

## Prevalence of *ermB* Gene among Clinical Isolates of *Staphylococcus aureus* in Shahrekord, Iran

Kourosh Naderi, M.sc. <sup>1</sup>, Fahimeh Ghanbari, Ph.D. <sup>2</sup>, Farzad Khademi, Ph.D. <sup>3</sup>, Behnam Zamanzad, Ph.D. <sup>4</sup>

1- Department of Microbiology, School of Medicine, Shahrekord University of Medical Sciences, Sharekord, Iran

2- Ph.D. student, Department of Genetic, School of Medicine, Shahid Sadooghi University of Medical Sciences, Yazd, Iran

3- Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

4- Department of Microbiology, School of Medicine, Shahrekord University of Medical Sciences, Sharekord, Iran (Corresponding author; microbial\_sci2013@yahoo.com)

Received: 28 August, 2017

Accepted: 15 February, 2018

### ARTICLE INFO

#### Article type:

Original article

#### Keywords:

*Staphylococcus aureus*

D-test

*Ern* gene

Macrolide-lincosamid-streptogramin

### Abstract

**Background:** Development of drug resistance to *Staphylococcus aureus* (*S. aureus*) has led to the use of older antibiotics such as macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) for the treatment of infections. MLS<sub>B</sub> resistance can be caused by several mechanisms, however, one of the predominant reasons is target modification mediated by *erm* genes. The objective of this study is to determine the prevalence of *erm* genes and the frequency of constitutive MLS<sub>B</sub> (cMLS<sub>B</sub>), inducible MLS<sub>B</sub> (iMLS<sub>B</sub>), and MS phenotypes using D-test and polymerase chain reaction (PCR) methods.

**Methods:** D-test was performed on 110 clinical specimens of *S. aureus* collected from Kashani and Hajar Hospitals in Shahrkord from October 2014 to May 2015. After sampling, DNA extraction was performed by simple boiling method and, in order to detect *erm* genes, multiplex PCR was carried out on erythromycin resistant isolates using specific primers.

**Results:** The result of this study revealed that among 110 *S. aureus* isolates examined, 35 (31.8%) were MRSA and frequency of cMLS<sub>B</sub>, iMLS<sub>B</sub>, and MS resistant phenotypes were 22 (20%), 9 (8.2%), and 2 (1.8%), respectively. The genes *ermA*, *ermB*, and *ermC* were detected in 27 (24.5%), 28 (25.4%), and 26 (23.6%) isolates.

**Conclusion:** This study demonstrated that cMLS<sub>B</sub> was the most common phenotype among isolated *S. aureus*. Moreover, another interesting point to notice in our study was the high frequency of the *ermB* gene in iMLS<sub>B</sub> resistant phenotypes.

### Introduction

*S. aureus* is the leading causes of opportunistic and device-related infections and is responsible for a wide spectrum of human and animal diseases ranging from mild folliculitis to potentially fatal systemic illnesses, such as endocarditis and bacteremia (1-3). The prevalence of antibiotic-resistant bacteria in hospitals has strikingly increased in the past decades, leading to an increase in costs and the probability of problematic

treatment of infections caused by this microorganism (4-6). Development of drug resistance of *S. aureus* has led to the use of older antibiotics such as macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) antibiotics (7). Clindamycin (a lincosamide) and erythromycin (a macrolide) represent two distinct classes of antimicrobial agents that cause protein synthesis inhibition by binding to 50S ribosomal subunits of bacterial cells (8). Resistance to both of these antibiotics

