Prevalence of Macrolide-Lincosamide-Streptogramin B (MLS<sub>B</sub>) Resistance in S. aureus Isolated from Patients in Tehran, Iran

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ABSTRACT

Background and Objectives: Staphylococcus aureus is an important cause of nosocomial and community-acquired infections in every region of the world. Clindamycin is one of the alternative agents used to treat S. aureus infections and accurate identification of clindamycin resistance is important to prevent therapeutic failure. Unfortunately, inducible clindamycin resistance is not detected by standard susceptibility tests. This study aimed to determine the prevalence of the macrolides-lincosamides-streptogramins B (MLS<sub>B</sub>) resistance in S. aureus isolated in four university hospitals in Tehran, Iran.

Material & Methods: Two hundreds and forty-four non-duplicate clinical isolates of S. aureus [133 methicillin resistant S. aureus (MRSA) and 111 methicillin susceptible (MSSA) S. aureus] were collected in 2008. Antimicrobial susceptibilities were determined by the D-test.

Results: Altogether, 68% and 61.1% of isolates were resistant to erythromycin and clindamycin, respectively; with higher resistance in MRSA isolates compared to MSSA isolates. The constitutive MLS<sub>B</sub> (cMLS<sub>B</sub>) resistance phenotype was recognized in 61.1%, while 5.3% had shown inducible MLS<sub>B</sub> (iMLS<sub>B</sub>) resistance phenotype. Constitutive MLS<sub>B</sub> resistance phenotype predominated over inducible MLS<sub>B</sub> resistance phenotype and susceptible phenotype (83.9, 9.3 and 6.8%, respectively) among the MRSA isolates, whereas susceptible phenotype predominated over constitutive MLS<sub>B</sub> resistance phenotype and inducible MLS<sub>B</sub> resistance phenotype (62.6, 31.3 and 2%, respectively) among the MSSA isolates.

Conclusion: Considering the higher prevalence of clindamycin resistance in MRSA isolates compared MSSA isolates, routine D-test of MRSA isolates is strongly recommended to prevent treatment failure.

Key words: Macrolides, Lincosamide, Streptogramin B, Prevalence, Antibiotic Resistance, Staphylococcus aureus
Introduction

Staphylococcus aureus is an important cause of nosocomial and community-acquired infections in every region of the world. The Macrolide-Lincosamide-Streptogramin B (MLSB) families of antibiotics are chemically distinct, but have similar inhibitory effects on bacterial protein synthesis by binding to the 50S ribosomal subunits. Erythromycin (a macrolide) and clindamycin (a lincosamide) are commonly used for treatment of S. aureus infections. Clindamycin is frequently used to treat skin, soft tissue and bone infections because of its tolerability, cost, oral form, excellent tissue penetration (except for the central nervous system), accumulation in abscesses and the fact that no renal dosing adjustment is necessary (1). It is an alternative in penicillin-allergic patients and good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy (2). However, widespread use of these antimicrobial agents has led to increase in the number of S. aureus strains resistant to them.

In S. aureus, an active efflux mechanism encoded by msrA gene confer resistance to macrolides and streptogramins B antibiotics (so called MS phenotype), and modification of ribosomal target encoded by erm genes cause resistance to macrolides, lincosamides and streptogramins B antibiotics, which called MLSB resistance. The latter mechanism can be constitutive (cMLS\textsubscript{B}); where the rRNA methylase is always produced, or can be inducible (iMLS\textsubscript{B}); where methylase is produced only in the presence of an inducing agent. Low levels of erythromycin are the most effective inducer of iMLS\textsubscript{B} resistance (3-5).

Previous reports indicated that treatment of patients harboring iMLS\textsubscript{B} resistant S. aureus with clindamycin might lead to development of cMLS\textsubscript{B} resistant strains and subsequently, therapeutic failure (6, 7). Unfortunately, the iMLS\textsubscript{B} phenotype cannot be recognized by using standard susceptibility test and require specific methods (2). A test known as disk approximation test or simply D-test detects MLS\textsubscript{B} resistance pattern of S. aureus. For this test, an erythromycin disk is placed 15 mm to 26 mm (edge to edge) from a clindamycin disk in a standard disk diffusion test. As the erythromycin diffuses through the agar, resistance to the clindamycin is induced, resulting in flattening of the clindamycin zone of inhibition (D-shape zone) adjacent to the erythromycin disk (2, 8). The NCCLS guidelines suggest that isolates with the iMLS\textsubscript{B} resistance phenotype should be reported as clindamycin resistant (8).

