Original Article

Distribution of *erm* genes among *Staphylococcus aureus* isolates with inducible resistance to clindamycin in Isfahan, Iran

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Abstract

Background: The rising frequency of methicillin resistant *Staphylococcus aureus* (MRSA) has led to an increased use of antibiotics such as macrolide, lincosamide, streptogramin B (MLS_B) for the treatment of *S. aureus* infections. Resistance to MLS_B in *S. aureus* is commonly encoded by *erm* genes, which can be constitutive MLS_B ($cMLS_B$) or inducible MLS_B ($iMLS_B$). The purpose of this study was to determine the frequency of $cMLS_B$, $iMLS_B$, and MS phenotypes using D-test and polymerase chain reaction (PCR) methods.

Materials and Methods: A total of 215 isolates of *S. aureus* were collected from January 2010 to May 2012 from Al-Zahra Hospital in Isfahan. PCR was performed for detection of *mecA* gene on all isolates using specific primers. The frequency of MLS_B-resistant isolates was determined using D-test, and then a multiplex PCR was performed for detection of *ermA*, *ermB,* and *ermC* genes.

Results: Among 215 *S. aureus* isolates examined, 82 (40.9%) were MRSA, and $iMLS_B$, $cMLS_B$, and MS resistance phenotypes had a frequency of 9 (4.18%), 58 (26.9%), and 11 (5.1%), respectively. Among nine isolates with $iMLS_B$ resistance phenotype, four isolates contained *ermC* gene, two isolates *ermB* gene, and one isolate *ermA* gene. Two isolates did not have any *erm* gene.

Conclusion: In the current study, $cMLS_B$ was the most frequent phenotype and *ermC* was the most common gene in $iMLS_B$ resistant phenotypes.

Key Words: Clindamycin, D-test, *erm* genes, inducible resistance, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is one of the most frequent pathogens that cause both community and hospital-acquired infections worldwide. Development of drug resistance in *S. aureus* has led to the use of older antibiotics such as macrolide, lincosamide, and streptogramin B (MLS_B) antibiotic.[1,2] However,

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extensive use of these antibiotics in serious staphylococcal infections has caused the emergence of *S. aureus* resistant to MLS_B antibiotics.[3] There are three different mechanisms of resistance to MLS_B antibiotics including: (1) Active efflux mechanism encoded by *msr* gene, (2) drug inactivation encoded by *lun* gene and (3) ribosomal binding site modification (by methylation or mutation in the *23s rRNA* gene) encoded by *erm* genes (*ermA*, *ermB*, *ermC*, and *ermF*) among which, *ermA* and *ermC* are predominant genes responsible for resistance to MLS_B antibiotics in staphylococci, which can be constitutive or inducible.[4-8] *In vitro*, *S. aureus* isolates with constitutive MLS_B ($cMLS_B$) resistance are resistant to erythromycin and clindamycin but isolates with inducible MLS_B ($iMLS_B$) resistance are resistant to erythromycin and susceptible to clindamycin. In this condition, treatment of patients with clindamycin can lead to the emergence of resistant mutants to $cMLS_B$ from $iMLS_B$-resistant strains and treatment failure.[3,6] On the other hand, assigning all erythromycin-resistant *S. aureus* as clindamycin resistant strains may cause to avoid the use of clindamycin in the treatment of *S. aureus* infections. For this reason, careful screening of $iMLS_B$-resistant strains is very important. While constitutive resistance is detectable by routine antimicrobial susceptibility tests, inducible resistance to clindamycin is not detectable by standard methods.[4,5] For detection of iMLS-resistant strains, Clinical and Laboratory Standards Institute (CLSI) developed a phenotypic method called the double disk diffusion test (D-test).[9-12] The aim of this study was to determine the frequency of inducible resistance to clindamycin using D-test and polymerase chain reaction (PCR) with specific primers to confirm the presence of the *erm* genes in these isolates.