occurs through methylation of their ribosomal target site. Clindamycin and erythromycin resistance are typically mediated by *erm* genes (*ermA*, *ermB*, *ermC*, or *ermF*) that codes 23s rRNA methylase (7, 9). Clindamycin is often used as an antimicrobial therapeutic drug for the treatment of skin and soft tissue infections caused by *S. aureus* strains (10). Clindamycin resistance in *S. aureus* can be either constitutive or inducible. *S. aureus* isolates with inducible resistance are resistant to erythromycin but seem susceptible to clindamycin. In this position, treatments with clindamycin may be selective for constitutive *erm* mutants, which can lead to clinical treatment failure inducible resistance could not be detected by the usual antimicrobial susceptibility tests (11). The Clinical and Laboratory Standards Institute (CLSI) recommends a test for inducible clindamycin resistance of *staphylococci* isolates through using a D-zone test, which is an important test for optimal treatment of patients (12, 13). The current study was aimed to investigate the antibiotic susceptibility profile and the prevalence of the *ermA*, *ermB*, and *ermC* genes among *S. aureus* strains isolated from different clinical samples in Shahrekord.

## Materials and Methods

### Bacterial strains

This study was conducted from October 2014 to May 2015 at Kashani and Hajar Hospitals, Shahrekord, Iran. During the study, 110 clinical isolates of *S. aureus*

were collected from various clinical specimens which were included wound, blood, urine, sputum and etc. Early identification was performed based on Gram-staining and biochemical tests such as catalase, coagulase, and DNase tests (14).

### Phenotypic antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out using disk diffusion method (MAST Diagnostic, UK) according to guidelines of Clinical Laboratory Standards Institute (CLSI-2012) (15). The list of the antibiotics used in the test is presented in Table 1. Strain of *S. aureus* ATCC 25923 was used as control. D-test method was performed according to CLSI using clindamycin (2 µg) and erythromycin (15µg) disks. For this purpose, suspensions of bacteria were prepared in the sterile saline (2 ml), equivalent to standard 0.5 McFarland. After culturing the bacteria, two antibiotic disks were placed on Muller-Hinton agar media in 15 mm distance (edge-to-edge) and then the plates were incubated at 35°C overnight. Strains with flat zones of growth inhibition of clindamycin near the erythromycin disk (D-shape) were classified as resistant phenotypes to iMLS<sub>B</sub> (D-test positive), while those with a circular zone were classified as MS resistant phenotypes (D-test negative). Methicillin-resistant *S. aureus* (MRSA) strains were identified by assessment of ceftioxin susceptibility (30 µg) disk (Mast, UK) using disc diffusion method according to CLSI.

**Table 1.** Antimicrobial-susceptibility for *S.aureus* strains

Antibiotics	Susceptibility of isolates					
	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Penicillin (10 U)	13	11.81	0	0	97	88.2
Erythromycin (15 µg)	75	68.2	2	1.8	33	30
Gentamicin (10 µg)	91	82.7	1	0.9	18	16.4
Clindamycin (2 µg)	81	73.6	5	4.5	24	21.8
Tetracycline (30 µg)	62	21.8	5	35/4	83	55/3
Quinupristin/dalfopristin	110	100	0	0	0	0
Cephalexin (30 µg)	78	70.9	1	0.9	31	28.2
Cefoxitin (30 µg)	75	68.2	0	0	35	31.8

### DNA extraction

DNA was extracted from 110 *S. aureus* isolates using simple boiling Method. 50 mg of bacterial biomass was suspended in 400 µl of TES (50 mM Tris hydrochloride [pH 8.0], 5 mM EDTA, 50 mM NaCl), and the suspension was heated at 95°C for 7 min and centrifuged at 10000 g for 10 min. The resulting supernatant was taken as DNA lysate and kept at -20°C for the polymerase chain reaction (PCR).

### Molecular Detection of *mecA* Gene

PCR was performed for the detection of *mecA* gene using primers displayed in Table 2. PCR conditions for detection of *mecA* were as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (72°C, 30 s), and a final elongation at 72°C for 2 min.

