© Med Sci Monit, 2011; 17(2): BR48-53
**PMID:** 21278685

www.MedSciMonit.com
Basic Research

**Received:** 2010.07.07
**Accepted:** 2010.10.04
**Published:** 2011.02.01

# Phenotypic and genotypic study of macrolide, lincosamide and streptogramin B ($MLS_B$) resistance in clinical isolates of *Staphylococcus aureus* in Tehran, Iran

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

**Horieh Saderi**[ABCDEFG], **Behzad Emadi**[BCDF], **Parviz Owlia**[ABCDEFG]

Department of Microbiology, School of Medicine, Shahed University, Tehran, Iran

**Source of support:** Research Council of Shahed University

## Summary

**Background:** Resistance to antimicrobial agents among *Staphylococcus aureus* is an increasing problem. Two common genes responsible for resistance to macrolide, lincosamide and streptogramin B ($MLS_B$) antibiotics are the *ermA* and *ermC* genes. Three resistance phenotypes have been detected to these antibiotics: strains containing $cMLS_B$ (constitutive $MLS_B$) and $iMLS_B$ (inducible $MLS_B$), which are resistant to macrolide, lincosamide and streptogramin B antibiotics, and MS, which is only resistant to macrolide and streptogramin B antibiotics. The aim of this study was to determine the prevalence of $MLS_B$ phenotypes and genotypes in erythromycin-resistant strains of *S. aureus* isolated from patients in 4 university hospitals in Tehran, Iran.

**Material/Methods:** *S. aureus* strains were isolated from various clinical specimens and identified by routine phenotypic methods and PCR for *nuc* gene. Erythromycin resistance was determined by disk diffusion testing. Prevalence of $MLS_B$ phenotypes was determined by use of the D-test. *ermA* and *ermC* genes were detected by PCR.

**Results:** Altogether, 126 erythromycin-resistant strains of *S. aureus* were detected. Prevalence of $cMLS_B$, $iMLS_B$ and MS resistance phenotypes were 92.8%, 6.4%, and 0.8%, respectively; 60.3% of strains had *ermA* gene and 54.8% *ermC* gene; 61 strains (48.4%) contained 2 studied *erm* genes and 42 strains (33.3%) did not have any studied *erm* genes.

**Conclusions:** Due to the high prevalence of clindamycin resistance among *S. aureus* isolated from patients in Iran, we recommend clindamycin therapy only after proper antimicrobial susceptibility testing.

**key words:** **clindamycin • D-test • *ermA* • *ermC* • erythromycin • *Staphylococcus aureus***

**Full-text PDF:** http://www.medscimonit.com/fulltxt.php?ICID=881386

**Word count:** 2608
**Tables:** 3
**Figures:** 1
**References:** 29

**Author's address:** Parviz Owlia, Department of Microbiology, School of Medicine, Shahed University, Abdollah Zade Ave., Keshavarz Blvd, Tehran, Iran, e-mail: owlia@yahoo.com

## Background

Macrolide, lincosamide and streptogramin B ($MLS_B$) antibiotics have different structure, but similar mode of action. These antibiotics inhibit bacterial protein synthesis by binding to 23s rRNA in 50S ribosomal subunits [1]. Erythromycin (a macrolide) and clindamycin (a lincosamide) are widely used in treatment of *Staphylococcus aureus* infections [2,3]. Clindamycin represents an attractive option for several reasons. Firstly, good oral absorption of clindamycin makes it suitable for outpatient therapy or as follow-up after intravenous therapy. Secondly, it has high tissue penetration (except for the central nervous system) and accumulation in abscesses, and no need for renal dosing adjustments. Thirdly, clindamycin can be used as an alternative antibiotic in patients allergic to penicillin. Fourthly, community-acquired methicillin-resistant *S. aureus*, which has rapidly emerged in recent years as a cause of skin and soft-tissue infections, has shown susceptibility to clindamycin. Finally, it has been shown that clindamycin inhibits the production of toxins and virulence factors in gram-positive organisms through inhibition of protein synthesis [2,4]. However, resistance to erythromycin and clindamycin is increasing among clinical isolates of *S. aureus* worldwide [3].