MATERIALS AND METHODS

Bacterial strains and phenotypic testing

A total of 215 clinical isolates of *S. aureus* were collected from Al-Zahra Hospital in Isfahan from January 2010 to May 2012. Bacterial isolates were obtained from various clinical specimens including: Wound, blood, urine, sputum, etc., Early identification was performed based on Gram-staining and positive biochemical reactions such as catalase, coagulase, and DNase tests. D-test method was performed according to the CLSI guidelines using clindamycin (2 μg) and erythromycin (15 μg) disks (Himedia-India). For this purpose, suspensions of bacteria were prepared in the sterile saline (2 ml) equivalent to standard 0.5 McFarland and then two antibiotic disks placed on Muller-Hinton agar media in 15 mm distance (edge-to-edge). Plates were incubated at 35°C overnight. Strains with flat zone of growth inhibition of clindamycin near the erythromycin disk (D-shape) were classified as resistant phenotypes to $iMLS_B$ (D-test positive), while those with a circular zone were classified as MS resistant phenotypes (D-test negative) [Figure 1].

Molecular detection of *mecA* gene

DNA was extracted from 215 *S. aureus* isolates using Fermentas K0512 DNA kit (Fermentas-USA) in accordance with the manufacturer's protocol. PCR reaction was carried out for the amplification of the 310 bp fragment of *mecA* gene using primers as exhibited in Table 1. PCR amplification reaction mixture (25 μL) contained 4 μL of DNA template, 2.5 μL of PCR buffer (×10), 0.75 μL $Mgcl_2$ (50 mM), 0.5 μL of dNTPs (10 mM), 1 μL of each primers (2 μL totally), 0.25 μL of Ex-Taq DNA polymerase (5u/μL) and 15 μL distill water. PCR conditions were as follows: Initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, and final extension at 72°C for 7 min.

Multiplex polymerase chain reaction for *erm* gene

Multiplex PCR was performed for detection of *erm* gene in D-test positive isolates using specific primers for the *ermA*, *B* and *C* genes as exhibited in Table 1. Each PCR was performed in a final volume of 25 μL consisting of 5 μL of DNA template, 2.5 μL of PCR buffer (×10), 1 μL $Mgcl_2$ (50 mM), 0.5 μL of dNTPs (10 mM), 0.75 μL of each primers (2 μL totally), 0.25 μL of Ex-Taq DNA polymerase (5 u/μL), 11.25 μL distill 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 10 min.

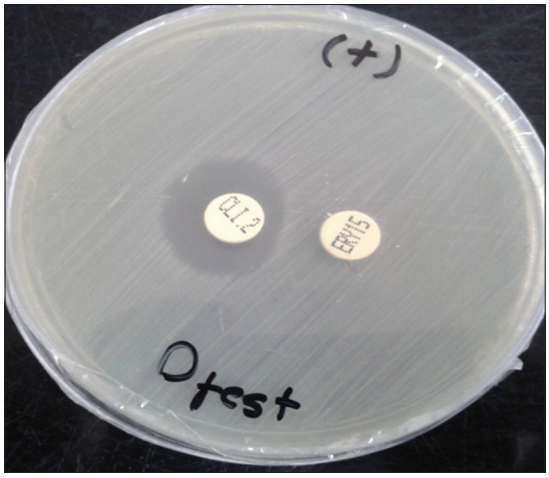

Figure 1: D-shape zone of growth inhibition around clindamycin disk (inducible macrolide, lincosamide, streptogramin B phenotype)