**Table 2.** Primers used in this study

Target	Primer	Sequence	Product size (bp)	Reference
<i>mecA</i>	F	AAAATCGATGGTAAAGGTTGGC	583	(35)
	R	AGTTCTGCAGTACCGGATTTG		
<i>ermA</i>	F	TATCTTATCGTTGAGAAGGGATT	139	(36)
	R	CTACACTTGGCTTAGGATGAAA		
<i>ermB</i>	F	CTATCTGATTGTTGAAGAAGGATT	142	(36)
	R	GTTTACTCTTGGTTTAGGATGAAA		
<i>ermC</i>	F	AATCGTCAATTCCTGCATGT	297	(36)
	R	TAATCGTGAATACGGGTTTG		

### Multiplex-PCR for erm genes

Multiplex PCR was performed to detect *erm* genes in erythromycin resistance isolates using specific primers for the *ermA*, *B*, and *C* genes as exhibited in Table 2. Each PCR was performed in a final volume of 25  $\mu$ L consisting 5  $\mu$ L of DNA template, 2.5  $\mu$ L of PCR buffer ( $\times 10$ ), 1  $\mu$ L  $MgCl_2$  (50 mM), 0.5  $\mu$ L of dNTPs (10 mM), 0.5  $\mu$ L of each primer (2  $\mu$ L totally), 0.25  $\mu$ L of Ex-Taq DNA polymerase (5 u/ $\mu$ L), and 11.25  $\mu$ L distilled water. DNA was amplified on a thermocycler (Ependorf-Germany), and PCR conditions were as follows: initial denaturation at 94 °C for 10 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 60 s followed by a final extension at 72 °C for 5 min. The PCR products were electrophoresed on a 2% agarose gel (Sina gene, Iran) with TBE buffer and subsequently stained with DNA Green Viewer (Pars Toos, Iran) to see the amplified DNA fragments under gel documentation system (Trans Illuminator, Germany).

### Statistical analysis

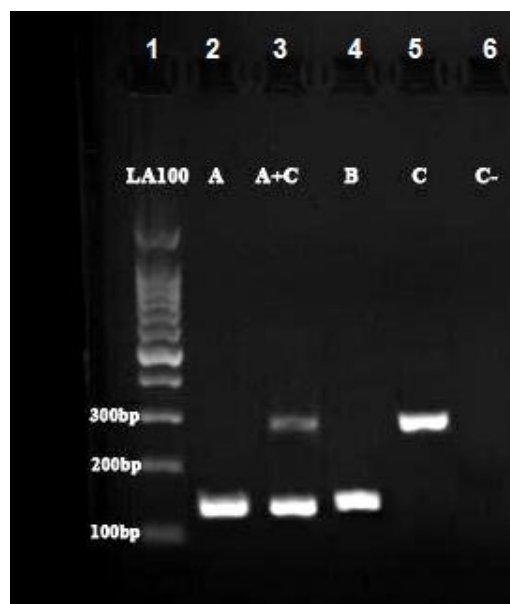
The data was analyzed by Chi-square test, using (SPSS) version v.15, and  $p < 0.05$  was considered as significant.

### Results

In this study, 110 isolates of *S. aureus* were collected from various clinical specimens, wound with 53 isolates (48.2%), blood with 23 (20.9%), sterile body fluids with 28 (25.4%), abscess with 2 (1.8%) and others with 4 cases (3.64%). Among the 110 *S. aureus* isolates, 64 (58.2%) were recovered from male patients and 46