Three mechanisms have been reported for resistance to $MLS_B$ antibiotics: target site modification, efflux of antibiotics, and drug modification [1]. Methylation of the A2058 residue, located in the conserved domain V of 23s rRNA, takes place in target-site modification and prevents the binding of $MLS_B$ antibiotics to their ribosomal target. This phenomenon leads to cross-resistance to these antibiotics, and produces the $MLS_B$ phenotype that was encoded by erythromycin ribosome methylases (*erm*) genes [5,6]. Among the 4 major classes of *erm* genes (*ermA*, *ermB*, *ermC* and *ermF*) in different bacteria, *ermA* and *ermC* are the primary genes responsible for $MLS_B$ resistance in *S. aureus* [1,2,5].

On the other hand, $MLS_B$ phenotype can be constitutive (rRNA methylase is always produced) or inducible (methylase is produced only in the presence of an inducing agent) [1,7]. While strains with constitutive $MLS_B$ resistance ($cMLS_B$) phenotypes can be detected by routine disk diffusion testing, strains with inducible $MLS_B$ resistance ($iMLS_B$) phenotypes show resistance to erythromycin and sensitivity to clindamycin, similar to strains containing the MS phenotype, which had resistance to only macrolide and streptogramin B, not to clindamycin. Therefore, a special disk diffusion method, the D-test, was developed for the detection of $iMLS_B$. In this test, an erythromycin disk was placed in close proximity to a clindamycin disk. In $iMLS_B$-resistant strains, resistance to clindamycin is induced by diffusion of erythromycin through the agar, and leads to flattening of the clindamycin zone of inhibition adjacent to the erythromycin disk (a D-shaped zone), while MS phenotype-containing strains form a circular zone around the clindamycin disk [2].

There are reports of clinical failures of clindamycin in treating patients with $iMLS_B$ resistance phenotype [8–12], attributed to selection for a mutation in the macrolide-responsive promoter region upstream of the *erm* gene and emergence of $cMLS_B$-resistant isolates [1], leading some investigators to recommend that clindamycin therapy be avoided for *S. aureus* isolates that display the $iMLS_B$ resistance phenotype [1,9,11]. On the other hand, labeling all erythromycin-resistant *S. aureus* as clindamycin-resistant may prevent the use of clindamycin in cases where it would be effective therapy [2]. Thus, accurate detection of $iMLS_B$-resistant strains is very important.

The present investigation was undertaken to determine the prevalence of $cMLS_B$, $iMLS_B$ and MS resistance phenotypes and primary *erm* genes (*ermA* and *ermC*) in 126 erythromycin-resistant *S. aureus* isolates from patients in Tehran, Iran.

## Material and Methods

### Bacterial strains

All *Staphylococcus aureus* isolates from various clinical samples (wounds, abscesses, urine, blood, sterile body fluids, and respiratory tract samples), identified from January to June 2008 in 4 university hospitals (3 general hospitals and 1 burn hospital) in Tehran, Iran, were included in this study. Multiple isolates from the same patient were excluded, even when the site of infection was different. Identification of the isolates as *S. aureus* was based on colony and microscopic morphology, growth on mannitol salt agar and fermentation of mannitol, and production of catalase, coagulase, and deoxyribonuclease. Moreover, amplification of the species-specific *nuc* gene was used to confirm phenotypic identification of *S. aureus* isolates, as described below. Confirmed *S. aureus* strains were stored at –70°C in nutrient broth plus 15% glycerol.

### Phenotypic determination of antibiotic resistance

Disk diffusion testing was used to determine antibiotic resistance according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), with *S. aureus* ATCC 25923 as control. For detecting erythromycin and clindamycin resistance, 15 µg erythromycin disks and 2 µg clindamycin disks (purchased from Mast Co., Merseyside, UK) were used. Interpretation of the diameters of zones of inhibition was as follows: for erythromycin ≥23 mm; S, 14–22 mm; I, ≤13 mm; R, and for clindamycin ≥21 mm; S, 15–20 mm; I, ≤14 mm; R [13]. Intermediate resistant strains were considered resistant. Erythromycin-resistant *S. aureus* strains were selected for further studies.