RESULTS

In this study, 215 isolates of *S. aureus* were collected from various clinical specimens, wound 53 (24.6%), blood 49 (22.79%), urinary tract infection 30 (13.9%), sputum 35 (16.27%), abscess 21 (9.76) and others 27 (12.55%), from Al-Zahra Hospital in Isfahan. The patient's average age was 47 years (ranged 1–88 years). The *mecA* gene screening in all isolates showed that 82 (40.9%) of the 215 tested isolates were methicillin resistant *S. aureus* (MRSA) and *mecA* positive [Figure 2]. Furthermore, double disk diffusion test results revealed that 134 (62.3%) of the isolates were susceptible to both clindamycin and erythromycin and 81 (37.7%) were shown to have four different resistance phenotypes in which 58 (26.9%) isolates were resistant phenotype to $cMLS_B$ (resistant to both erythromycin and clindamycin), 9 (4.18%) isolates were resistant phenotype to $iMLS_B$ (resistant to erythromycin and susceptible to clindamycin), 11 (5.1%) isolates were MS resistance phenotype (susceptible to clindamycin and resistant to erythromycin) and finally, 3 (1.39%) isolates were susceptible to erythromycin and resistant to clindamycin [Figure 3]. Among nine isolates with $iMLS_B$ resistance phenotype, 5 (55.5%) were MRSA. Nine staphylococcal isolates with $iMLS_B$ resistance phenotype were tested for the presence of the *erm* genes, the *ermA* gene in 1 (11.1%) isolate, the *ermB* gene in 2 (22.2%) isolates, the *ermC* gene in 4 (44.4%) isolates was detected and two isolates did not have any *erm* genes [Figure 3].

Table 1: Primers used in this study

Target	Sequence	Product size (bp)	References
ermA	GTTCAAGAAC AATCAATACAGAG GGATCAGGAA AAGGACATTTTAC	421	[13]
ermB	CCGTTTACGA AATTGGAACA GGTAAAGGGC GAATCGAGAC TTGAGTGTGC	359	[13]
ermC	GCTAATATTG TTTAAATCGT CAATTCC GGATCAGGAA AAGGACATTT TAC	572	[13]
mecA	AAAATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTG	310	[14]

DISCUSSION

D-test results in our study demonstrated that 134 (62.3%) isolates were sensitive to both erythromycin and clindamycin; the frequency of cMLSB, $iMLS_B$, and MS phenotypes were found to be 58 (26.9%), 9 (4.18%), and 11 (5.1%), respectively. In addition, the frequency of ermC, ermB, and ermA genes among isolates with $iMLS_B$ phenotype was determined to be 44.4%, 22.2%, and 11.1% respectively. Clindamycin due to its advantages including low-cost, low side effects, and good tissue penetration is used for the treatment of *S. aureus* infections. Although it is a good alternative in allergic patients instead of β-lactam antibiotics;[1,9,14,15] however, excessive use of this antibiotic has an important role in bacterial resistance to clindamycin. Since the treatment of infected patients with resistant strains to $iMLS_B$ can lead to the expansion of constitutive resistance ($cMLS_B$) and therapy failure with clindamycin, detection of resistant strains to $iMLS_B$ is important from other resistance phenotypes. Since the frequency of $cMLS_B$, $iMLS_B$, and MS phenotypes varies in different geographical areas, even among different hospitals, awareness of regional frequency of MLS_B resistant isolates is important for laboratories to decide for performing the D-test routinely or reporting all erythromycin-resistant *S. aureus* as clindamycin resistant.[7,10,12,16]

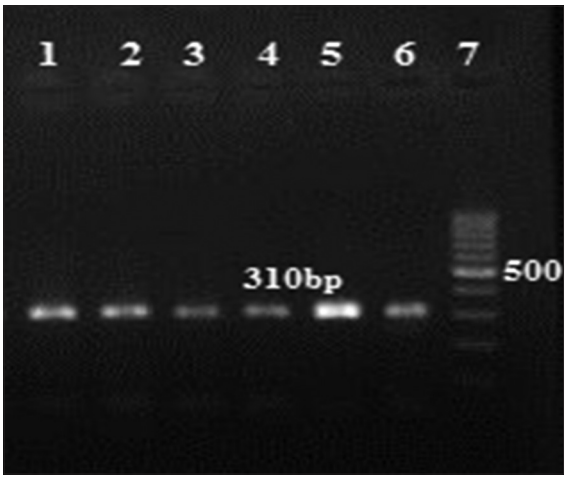

Figure 2: Gel electrophoresis of *mecA* gene. Lanes 1–5: 310 bp fragment, Lane 6: positive control of methicillin resistant *Staphylococcus aureus* strains ATCC 33591, Lane 7: DNA Ladder 100 bp