(41.8%) from females. The patient's average age was 41.9 years. The antibiotic resistance profile for all isolates to macrolides as well as other tested antibiotics is listed in Table 1. The majority of isolates expressed resistance to penicillin (97%). However, no resistance to Quinupristin-Dalfopristin was noted. 31.8% of isolates were resistant to ceftiofloxacin. In disk diffusion testing, 33 (30%) strains showed resistance to erythromycin and were selected for further study. Furthermore, double disk diffusion test results revealed that 69 (62.7%) of the isolates were susceptible to both clindamycin and erythromycin and the prevalence of cMLS<sub>B</sub>, iMLS<sub>B</sub> and MS resistance phenotypes were 20%, 8.18%, and 1.8%, respectively. Finally, 2 (1.8%) isolates were susceptible to erythromycin and resistant to clindamycin. Among nine isolates with iMLS<sub>B</sub> resistance phenotype, three isolates were MRSA. Thirty-three *S. aureus* isolates with erythromycin resistance phenotype were tested for the presence of the *erm* genes. In PCR testing, 5 (15.1%), 1 (3%) and 1 (3%) strains showed *ermB*, *ermA* and *ermC* genes amplification, respectively; 26 strains (78.7%) contained 2 or 3 of the studied *erm* genes. Both (*ermA* and *ermC*) genes were co-presented in 3 (9.1%) strains, (*ermA* and *ermB*) genes were in 1 (3%) strain and (*ermA*, *B* and *C* genes) were in 22 (66.7%) strains (Figure 1). Table 3 and 4 show the difference in MLS<sub>B</sub> resistance phenotypes in relation to the presence of *ermA* and *ermC* genes. Most strains with the cMLS<sub>B</sub> resistance phenotype, 18 (81.8%), were MRSA. In addition, among 9 strains with iMLS<sub>B</sub> resistance phenotypes, 3 strains were MRSA, while all strains with the MS resistance phenotype were methicillin-

susceptible *S. aureus* (MSSA). Of 35 MRSA strains, 1 strain contained the *ermB* gene, while in 75 MSSA strains 4 strains contained this gene. Furthermore, only 1 MSSA strain contained *ermC* gene. Prevalence of the *ermA* gene in MRSA and MSSA strains was 0 and 1, respectively. *ErmA*, *B* and *C* genes were co-presented in 18 of MRSA strains and 4 of the MSSA strains. Therefore, *ermA*, *B* and *C* genes were more common in MRSA erythromycin-resistant strains than the MSSA strains.



**Figure 1.** Gel electrophoresis of *erm* genes. Lane 1: ladder, Lane 2: *ermA* positive (139 bp), Lane 3 : *ermA+C* positive (139+297 bp), Lane 4: *ermB* positive (142 bp), Lane 5: *ermC* (297bp).

**Table 3.** Distribution of *erm* genes (*ermA*, *B* and *C*) based on resistance to methicillin among *S. aureus* isolates

Gene	MRSA (n=)	MSSA (n=)	Total strains (n=33)
<i>ermA</i>	0	1	1(3%)
<i>ermC</i>	0	1	1(3%)
<i>ermB</i>	1	4	5(15.1%)
<i>ermA + C</i>	1	2	3(9.1%)
<i>ermA + B</i>	1	0	1(3%)
<i>ermB + C</i>	0	0	0(0%)
<i>ermA + C+B</i>	18	4	22 (66.7%)

**Table 4.** Prevalence of *ermA*, *B* and *C* genes based on phenotypic test (D test)

<i>ermA+B+C</i>	<i>ermB+C</i>	<i>ermA+C</i>	Genotypes				<i>ermA</i>	Gene	D <sup>+</sup>
			<i>ermA+B</i>	<i>ermC</i>	<i>ermB</i>				
22	0	3	1	1	5	1	NO		
66.7%	0%	9.1%	3%	3%	15.1%	3%	%		
3	0	1	1	0	3	1	NO		
33.3%	0%	11.1%	11.1%	0%	33.3%	11.1%	%		
				C	B	A	Total		
				26	28	27	NO		
				32.1%	34.5%	33.3%	%		