D-testing was performed for erythromycin-resistant *S. aureus* strains according to the guidelines of the CLSI. Suspension equivalent to 0.5 McFarland of each freshly cultured isolate in normal saline was prepared and used for inoculation of Mueller-Hinton agar (Merck-Hampshire, England) plates. Erythromycin and clindamycin disks were placed on inoculated plates 15 mm apart (edge-to-edge). Plates were read after 18 h of incubation at 35°C and the shape of the clindamycin zone was verified. Strains resistant to both antibiotics were considered to have $cMLS_B$ resistance. Strains with flattening of the susceptible zone of inhibition to clindamycin adjacent to the erythromycin disk (D-shape) were considered to contain $iMLS_B$-resistance, while strains with circular zones were considered to contain MS resistance [2].

### Polymerase Chain Reaction (PCR)

DNA was extracted from all erythromycin-resistant *S. aureus* strains by rapid DNA extraction method [14]. Five colonies

**Table 1.** Primers sequence, thermal cycling profile and size of amplified PCR fragment in each PCR reaction.

| Gene | Primers sequences | No. of PCR cycles (condition) | No. of nucleotide for amplified PCR fragment | Reference |
|---|---|---|---|---|
| *nuc* | 5′-GCGATTGATGGTGATACGGTT-3′<br>5′-AGCCAAGCCTTGACGAACTAAAGC-3′ | 30 (1 min at 94°C, 1 min at 50°C, 2 min at 72°C) | 279 | [15] |
| *mecA* | 5′-GTAGAAATGACTGAACGTCCGATAA-3′<br>5′-CCAATTCCACATTGTTTCGGTCTAA-3′ | 30 (30 s at 94°C, 30 s at 52°C, 30 s at 72°C) | 310 | [15] |
| *ermA* | 5′-GTTCAAGAACAATCAATACAGAG-3′<br>5′-GGATCAGGAAAAGGACATTTTAC-3′ | 35 (30 s at 94°C, 30 s at 52°C, 1 min at 72°C) | 421 | [3,16] |
| *ermC* | 5′-GCTAATATTGTTTAAATCGTCAATTCC-3′<br>5′-GGATCAGGAAAAGGACATTTTAC-3′ | 35 (30 s at 94°C, 30 s at 52°C, 1 min at 72°C) | 572 | [3,16] |

of each isolate's overnight growth on brain-heart infusion agar were suspended in 300 µl of sterile distilled water and heated for 15 min at 100°C. After centrifugation at 14 000 rpm for 5 min, supernatant was collected and used as the DNA template in each PCR run.

PCR was performed with primers specific for *ermA*, *ermC*, *mecA* and *nuc* genes according to previous studies [3,15,16]. Primers were purchased from Cinnagen, Iran, and their sequences, thermal cycling profile and PCR fragment size are shown in Table 1. PCR reaction was performed in a 20 µl volume, and 2 µl of DNA template was added to 18 µl of PCR mixture consisting of 2 µl of PCR buffer (10×), 1 µl of $MgCl_2$ (50mM), 4 µl of dNTPs (1mM), 1 µl of each primers (10 Pmol), 0.25 µl of Taq DNA polymerase (5 u/µl) and 8.75 µl of double-distilled water. DNA amplification was carried out in a thermocycler (Touchgene Gradient, Techne, UK). In each thermal cycling profile, there was an initial denaturation step at 94°C for 5 min, and a final extension step at 72°C for 5 min. After PCR amplification, 5 µl of PCR product was subjected to agarose gel electrophoresis (2% agarose, 1× Tris-acetate-EDTA, 100 V, 100 min). The gel was stained with ethidium bromide, and a PCR fragment was visualized using a gel documentation system by comparison with a molecular size marker (100 bp ladder, Eurobio, UK). Positive control strains for *ermA* and *ermC* donated by Mohammad Emaneini (Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran) and molecular grade water instead of template DNA as the negative control were included in each run. *S. aureus* ATCC29213 (without *ermA*, *ermC*, and *mecA* genes) was also used as a negative control.