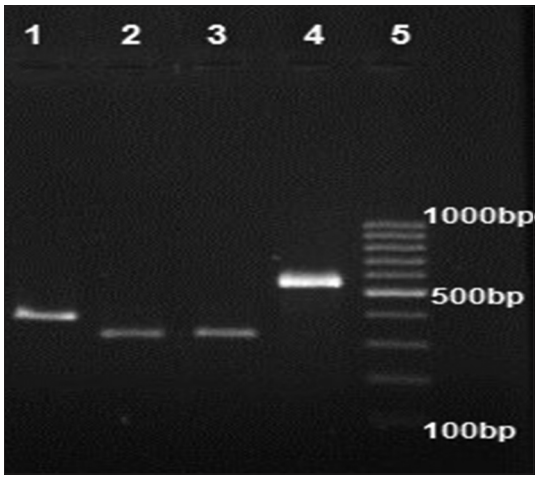

Figure 3: Gel electrophoresis of *erm* genes. Lane 1: *ermA* positive (421 bp), Lane 2 and 3: *ermB* positive (359 bp), Lane 4: *ermC* positive (572 bp), Lane 5: DNA Ladder 100 bp

In the current study, 82 (40.9%) isolates were found to be MRSA that is, comparable with a study conducted by Seifi *et al*.[5] Also, 6.09% of MRSA isolates had resistant phenotype to $iMLS_B$, which is lower than those reported by Shoja *et al*.[17] In the current study, 134 (62.3%) isolates were sensitive to both erythromycin and clindamycin and the frequency of $cMLS_B$, $iMLS_B$ and MS phenotypes were found to be 58 (26.9%), 9 (4.18%), and 11 (5.1%) respectively. Similar results were reported by Aslanimehr *et al*.[18] In the present study, the frequency of $cMLS_B$ phenotype was higher than $iMLS_B$ phenotype. Similar results were obtained by Memarian *et al*.[19] and Mahesh *et al*.[20] In contrast, Reddy and Suresh found the frequency of $iMLS_B$ phenotype to be higher than $cMLS_B$ phenotype.[3] In our study, the frequency of MS resistance phenotype was shown to be higher than $iMLS_B$ phenotype, which was concordant to some previous studies.[3,5,7] Incidentally, we detected 3 (1.39%) isolates resistant to clindamycin and susceptible to erythromycin, similar results were also obtained by Coutinho *et al*.[10] In addition, Seifi *et al*. reported 6 (2.84%) *S. aureus* isolates with such a phenotype.[5] This phenotype can be created by lincosamide nucleotide transferase enzyme that only inactivates lincosamide (clindamycin). Therefore, we investigated *erm* gene distribution among isolates with $iMLS_B$ phenotype. Our results revealed the frequency of *ermC*, *ermB*, and *ermA* genes among isolates with $iMLS_B$ phenotype to be 44.4%, 22.2%, and 11.1%, respectively. Two isolates with $iMLS_B$ phenotype were negative in genotypic test.

It must be noted that the frequency of *erm* genes is variable in different studies. According to our findings, the *ermC* gene was the most prevalent gene, similar study was performed by Aktas *et al*. in Turkey,[7] while in a study conducted by Saderi *et al*. *ermA* gene was prevalent (60%) among erythromycin-resistant *S. aureus*.[2] An interesting point to notice in our study was the high frequency of *ermB* gene, Similar results were shown in some studies.[21,22]

CONCLUSION

This report has investigated the frequency of inducible resistance to clindamycin using D-test and PCR methods. This was the first study to investigate the frequency of MLS_B phenotypes in Isfahan which demonstrated $cMLS_B$ resistance to be the most prevalent resistance phenotype, *ermC* gene as the most common gene among iMLS-resistant *S. aureus* and $iMLS_B$ phenotype having a low frequency. Therefore, we do not recommend the routine performance of D-test but since the frequency of different resistance phenotype may change through time with the emergence of strains with different antibiotic susceptibility patterns, it is recommended that local periodic survey be performed.

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Conflicts of interest
There are no conflicts of interest.

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