## Discussion

*S. aureus* is responsible for an increasing number of serious hospital and community acquired infections. Recent studies have documented that *S. aureus* has become resistant to most available antibiotics. Additionally, antimicrobial resistance of *S. aureus* infections has changed, which in turn led to renewed interest in the use of MLS<sub>B</sub> antibiotics, particularly clindamycin, for the treatment of these infections (16, 17). However, widespread use of these antibiotics without performing the D-test can lead to the emergence of resistant mutants to cMLS<sub>B</sub> from iMLS<sub>B</sub>-resistant strains, which in turn results in failure of clindamycin therapy. Therefore, it is important for laboratories to declare the local prevalence of iMLS<sub>B</sub> isolates to ensure the determination of false susceptibility results of clindamycin and also to make accurate decision regarding the treatment based on the application of D-test (17-19). In the current study, we investigated the antibiotic resistance of *S. aureus* to penicillin, erythromycin, cephalixin, quinupristin/dalfopristin, tetracycline, ceftioxin, clindamycin and gentamicin through disk diffusion method. Results of resistance of *S. aureus* to antibiotics were as follows: penicillin 88.2%, erythromycin 30%, quinupristin/dalfopristin 0%, cephalixin 28.2%, tetracycline 55/3%, ceftioxin 31.8%, clindamycin 21.8% and gentamicin 16.4%. Results revealed that resistance to methicillin was lower in this study than those reported from shahrekord and other cities in Iran, This seems to be due to the variable drug administration and the different consumption of antibiotics in these hospitals (17, 20-23), but was comparable with a study conducted by Vivek *et al.* in India, in 2011 (1).

Moreover, resistance to erythromycin 33(30%) was lower than the other studies from Iran and other countries (4, 7, 20, 24). Our results were in agreement with those studies conducted by Schmitz *et al.* on resistance to erythromycin in clinical isolates of *S. aureus* isolated from patients in 20 European university hospitals. They found that resistance to erythromycin was low (39%) (25). In the current study, most erythromycin-resistant strains showed resistance to clindamycin and were of MRSA 21 type (19%). These results suggested that there is probably a correlation between the *mecA* gene and macrolide-resistance genes, in *S. aureus* isolates in our study; similar data were reported by Fasihi *et al.* (26). The prevalence of cMLS<sub>B</sub>, iMLS<sub>B</sub> and MS resistance phenotypes among erythromycin-resistant *S. aureus* were 20%, 8.2%, and 1.8%, respectively. Two isolates were also sensitive to both erythromycin and clindamycin. Three of MRSA isolates had a resistant phenotype to iMLS<sub>B</sub>, which was lower than those reported by Moosavian *et al.* (17). In another study done by Naderinasab *et al.*, 0.7% of methicillin resistant *staphylococci* isolates represented the iMLS<sub>B</sub> phenotype (21). The frequency of MLS<sub>B</sub> resistance phenotypes has been reported variously in different countries, as some of these reports showed iMLS<sub>B</sub> rates ranging from 3.3% to 96.3% (4, 7, 17, 23, 27). For example, in a study conducted by Ghanbari *et al.* on 215 *S. aureus* strains isolated from Al-Zahra Hospital in Isfahan, the prevalence of iMLS<sub>B</sub>, cMLS<sub>B</sub>, and MS resistance phenotypes were estimated to be 9 (4.18%), 58 (26.9%), and 11 (5.1%), respectively (28). In Mashhad, Seifi *et al.* reported the prevalence of iMLS<sub>B</sub>, cMLS<sub>B</sub> and MS resistance phenotypes as 11.37%, 26.07% and 12.32%, respectively (29). In India, the prevalence of