## Results

During the 6-month study period, 186 *Staphylococcus aureus* strains were isolated from patients admitted to 4 university hospitals in Tehran, Iran. As expected, all phenotypically detected *S. aureus* strains showed species-specific *nuc* gene amplification in PCR, as reported by Zhang K., et al. [15]. In disk diffusion testing, 126 strains (67.7%) showed resistance to erythromycin and were selected for further study. From these, 117 strains (92.9%) showed resistance to clindamycin in disk diffusion testing and 9 strains were clindamycin-susceptible. In D-testing, 117 strains (92.9%) exhibited $cMLS_B$ resistance, 8 strains (6.3%) $iMLS_B$, and 1 strain (0.8%) had MS resistance phenotype (Figure 1).

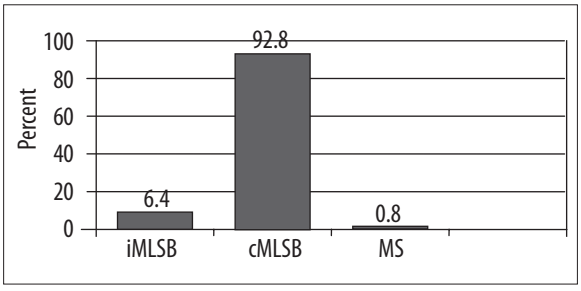


**Figure 1.** The result of D-test for erythromycin-resistant *S. aureus* strains.

Therefore, from 9 clindamycin susceptible strains in disk diffusion testing, 8 strains had $iMLS_B$ and 1 strain had MS resistance phenotypes.

In PCR testing, 76 and 69 strains (60.3% and 54.8%) showed *ermA* and *ermC* genes amplification, respectively; 84 strains (66.7%) contained 1 or 2 of the studied *erm* genes and 42 strains (33.3%) did not contain any studied *erm* genes. Both genes (*ermA* and *ermC*) were co-present in 61 strains (48.4%). Table 2 shows the difference in $MLS_B$ resistance phenotypes in relation to presence of *ermA* and *ermC* genes. In strains with $cMLS_B$ and $iMLS_B$ resistance phenotypes, the most prevalent genotype was *ermA* + *ermC*, while strains with the MS resistance phenotype had only the *ermA* gene.

The *mecA* gene was detected in 86 strains by PCR, thus 68.3% of strains were considered methicillin-resistant *Staphylococcus aureus* (MRSA). Most strains with the $cMLS_B$ resistance phenotype were MRSA (69.2%). Also, among 8 strains with $iMLS_B$ resistance phenotypes, 5 strains were MRSA, while 1 strain with the MS resistance phenotype was MSSA.

Of 86 MRSA strains, 70 strains contained the *ermA* gene, while in 40 MSSA strains only 6 strains contained this gene. In addition, 64 MRSA strains and 5 MSSA strains contained *ermC* genes. Prevalence of the *ermA* gene in MRSA and MSSA strains was 81.4% and 15%, respectively, and for the *ermC* gene prevalence was 74.4% and 12.5%, respectively. *ermA* and *ermC* genes were co-present in 58 (67.4%) of MRSA strains and in 3 (7.5%) of the MSSA strains. Therefore, *ermA* and *ermC* genes were more common in MRSA erythromycin-resistant strains than in MSSA strains (Table 3).

