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Original Article

Prevalence of Macrolide-Lincosamide-Streptogramin B (MLS_B) Resistance in *Staphybcoccus* 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_B) 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_B ($cMLS_B$) resistance phenotype was recognized in 61.1%, while 5.3% had shown inducible MLS_B ($iMLS_B$) resistance phenotype. Constitutive MLS_B resistance phenotype predominated over inducible MLS_B resistance phenotype and susceptible phenotype (83.9, 9.3 and 6.8%, respectively) among the MRSA isolates, whereas susceptible phenotype predominated over constitutive MLS_B resistance phenotype and inducible MLS_B 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*

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Introduction

Staphylococcus aureus is an important cause of nosocomial and community-acquired infections in every region of the world. The Macrolide-Lincosamide-Streptogramin B (MLS_B) 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 MLS_B resistance. The latter mechanism can be constitutive ($cMLS_B$); where the rRNA methylase is always produced, or can be inducible ($iMLS_B$); where methylase is produced only in the presence of an inducing agent. Low levels of erythromycin are the most effective inducer of $iMLS_B$ resistance (3-5).

Previous reports indicated that treatment of patients harboring $iMLS_B$ resistant *S. aureus* with clindamycin might lead to development of $cMLS_B$ resistant strains and subsequently, therapeutic failure (6, 7). Unfortunately, the $iMLS_B$ 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_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 $iMLS_B$ resistance phenotype should be reported as clindamycin resistant (8).

There is no published data on the prevalence of MLS_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 $iMLS_B$ resistance (strains with flattening of the clindamycin zone adjacent to the erythromycin disk), $cMLS_B$ 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 MLS_B resistance were shown in 61.1% (n=149) of studied isolates. Prevalence of $iMLS_B$ resistance in all *S. aureus* isolates was found to be 5.3% (13 of 244). Prevalence of $cMLS_B$, $iMLS_B$ and MS phenotype among 166 erythromycin resistant strains of *S. aureus* was 89.8%, 7.8%, and 2.4%, respectively.

MLS_B 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 $cMLS_B$ 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 $iMLS_B$ resistance, 7.3% and 6.3% of strains isolated from patients of intensive care unit and burn ward, respectively, had shown $iMLS_B$ 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: MLS_B resistance pattern of isolated *S. aureus* according to sex of patients, type of clinical specimens, hospitals and wards

	Frequency (%) of strains with phenotype				
	$iMLS_B$	**$cMLS_B$**	**MS**	**S**	**Total**
Hospitals					
A	3 (3.6)	63 (75.9)	1 (1.2)	16 (19.3)	83
B	3 (4.9)	31 (50.8)	1 (1.6)	26 (42.6)	61
C	3 (9.1)	10 (30.3)	0 (0)	20 (60.6)	33
D	4 (6)	45 (67.2)	2 (3)	16 (23.9)	67
Clinical specimens					
wound	6 (5.7)	66 (62.3)	2 (1.9)	32 (30.2)	106
respiratory tract	3 (3.8)	65 (83.3)	1 (1.3)	9 (11.5)	78
blood	1 (5)	9 (45)	1 (5)	9 (45)	20
Sex of patients					
female	4 (3.6)	74 (67.3)	2 (1.8)	30 (27.3)	110
male	9 (6.7)	77 (57.5)	2 (1.5)	46 (34.3)	134
Wards					
burn	6 (6.3)	64 (67.4)	3 (3.2)	22 (23.2)	95
intensive care unit	3 (7.3)	33 (80.5)	0 (0)	5 (12.2)	41
surgery	0 (0)	15 (83.3)	0 (0)	3 (16.7)	18
emergency	0 (0)	6 (46.2)	1 (7.7)	6 (46.2)	13

$iMLS_B$: inducible macrolides-lincosamides-streptogramins

$cMLS_B$: constitutive macrolides-lincosamides-streptogramins

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. MLS_B resistance pattern was different between MRSA and MSSA isolates (Fig. 1). Inducible and constitutive MLS_B 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%).

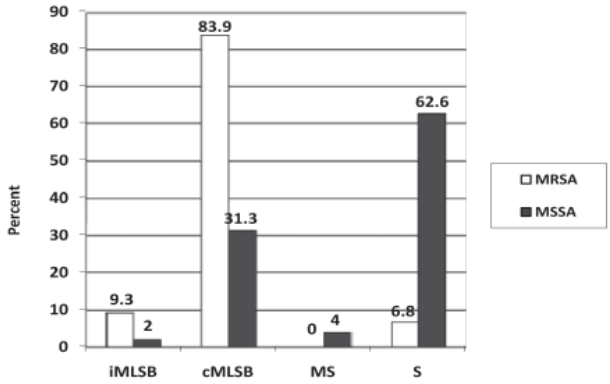

Fig. 1: MLS_B resistance pattern of isolated *S. aureus* according to MRSA and MSSA

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 MLS_B 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 $iMLS_B$ 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 $iMLS_B$ 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 MLS_B 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 MLS_B 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 MLS_B resistance of *S. aureus* isolates for comparison. In two studies performed in Turkey (10, 16), prevalence of $iMLS_B$ 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 $cMLS_B$ 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, $cMLS_B$ resistance was more common than $iMLS_B$ 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_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 *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_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 $iMLS_B$ 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.

Conclusion

High prevalence of clindamycin resistance, especially $cMLS_B$ resistance, in our community shows that antimicrobial susceptibility test is essential when clindamycin is an option for therapy of *S. aureus* infection.

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