There is no published data on the prevalence of MLS\textsubscript{B} resistance among clinical isolate of S. aureus in Tehran, Iran. The purpose of this study was to determine accurately the prevalence of resistance to erythromycin and clindamycin in S. aureus isolated from various infections in four university hospitals in Tehran, Iran, in order to assist clinicians in treatment of these infections by these groups of antibiotics.

Material and Methods

S. aureus isolates were collected from various clinical specimens (wounds, abscesses, urine, blood, sterile body fluids, and respiratory tract samples) from January 1 to June 28, 2008 in 4 university hospitals (A to D hospitals) in Tehran, Iran. A, B and C hospitals are general hospitals, while D hospital is a burn center. Duplicate isolates were not included. The isolated S. aureus were stored in freezing medium (contain glycerol) at -70°C until batch testing in July 2008.

For performing D-test, suspension equivalent to 0.5 McFarland of each freshly cultured isolate in normal saline was prepared and inoculated onto a Mueller–Hinton agar plate as described in the NCCLS recommendations (8). Clindamycin (2 μg) and erythromycin (15 μg) discs (purchased from Mast Co., Merseyside, UK) were manually placed 15 mm apart (edge to edge) on the Mueller–Hinton agar plate. Plates were read after 18 h of incubation at 35°C. Quality control was performed with S. aureus ATCC 25923. Interpretation of the diameters of zones of inhibition was according to NCCLS guideline as follows (8): 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. Intermediate resistant strains were considered resistant. Results were recorded as iMLS\textsubscript{B} resistance (strains with flattening of the clindamycin zone adjacent to the erythromycin disk), cMLS\textsubscript{B} resistance (strains resistant to both antibiotics), MS phenotype (strains resistant to erythromycin but susceptible to clindamycin with no D-shape zone), and S phenotype (strains susceptible to both antibiotics) (9).
Methicillin resistance was determined by disk diffusion test with oxacillin disk according to standard method (8).

**Results**

During the study period, 244 S. aureus were isolated from patients in four university hospitals A to D (83, 61, 33 and 67 isolates, respectively) in Tehran, Iran. One hundred and ten S. aureus were isolated from female and 134 were from male patients. Wound infections were the most prevalent clinical specimens for isolation of S. aureus, followed by respiratory tract samples and blood (106, 78 and 20, respectively). The remaining 40 strains were isolated from other specimens. Distribution of S. aureus among wards was different; most of strains were recovered from burn ward patients, then intensive care unit, surgery ward and emergency department (95, 41, 18, and 13 strains), respectively. The remaining 77 strains were isolated from other wards. 54.5% (n=133) of all isolates were recognized as methicillin resistant S. aureus (MRSA) and 45.5% (n=111) as methicillin susceptible S. aureus (MSSA).

Of the 244 S. aureus isolates, 68% (n=166) were resistant to erythromycin and 61.1% (n=149) to clindamycin. MS phenotype was recognized in 1.6% (n=4), while 32% (n=78) of isolates were sensitive to both antibiotics. Constitutive MLSₜₗ resistance were shown in 61.1% (n=149) of studied isolates. Prevalence of MLSₜₗ resistance in all S. aureus isolates was found to be 5.3% (13 of 244). Prevalence of cMLSₜₗ, iMLSₜₗ and MS phenotype among 166 erythromycin resistant strains of S. aureus was 89.8%, 7.8%, and 2.4%, respectively.

MLSₜₗ resistance pattern of isolated S. aureus according to sex of patients, type of clinical specimens, hospitals and wards were shown in Table 1. This resistance pattern varied among four studied hospitals. The S. aureus isolates from respiratory tract samples showed higher cMLSₜₗ resistance (83.3%) and lower sensitivity (11.5%) to both antibiotics. While no strain of S. aureus isolated from patients of surgery ward and emergency department showed iMLSₜₗ resistance, 7.3% and 6.3% of strains isolated from patients of intensive care unit and burn ward, respectively, had shown iMLSₜₗ resistance. In patients of intensive care unit, only 12.2% had erythromycin-clindamycin susceptible strains, while this was 46.2% for strains of patients in emergency department.