**Table 2.** Difference of $MLS_B$ resistance phenotypes regarding presence of studied *erm* genes.

| Gene | No. of strains with resistance phenotype | | |
|---|---|---|---|
| | $cMLS_B$ (n=116) | $iMLS_B$ (n=8) | MS (n=1) |
| *ermA* alone | 10 | 2 | 1 |
| *ermC* alone | 7 | 0 | 0 |
| *ermA* + *ermC* | 61 | 5 | 0 |
| without *ermA* and *ermC* | 39 | 1 | 0 |

**Table 3.** Distribution of *ermA* and *ermC* genes among studied strains.

| Gene | No. (%) in | | |
|---|---|---|---|
| | MRSA (n=86) | MSSA (n=40) | total strains (n=126) |
| *ermA* alone | 12 (14%) | 3 (7.5%) | 15 (11.9%) |
| *ermC* alone | 6 (7%) | 2 (5%) | 8 (6.4%) |
| *ermA* + *ermC* | 58 (67.4%) | 3 (7.5%) | 61 (48.4%) |
| without *ermA* and *ermC* | 10 (11.6%) | 32 (80%) | 42 (33.3%) |

## Discussion

The increasing frequency of *S. aureus* infections and their antimicrobial resistance have led to renewed interest in the use of $MLS_B$ antibiotics, especially clindamycin, for treatment of these infections in many countries [17]. For appropriate therapy decision-making, accurate susceptibility data are important. However, only a few published articles are available on the prevalence of erythromycin and clindamycin resistance in Iranian isolates of *S. aureus*. Moreover, false susceptibility results for clindamycin may be obtained if isolates are not tested for $iMLS_B$ resistance by D-testing, because it cannot be determined using standard susceptibility tests [2]. Recognition of this type of resistance is important because treatment of patients harboring $iMLS_B$-resistant *S. aureus* with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure [8–12].

In this study, resistance to erythromycin (67.7%) was higher than in studies from other countries, such as the study of Schmitz et al. on *S. aureus* isolated from patients in 20 European university hospitals with rates of 39% [18]. In another study in Tehran, resistance to erythromycin in clinical isolates of *S. aureus* was also high (56.2%) [19]. Most erythromycin-resistant strains (92.9%) in this study also showed clindamycin resistance and were MRSA (68.3%).

In the present study, prevalence of $cMLS_B$, $iMLS_B$ and MS resistance phenotypes among erythromycin-resistant *S. aureus* was 92.9%, 6.3% and 0.8%, respectively. There is only 1 previous study of $MLS_B$ resistance among *S. aureus* isolated in Iran for comparison [20], reporting 9.7% of *S. aureus* strains isolated from patients in Milad Hospital, Tehran, had the $iMLS_B$ resistance phenotype. In the present study, the $cMLS_B$ resistance phenotype was more common than the $iMLS_B$ resistance phenotype among erythromycin – resistant *S. aureus* isolates, in agreement with the results of the 3-year study by Spiliopoulou et al. on 173 erythromycin-resistant *S. aureus* strains isolated from patients in a university hospital in Greece, which reported 61.3%, 30.6% and 7.5% prevalence to $cMLS_B$, $iMLS_B$ and MS resistance phenotypes, respectively [16]. Higher prevalence of $cMLS_B$ than $iMLS_B$ resistance phenotype among *S. aureus* isolates were also reported in other studies [2,4,18]. Low prevalence of the MS resistance phenotype seen in the present study has also been shown in other studies performed in Turkey, the neighboring country of Iran [17,21]; although in the previously mentioned European study [18], it was relatively high (13%). Such differences in the $MLS_B$ resistance pattern could be caused by differences in guidelines for drug usage in Iran, where $MLS_B$ antibiotics are widely used in treating *S. aureus* infections. However, since the occurrence of $iMLS_B$ varies widely by hospital and geographic region [22], it is necessary to perform the D-zone test for erythromycin-resistant, clindamycin-susceptible *S. aureus* isolates [23].