iMLS<sub>B</sub>, cMLS<sub>B</sub>, and MS phenotypes among *S. aureus* strains were 21%, 26.5%, and 12%, respectively; both the iMLS<sub>B</sub> and cMLS<sub>B</sub> resistant phenotypes were known to be significantly higher in MRSA isolates as compared to MSSA (30). Similar to our results, in a study conducted by Spode Coutinho *et al.* the cMLS<sub>B</sub> resistant phenotype was the most prevalent phenotype (46.7%) and the iMLS<sub>B</sub> was found just in 3.3% of the isolates, indicating a difference in relation to iMLS<sub>B</sub> data in the present study (7). In our study, MS phenotype was found only among the MSSA isolates. Low prevalence of the MS resistant phenotype in the present study was similar to previous studies from Iran (16, 20, 28). Osman *et al.* reported a high prevalence of MS in Lebanon (31). The results of PCR in our study showed that *ermB* (77.8%), *ermC* (44.4%) and *ermA* (66.8%) were the most prevalent genes in erythromycin-resistant *S. aureus* isolates. An interesting point to notice in the present study was the high frequency of *ermB* gene which is in contrast with other studies from Iran and other countries (10, 20, 24, 27). According to the published literature, *ermB* is primarily originated from animal strains. It is the most prevalent gene among *enterococci* strains and is rarely identified in *staphylococci*. Shahrekord is a city with animal husbandry industry. Since the resistance to macrolides can spread from animals to human by plasmid, it seems that *ermB* has been transferred from animals to human in this region (20, 32, 33). Similar results demonstrated by Zmantar *et al.* in two different studies revealed *ermB* (45.7%) was the most common gene during the evaluation of 81 *S. aureus* strains

isolated from human auricular. These results are consistent with those reported by Goudarzi *et al.* that stated the most prevalent gene in *S. aureus* strains isolated from anterior nares was *ermC* (13.7%) (34). While Duran *et al.* reported that the *ermA* and *ermB* genes were more prevalent than the other erythromycin resistant genes in *S. aureus* isolates (5). In a study conducted by Emaneini *et al.* *ermC* (55%) gene was the prevalent gene and was predominant in MRSA, whereas *ermA* was common in MSSA (27). In another study conducted by Schmitz *et al.*, *ermA* was more common in MRSA isolates and *ermC* was more common in MSSA (25). In addition, a notable finding of the present study was the coexistence of *ermA*, *B* and *C* genes in a considerable number of erythromycin-resistant *S. aureus* strains, including 22 cases (66.7%).

### Conclusion

Our study is the first study to determine the phenotypic and genotypic characteristics of MLS<sub>B</sub> resistant *S. aureus* isolates from Shahrekord area. The cMLS<sub>B</sub> resistance phenotype was the most common resistant phenotype and *ermB* gene was the most common gene among iMLS-resistant *S. aureus* and iMLS<sub>B</sub> phenotypes, with a low frequency. Since the rate of iMLS<sub>B</sub> resistance of *staphylococci* varies greatly in different geographical regions, it is required to perform the D-test for erythromycin-resistance, clindamycin-susceptible *S. aureus* isolates for appropriate therapy and wise use of antibiotics.