**Table 1:** MLSₜₗ resistance pattern of isolated S. aureus according to sex of patients, type of clinical specimens, hospitals and wards

<table>
<thead>
<tr>
<th>Frequency (%) of strains with phenotype</th>
<th>iMLSₜₗ</th>
<th>cMLSₜₗ</th>
<th>MS</th>
<th>S</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitals</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>3 (3.6)</td>
<td>63 (75.9)</td>
<td>1 (1.2)</td>
<td>16 (19.3)</td>
<td>83</td>
</tr>
<tr>
<td>B</td>
<td>3 (4.9)</td>
<td>31 (50.8)</td>
<td>1 (1.6)</td>
<td>26 (42.6)</td>
<td>61</td>
</tr>
<tr>
<td>C</td>
<td>3 (9.1)</td>
<td>10 (30.3)</td>
<td>0 (0)</td>
<td>20 (60.6)</td>
<td>33</td>
</tr>
<tr>
<td>D</td>
<td>4 (6)</td>
<td>45 (67.2)</td>
<td>2 (3)</td>
<td>16 (23.9)</td>
<td>67</td>
</tr>
<tr>
<td>Clinical specimens</td>
<td></td>
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<tr>
<td>wound</td>
<td>6 (5.7)</td>
<td>66 (62.3)</td>
<td>2 (1.9)</td>
<td>32 (30.2)</td>
<td>106</td>
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<tr>
<td>respiratory tract</td>
<td>3 (3.8)</td>
<td>65 (83.3)</td>
<td>1 (1.3)</td>
<td>9 (11.5)</td>
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<td>blood</td>
<td>1 (5)</td>
<td>9 (45)</td>
<td>1 (5)</td>
<td>9 (45)</td>
<td>20</td>
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<td>Sex of patients</td>
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<tr>
<td>female</td>
<td>4 (3.6)</td>
<td>74 (67.3)</td>
<td>2 (1.8)</td>
<td>30 (27.3)</td>
<td>110</td>
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<tr>
<td>male</td>
<td>9 (6.7)</td>
<td>77 (57.5)</td>
<td>2 (1.5)</td>
<td>46 (34.3)</td>
<td>134</td>
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<tr>
<td>burn</td>
<td>6 (6.3)</td>
<td>64 (67.4)</td>
<td>3 (3.2)</td>
<td>22 (23.2)</td>
<td>95</td>
</tr>
<tr>
<td>intensive care unit</td>
<td>3 (7.3)</td>
<td>33 (80.5)</td>
<td>0 (0)</td>
<td>5 (12.2)</td>
<td>41</td>
</tr>
<tr>
<td>surgery</td>
<td>0 (0)</td>
<td>15 (83.3)</td>
<td>0 (0)</td>
<td>3 (16.7)</td>
<td>18</td>
</tr>
<tr>
<td>emergency</td>
<td>0 (0)</td>
<td>6 (46.2)</td>
<td>1 (7.7)</td>
<td>6 (46.2)</td>
<td>13</td>
</tr>
</tbody>
</table>

iMLSₜₗ: Cons  aCrolide-hinCosamide -Streprogramin

cMLSₜₗ:Nbucible  aCrolide-hinCosamide -Streprogramin

MS:
37.4% and 31.3% of the MSSA isolates were resistant to erythromycin and clindamycin, respectively, whereas MRSA isolates had higher resistance (93.2% and 83.9%), respectively. MLSB resistance pattern was different between MRSA and MSSA isolates (Fig. 1). Inducible and constitutive MLSB resistances were higher in MRSA (9.3% and 83.9%, respectively) as compared to MSSA (2% and 31.3%, respectively). Sensitivity to both erythromycin and clindamycin was significantly higher in MSSA than in MRSA isolates (62.6% compared to 6.8%).