We studied the distribution of 2 *erm* genes (*ermA* and *ermC*) among erythromycin-resistant *S. aureus* isolates. These genes were reported as being the most prevalent genes responsible for resistance to $MLS_B$ antibiotics within *S. aureus* strains in other studies, including the study by Lina et al in French hospitals [3]. In the present study, *ermA* and *ermC* were also prevalent in erythromycin-resistant *S. aureus* isolates (60.3% and 54.8%, respectively), although there was no significant difference between their presence. It should be noted the prevalences of these genes were variable in different studies, and in some studies *ermA* was more prevalent than *ermC*, while in other studies the reverse was found. In research by Martineau et al, the prevalence of *ermA* and *ermC* in erythromycin-resistant *S. aureus* strains was 21% and 10.2%, respectively [24]. Schmitz et al. [25] found *ermA* was more prevalent than *ermC* (67% and 23%, respectively). On the other

hand, 16% and 84% of *S. aureus* strains isolated in Denmark were carrying *erm*A and *ermC*, respectively [26]. In the study by Spiliopoulou et al. [16], *ermC* was more prevalent than *ermA* (70% and 22%, respectively).

A notable finding of the present study was the co-existence of *ermA* and *ermC* in a significant number of erythromycin-resistant *S. aureus* strains (48.4%), similar to results from 2 studies in Turkey (37.5% and 18.6%), and in contrast to 2 studies in European countries (0.6% and 3%) [16,25,27,28]. We also found that a significant number of erythromycin-resistant *S. aureus* isolates (33.3%) did not carry *ermA* and *ermC*, therefore other genes also have a significant role in resistance to erythromycin.

We found prevalence of 81.4% for MRSA strains and 15% for MSSA isolates for *ermA*. Prevalence of *ermC* in MRSA and MSSA isolates were 74.4% and 12.5%, respectively; therefore, both *ermA* and *ermC* were more prevalent among MRSA than MSSA isolates in Tehran, Iran. These results regarding predominance of the *ermA* among MRSA isolates are consistent with previous reports [25,29], but predominance of the *ermC* among MRSA isolates has not been reported, except from Greece [16]. However, prevalence of these genes among MRSA and MSSA strains were variable in different studies. A multicenter study in 24 European university hospitals [25] revealed that in *S. aureus*, the *ermA* gene was more common among MRSA isolates (88%) than in MSSA isolates (38%). In contrast, *ermC* was more common in MSSA (47%) than in MRSA (5%). Otsuka et al. [29] found the *ermA* gene was also predominant among erythromycin-resistant isolates of MRSA compared to MSSA strains (95.0% and 53.3%, respectively), while the *ermC* gene were more prevalent among MSSA than MRSA strains (42.0% and 11.5%, respectively). High prevalence of *erm* genes in MRSA strains emphasizes the need for performing antimicrobial susceptibility testing when clindamycin is considered for use in treatment of infections caused by MRSA.

## Conclusions

Although there are some reports of $MLS_B$ resistance phenotypes in Iranian isolates of *S. aureus*, to our knowledge this is the first report of the involved genes in Iran. We found *ermA* + *ermC* related resistance was the most prevalent in erythromycin-resistant *S. aureus* isolates, and constitutive resistance was particularly predominant among MRSA strains.

**Acknowledgements**

This paper is the result of an M. Sc. student thesis and was financially supported by the Research Council of Shahed University. We also thank Dr. Mohammad Emaneini, Faculty member of Tehran University of Medical Sciences, Tehran, Iran, for providing control strains.