## References

1. Goudarzi M, Seyedjavadi SS, Azad M, Goudarzi H, Azimi H. Distribution of spa types, integrons and associated gene cassettes in staphylococcus aureus strains isolated from intensive care units of hospitals in Tehran, Iran. *Arch Clin Infect Dis* 2016;11(4):e38813.
2. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect Control Hosp Epidemiol* 2005;26(2):166-74.
3. Khademi F, Ghanbari F, Mellmann A, Najafzadeh MJ, Khaledi A. Phylogenetic relationships among staphylococcus aureus isolated from clinical samples in Mashhad, Iran. *J Infect Public Health* 2016;9(5):639-44.
4. Vivek JS, Mukesh S, Manpreet K, Misra R, Matnani G, Ujagare M, et al. Prevalence of inducible Clindamycin resistance among community-and hospital-associated staphylococcus aureus isolates in a tertiary care hospital in India. *Biomedical Research* 2011;22(4): 465-9.
5. Duran N, Ozer B, Duran GG, Onlen Y, Demir C. Antibiotic resistance genes & susceptibility patterns in staphylococci. *Indian J Med Res* 2012;135:389-96.
6. Abbas A, Srivastava P, Nirwan PS. Prevalence of MLSB resistance and observation of erm A & erm C genes at a tertiary care hospital. *J Clin Diagn Res* 2015;9(6):DC08-10.
7. Coutinho Vde L, Paiva RM, Reiter KC, de-Paris F, Barth AL, Machado AB. Distribution of erm genes and low prevalence of inducible resistance to clindamycin among staphylococci isolates. *Braz J Infect Dis* 2010;14(6):564-8.
8. Novotna G, Adamkova V, Janata J, Melter O, Spizek J. Prevalence of resistance mechanisms against macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic and occurrence of an undefined mechanism of resistance to lincosamides. *Antimicrob Agents Chemother* 2005;49(8):3586-9.
9. Reddy PS, Suresh R. Phenotypic detection of inducible clindamycin resistance among the clinical isolates of *Staphylococcus aureus* by using the lower limit of inter disk space. *Journal of Microbiology and Biotechnology Research* 2017;2(2):258-64.
10. Aktas Z, Aridogan A, Kayacan CB, Aydin D. Resistance to macrolide, lincosamide and streptogramin antibiotics in staphylococci isolated in Istanbul, Turkey. *J Microbiol* 2007;45(4):286-90.
11. Nikbakht M, Ahangarzadeh Rezaee M, Hasani A, Nahaei MR, Sadeghi J, Jedari Seifi S. Phenotypic and genotypic study of inducible clindamycin resistance in clinical isolates of staphylococcus aureus in Tabriz, Northwest Iran. *Jundishapur J Microbiol* 2017;10(1):e39274.
12. Lim HS, Lee H, Roh KH, Yum JH, Yong D, Lee K, et al. Prevalence of inducible clindamycin resistance in staphylococcal isolates at a Korean tertiary care hospital. *Yonsei Med J* 2006;47(4):480-4.
13. Mansouri S, Sadeghi J. Inducible clindamycin resistance in methicillin-resistant and-susceptible staphylococcus aureus isolated from South East of Iran. *Jundishapur J Microbiol* 2014;7(12):e11868.
14. Mahon CR, Lehman DC, Manuselis G. *Textbook of Diagnostic Microbiology - E-Book*. 4th ed. Elsevier Health Sciences; 2014.