**Fig. 1:** MLSB resistance pattern of isolated S. aureus according to MRSA and MSSA

**Discussion**

The increasing frequency of S. aureus infections among patients and the pattern of their antimicrobial resistance have led to renewed interest in the use of MLSB antibiotics, especially clindamycin, for therapy of such infections in many countries (10). For appropriate therapy decision making, accurate susceptibility data are important. However, only a few published articles are available about 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 iMLSB resistance. This type of resistance cannot be determined using standard susceptibility test and need the use of D-test (2). Recognition of this type of resistance is important because treatment of patients harboring iMLS\textsubscript{B} resistant S. aureus with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure (6, 7, 11). Nevertheless, reports of successful treatment of these infections by some investigators exist in the literature (12, 13).

In our isolates, resistance to erythromycin and clindamycin (68% and 61.1%, respectively) were higher than studies in other countries; such as the study of Schmitz et al. (14) on S. aureus isolated from patients in 20 European university hospitals with rates of 39% and 27%, respectively. In another study in Tehran (15), resistance to erythromycin and clindamycin in clinical isolates of S. aureus were also high (56.2% and 53.1%, respectively). In addition, we found different resistance rate between MRSA and MSSA to erythromycin and clindamycin (37.4% and 31.3% for MSSA isolates), compared to 93.2% and 83.9% for MRSA isolates, respectively. These differences were shown in other studies; for example, 9.7% resistance to clindamycin among MSSA and 89.4% among MRSA isolates (14) no resistance to clindamycin among MSSA and 43% among MRSA isolates (16).

This study has shown different MLSB resistance pattern for Iranian isolates of S. aureus from studies performed in other countries. In addition, the resistance pattern was variable and related to the type of hospitals and wards, source of specimens, sex of patients and the isolates (MRSA or MSSA). The prevalence of constitutive and inducible MLSB resistance of S. aureus resistance of S. aureus varies by geographic region and even from hospital to hospital, patient group, and antibiotic susceptibility profile. These variations may be associated with variable use of these antibiotics in each country and/or may depend on the source of the strains such as nosocomial or community acquired, patient age, and sample origin (17). There was no previous report of Iran regarding MLSB resistance of S. aureus isolates for comparison. In two studies performed in Turkey (10, 16), prevalence of iMLS\textsubscript{B} resistance among S. aureus isolates were found higher than those in this study (19.8% in the study of Yilmaz et al. and 7.8% in the study of Delialioglu et al. compared to 5.3% in our study). In contrast, the cMLS\textsubscript{B} resistance of our isolates (61.1%) was higher than two mentioned studies (25.4% in the study of Yilmaz et al. and 24.3% in the study of Delialioglu et al.) (10, 16).

In this study, cMLS\textsubscript{B} resistance was more common than iMLS\textsubscript{B} resistance, same as the earlier studies (14, 16, 2) and in contrast with some other studies (18-20). It has also been reported that clinical isolates
that are constitutively resistant to MLS\textsubscript{B} antibiotics are widespread, particularly among the methicillin resistant strains (9), as that was seen in 83.9\% of our studied MRSA isolates.

In this study, prevalence of MS phenotype among all isolates was low (1.6\%). Low prevalence of MS phenotype was shown in some studies, as 4.4\% (10) and in the study of Delialioglu et al. (16), no isolate showed this phenotype. However, it was 13\% in a recent European study (14). This phenotype, caused by efflux mechanism encoded by \textit{msrA} gene, is increasingly found in MSSA isolates (14). It is suggested that in the area with low prevalence of MS phenotype (like our study) routine D-test are not needed and simply reporting of all erythromycin resistant isolates as clindamycin resistant is sufficient to avoid failure in clindamycin therapy (21). However, it is noteworthy to consider that we found significant differences in MLS\textsubscript{B} resistance pattern between MRSA and MSSA, as shown in other studies (6, 10, 14, 16, 22). In this study, among MRSA strains, 9.3\% had shown iMLSB but no strain with MS phenotype was recognized (compared with 2 and 4\%, respectively, in MSSA). Therefore, these results suggest that where infections with MRSA strains are suspected, routine D-test is necessary to detect clindamycin resistant strains. Nevertheless, when MSSA strains are suspected, empirical therapy with clindamycin is successful in many circumstances.

\textbf{Conclusion}

High prevalence of clindamycin resistance, especially cMLS\textsubscript{B} resistance, in our community shows that antimicrobial susceptibility test is essential when clindamycin is an option for therapy of \textit{S. aureus} infection.

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\textbf{References}


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