## References:

1. Leclercq R: Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. Clin Infect Dis, 2002; 34: 482–92
2. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH: Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative *staphylococci*. J Clin Microbiol, 2003; 41: 4740–44
3. Lina G, Quaglia A, Reverdy ME et al: Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother, 1999; 43(5): 1062–66
4. Kasten MJ: Clindamycin, metronidazole, and chloramphenicol. Mayo Clin Proc, 1999; 74: 825–33
5. Weisblum B: Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother, 1995; 39: 577–85
6. Roberts MC, Sutcliffe J, Courvalin P et al: Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob Agents Chemother, 1999; 43: 2823–30
7. Daurel C, Huet C, Dhalluin A et al: Differences in potential for selection of clindamycin-resistant mutants between inducible erm(A) and erm(C) *Staphylococcus aureus* genes. J Clin Microbiol, 2008; 46(2): 546–50
8. Levin TP, Suh B, Axelrod P et al: Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant *Staphylococcus aureus*: report of a clinical failure. Antimicrob Agents Chemother, 2005; 49(3): 1222–24
9. Drinkovic D, Fuller ER, Shore KP et al: Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother, 2001; 48(2): 315–16
10. Frank AL, Marcinak JF, Mangat PD et al: Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J, 2002; 21: 530–34
11. Siberry GK, Tekle T, Carroll K, Dick J: Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance *in vitro*. Clin Infect Dis, 2003; 37(9): 1257–60
12. Panagea S, Perry JD, Kate Gould F: Should clindamycin be used as treatment of patients with infections caused by erythromycin-resistant *staphylococci*? J Antimicrob Chemother, 1999; 44: 581–82
13. Clinical and Laboratory Standards Institute (CLSI). 2005. Performance standards for antimicrobial susceptibility tests; Fifteenth informational supplement. CLSI document M100–S15. Vol. 25, No. 1. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA
14. Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S: Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J Clin Microb, 2001; 39: 4037–41
15. Zhang K, Sparling J, Chow BL et al: New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative *staphylococci*. J Clin Microbiol, 2004; 42(11): 4947–55
16. Spiliopoulou I, Petinaki E, Papandreou P, Dimitracopoulos G: erm(C) is the predominant genetic determinant for the expression of resistance to macrolides among methicillin-resistant *Staphylococcus aureus* clinical isolates in Greece. J Antimicrob Chemother, 2004; 53(5): 814–17
17. Yilmaz G, Aydin K, Iskender S et al: Detection and prevalence of inducible clindamycin resistance in *staphylococci*. J Med Microbiol, 2007; 56: 342–45
18. Schmitz FJ, Verhoef J, Fluit AC: Prevalence of resistance to MLS antibiotics in 20 European university hospitals participating in the European SENTRY surveillance programme. Sentry Participants Group. J Antimicrob Chemother, 1999; 43: 783–92
19. Saderi H, Owlia P, Habibi M: Mupirocin resistance among Iranian isolates of *Staphylococcus aureus*. Med Sci Monit, 2008; 14(10): BR210–13
20. Rahbar M, Hajia M: Inducible clindamycin resistance in *Staphylococcus aureus*: a cross-sectional report. Pak J Biol Sci, 2007; 10(1): 189–92
21. Delialioglu N, Aslan G, Ozturk C et al: Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis, 2005; 58: 104–6
22. Schreckenberger PC, Ilendo E, Ristow KL: Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative *staphylococci* in a community and a tertiary care hospital. J Clin Microbiol, 2004; 42: 2777–79
23. Zelazny AM, Ferraro MJ, Glennen A et al: Selection of strains for quality assessment of the disk induction method for detection of inducible clindamycin resistance in *Staphylococci*: a CLSI collaborative study. J Clin Microbiol, 2005; 43(6): 2613–15
24. Martineau F, Picard FJ, Lansac N 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): 231–38
25. Schmitz FJ, Sadurski R, Kray A 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): 891–94

26. Westh H, Hougaard DM, Vuust J, Rosdahl VT: Erm genes in erythromycin-resistant *Staphylococcus aureus* and coagulase-negative *staphylococci.* APMIS, 1995; 103: 225–32

27. 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

28. Gul HC, Kilic A, Guclu AU et al: Macrolide-lincosamide-streptogramin B resistant phenotypes and genotypes for methicillin-resistant *Staphylococcus aureus* in Turkey, from 2003 to 2006. Pol J Microbiol, 2008; 57(4): 307–12

29. Otsuka T, Zaraket H, Takano T et al: Macrolide-lincosamide-streptogramin B resistance phenotypes and genotypes among *Staphylococcus aureus* clinical isolates in Japan. Clin Microbiol Infect, 2007; 13(3): 325–27