15. Institute CalS. Performance Standards for antimicrobial Susceptibility Testing. 21 ed 2011.
16. Kareem SM, Al- Jubori SS, Ali M. Prevalence of erm genes among methicillin resistant staphylococcus aureus MRSA Iraqi Isolates. *Int J Curr Microbiol App Sci* 2015;4(5):575-85.
17. Moosavian M, Shoja S, Rostami S, Torabipour M, Farshadzadeh Z. Inducible clindamycin resistance in clinical isolates of staphylococcus aureus due to erm genes, Iran. *Iran J Microbiol* 2014;6(6):421-7.
18. O'Sullivan MV, Cai Y, Kong F, Zeng X, Gilbert GL. Influence of disk separation distance on accuracy of the disk approximation test for detection of inducible clindamycin resistance in staphylococcus spp. *J Clin Microbiol* 2006;44(11):4072-6.
19. Zmantar T, Chaieb K, Ben Abdallah F, Ben Kahla-Nakbi A, Ben Hassen A, Mahdouani K, et al. Multiplex PCR detection of the antibiotic resistance genes in Staphylococcus aureus strains isolated from auricular infections. *Folia Microbiol (Praha)* 2008;53(4):357-62.
20. Sadari H, Emadi B, Owlia P. Phenotypic and genotypic study of Macrolide, Lincosamide and Streptogramin B (MLSB) resistance in clinical isolates of staphylococcus aureus in Tehran, Iran. *Med Sci Monit* 2011;17(2):BR48-53.
21. Naderinasab M, Farshadzadeh Z, Yousefi F. Determine the inducible resistance phenotype in methicillin resistance staphylococcus aureus and coagulase negative staphylococci. *Iran J Med Microbiol* 2007; 1(3):25-31. In Persian
22. Shariati L, Validi M, Hasheminia AM, Ghasemikhah R, Kianpour F, Karimi A, et al. Staphylococcus aureus isolates carrying panton-valentine leucocidin genes: their frequency, antimicrobial patterns, and association with infectious disease in Shahrekord city, Southwest Iran. *Jundishapur J Microbiol* 2016; 9(1): e28291.
23. Hosseini SS, Niakan M, Sadari H, Motallebi M, Taherikalani M, Asadollahi K, et al. Frequency of genes encoding erythromycin ribosomal methylases among Staphylococcus aureus clinical isolates with different D-phenotypes in Tehran, Iran. *Iran J Microbiol* 2016;8(3):161-7.
24. Mohammadi S, Sekawi Z, Monjezi A, Maleki MH, Soroush S, Sadeghifard N, et al. Emergence of SCCmec type III with variable antimicrobial resistance profiles and spa types among methicillin-resistant Staphylococcus aureus isolated from healthcare- and community-acquired infections in the west of Iran. *Int J Infect Dis* 2014;25:152-8.
25. Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Kohrer K, et al. Prevalence of macrolide-resistance genes in Staphylococcus aureus and enterococcus faecium isolates from 24 European university hospitals. *J Antimicrob Chemother* 2000;45(6):891-4.
26. Fasihi Y, Saffari F, Kandehkar Ghahraman MR, Kalantar-Neyestanaki D. Molecular detection of macrolide and lincosamide-resistance genes in clinical methicillin-resistant staphylococcus aureus isolates from Kerman, Iran. *Arch Pediatr Infect Dis* 2017;5(1):e37761.
27. Emaneini M, Eslampour MA, Sedaghat H, Aligholi M, Jabalameli F, Shahsavan S, et al. Characterization of phenotypic and genotypic inducible macrolide resistance in staphylococci in Tehran, Iran. *J Chemother* 2009;21(5):595-7.
28. Ghanbari F, Ghajavand H, Havaei R, Jami MS, Khademi F, Heydari L, et al. Distribution of erm genes among Staphylococcus aureus isolates with inducible resistance to clindamycin in Isfahan, Iran. *Adv Biomed Res* 2016;5:62.

29. Seifi N, Kahani N, Askari E, Mahdipour S, Naderi NM. Inducible clindamycin resistance in *Staphylococcus aureus* isolates recovered from Mashhad, Iran. *Iran J Microbiol* 2012;4(2):82-6.
30. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry R. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. *Indian J Med Res* 2006;123(4):571-3.
31. Osman M, Nasbeh AA, Rafei R, Mallat H, Achkar M, Dabboussi F, et al. Characterization of resistance genes to Macrolides, Lincosamides and Streptogramins (MLS) among clinical isolates of *Staphylococcus aureus* in North Lebanon. *The International Arabic Journal of Antimicrobial Agents* 2016;5(4):1-8.
32. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother* 1999;43(5):1062-6.
33. Jensen LB, Frimodt-Moller N, Aarestrup FM. Presence of erm gene classes in gram-positive bacteria of animal and human origin in Denmark. *FEMS Microbiol Lett* 1999;170(1):151-8.
34. Goudarzi G, Tahmasbi F, Anbari K, Ghafarzadeh M. Distribution of genes encoding resistance to macrolides among staphylococci isolated from the nasal cavity of hospital employees in Khorramabad, Iran. *Iran Red Crescent Med J* 2016; 18(2): e25701.
35. Ghanbari F, Saberianpour S, Zarkesh-Esfahani FS, Ghanbari N, Taraghian A, Khademi F. Staphylococcal Cassette Chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated from community-and hospital-acquired infections. *Avicenna J Clin Microb Infec* 2017;4(2):e42244.
36. Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M, et al. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 2000;44(2):231-8.