA	F	222	47.3	2
	R	222	47.7	2

F; forward, R; reverse, bp; base pair, GC; guanine + cytosine, ORF; Open Reading Frames.

Discussion

In this study, *S. aureus* was isolated from urine samples at a high rate (80.4%), while it was not detected in catheter or stretcher samples (0%). *S. epidermidis* was also isolated at a high rate from urine (77.5%), but it was not found in thermometer or wound samples (0%). The frequency percentage of urinary *S. aureus* and *S. epidermidis* isolates was significantly different in comparison with other clinical samples. The present findings are not consistent with the results reported by Adhikari *et al.* from Nepal (2017), as they found that *S. aureus* was isolated from pus specimens at a high rate (54.4%), while *S. aureus* was isolated from 7.4% of urine specimens [11]. Moreover, Khashei *et al.* in a study from Iran

(2018) reported that 46.5% of *S. aureus* and *S. epidermidis* strains isolated from the specimens were related to bloodstream infections, respectively. In the study by Khashei *et al.*, the prevalence of *S. aureus* and *S. epidermidis* strains in urine specimens was nearly 15.1% [12]. Presumably, the high rate of urinary isolates of *S. aureus* and *S. epidermidis* in our study is related to the high numbers of urinary samples rather than the other samples.

According to the results of disk diffusion test, resistance of *S. aureus* and *S. epidermidis* bacteria to beta-lactam antibiotics (oxacillin and cefoxitin) was the highest, while linezolid showed the greatest antibacterial activity against *S. aureus* and *S. epidermidis*. The increased antimicrobial resistance of *Staphylococcus* strains to beta-lactams may be attributed to the expansion of resistance genes in *S. aureus* and *S. epidermidis* strains. In this regard, Connor *et al.* (2015) have reported that <1% of *S. aureus* and 2% of coagulase-negative *Staphylococcus* spp. (CoNS) are resistant to linezolid [13]. In another study by Yoo (2020) in Korea, the resistance of *S. aureus* was 14.8% [14]. The low rate of resistance to linezolid among *S. aureus* and *S. epidermidis* isolates is probably related to the low frequency of mutations in the binding site of ribosome 50s subunit [15]. Also, an RNA methyl transferase gene (*cfr* gene) has been recently described in both MRSA and coagulase-negative *Staphylococci* isolates in the United States and other countries [16].

The results of D-test revealed that R-phenotype was the most common in both *S. aureus* and *S. epidermidis* strains, while HD type had the lowest rate.

Phenotypic conversion from D to D⁺ and from HD to R increased, while conversion from D⁺ to HD decreased among *S. aureus* and *S. epidermidis* strains; this finding shows that some *S. aureus* and *S. epidermidis* strains do not tend to convert from induced resistance to constitutive resistance (Fig. 2). Our results are in agreement with a report by Majhi *et al.* [17]. Moreover, Ejikeugwu *et al.* reported variations in induced and constitutive resistance phenotypes among strains isolated from different locations [18]. In our study, *Staphylococcus* strains (*S. aureus* and *S. epidermidis*) with R phenotypes constituted the majority of strains. This may be a source of concern in health centers as these strains acquire and express individual resistance genes involving clindamycin and erythromycin.

Based on the PCR results, *erm* (*ermB* and *ermC*) and *msrA* genes were detected in 100% of *S. aureus* and *S. epidermidis* strains with HD phenotypes and constitutive resistance. Nearly 77–78% of strains with R phenotypes exhibited *erm* genes (*ermB* and *ermC*) and *msrA* gene (Fig. 3). The number of *S. aureus* and *S. epidermidis* strains with D and D⁺ phenotypes (inducible resistance), as well as *erm* (*ermB* and *ermC*) and *msrA* genes, was lower than the number of strains with HD and R phenotypes. In all inducible and constitutive phenotypes (D, D⁺, HD and R), *ermA* gene was detected in only a few strains of *S. aureus* and *S. epidermidis* (< 25%). Also, *ermB* gene was the most frequent gene among MRSA strains, followed by *ermC* gene.

In MRSE strains, *ermC* gene was dominant, followed by *ermB* gene. Conversely, *ermA* gene had the lowest frequency among MRSA and MRSE strains. The *msrA* gene showed a frequency rate of >65% among all D-phenotypes of *S. aureus* and *S. epidermidis* strains.

Additionally, in a study by Jarajreh *et al.*, the frequency of *ermC*, *ermB*, and *ermA* genes among *S. aureus* strains was reported to be 82.4%, 58.8%, and 29.4%, respectively [19]. In a study by Paz Pereira from Brazil (2016), *ermA* and *ermC* genes were found individually in 9.1% and 38.6% of isolates with MLS_B resistance phenotypes of *Staphylococcus* strains, respectively [20]. In another study by Talebi *et al.* from Iran (2019), the frequency of *ermC*, *ermB*, and *ermA* genes in *S. aureus* strains was 10%, 7.5%, and 5%, respectively. In the study by Talebi *et al.*, the frequency of *ermC* gene strains was the highest among resistant strains of *S. aureus*; this finding is in disagreement with our results [10]. In previous studies, the frequency distribution of *ermC*, *ermB*, and *ermA* genes in relation to D-phenotypes has not been investigated; therefore, it is recommended to study the distribution of D-phenotype-positive strains of *Staphylococcus* in different parts of the world. In conclusion, based on the findings, phenotypic conversion increased from D to D⁺ and from HD to R, whereas conversion from D⁺ to HD decreased in *S. aureus* and *S. epidermidis* strains. It seems that, some *S. aureus* and *S. epidermidis* strains did not tend to convert from induced resistance to constitutive resistance. The *ermA* gene showed the lowest prevalence (<25%) among all inducible and constitutive phenotypes of *S. aureus* and *S. epidermidis* (D, D⁺, HD, and R). Conversely, *ermB* and *ermC* genes were found to

be the most frequent genes among MRSA and MRSE strains, respectively. Spread and distribution of inducible resistance genes among *S. aureus* and *S. epidermidis* strains can lead to an increase in constitutive resistance to erythromycin, clindamycin, and other similar antibiotics; therefore, treatment of infections can be challenging due to the presence of these resistant strains. We suggest both molecular and phenotypic methods as routine procedures can be used to detect inducible resistance in *S. aureus* and *S. epidermidis* strains in clinical microbiology laboratories.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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