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Patterns of Constitutive and Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolated from Clinical Samples by D-test Method, Shiraz, Southwest of Iran

Mohammad Motamedifar^{1,2}, Hadi Sedigh Ebrahim Sarai^{1✉}, Davood Mansury¹¹Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.²Shiraz HIV/AIDS Research Center, Shiraz University of Medical Science, Shiraz, Iran.

Abstract

Background: Macrolides, Lincosamides and type B Streptogramins (MLSB) are commonly used for the treatment of Staphylococcal infections. Inducible MLSB resistance (iMLSB) cannot be identified by standard methods of antibiotic susceptibility testing. D-test appears to be a reliable indicator of iMLSB strains. The aim of this study was to determine the prevalence of Clindamycin resistance phenotypes in *Staphylococcus aureus* (S.aureus) isolated from clinical samples in Shiraz, southwest of Iran. **Materials and Methods:** This cross-sectional study was performed on a total of 302 S. aureus isolates which were collected from two teaching hospitals in Shiraz during 2012. Methicillin resistant *Staphylococcus aureus* (MRSA) were screened based on their resistance to 30µg Cefoxitin disk. 168 Methicillin-sensitive *Staphylococcus aureus* (MSSA) and 134 MRSA isolates were tested in this study. The isolates were tested for susceptibility to Clindamycin (2 µg) and Erythromycin (15 µg) by Clinical and Laboratory Standards Institute (CLSI) recommended disk diffusion test. **Results:** Of 302 collected S. aureus isolates, 134 (44.4%) were MRSA and 168 (55.6%) were MSSA. Inducible MLSB resistance was observed in 10.4% of all recovered MRSA and 3% of all MSSA isolates. The majority of MRSA isolates (77.6%) constituted MLSB phenotype (cMLSB); this phenotype was seen in 4.1% of our tested MSSA isolates. Finally, 12.0% of MRSA isolates and 89.9% of MSSA showed sensitivity to both Erythromycin and Clindamycin. **Conclusion:** Different resistance patterns in hospitals indicated that performing routine D-test for S. aureus infections is highly recommended for each medical center. [GMJ. 2014;3(4):216-21]

Key Words: Microbiology; Drug Resistance; *Staphylococcus Aureus*; Clindamycin; Erythromycin

Correspondence to: Hadi Sedigh Ebrahim Sarai, Department of
Bacteriology and Virology, School of Medicine, Shiraz
University of Medical Sciences
Telephone Number: (+98) 9368829763
Email Address: Seddigh.hadi@gmail.com

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most important causes of bacterial infections in the world and is responsible for a wide range of diseases. *S. aureus* infections are increasingly reported around the world [1]. Although *S. aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. National Nosocomial Infection Surveillance (NNIS) system data demonstrate a steady increase in the incidence of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) among ICU patients [2].

Bacterial resistance mechanisms to antimicrobial agents are mainly through drug inactivation, shifting drug targets or drug impermeability which depends on efflux pumps [3]. Antibiotic resistance to macrolides family in *S. aureus* and coagulase-negative *Staphylococci* (CoNS) may be due to activation of efflux pumps leading to resistance to macrolides and type B Streptogramins or may result from a modification in the ribosomal target, which would lead to resistance to macrolides, Lincosamides and type B Streptogramins (MLSB) [4]. MLSB antibiotics are commonly used for the treatment of staphylococcal infections. Clindamycin is a common choice for some of staphylococcal infections, especially for skin and soft tissue infections; it is an alternative antibiotic for allergic patients to Penicillin [5]. Inducible MLSB (iMLSB) resistance cannot be identified by standard methods of antibiotic susceptibility testing. Failure in detection of iMLSB resistance may be a clinical failure to Clindamycin treatment. On the other hand, all Erythromycin-resistant staphylococci are routinely considered to be resistant to Clindamycin. It may result in avoiding Clindamycin prescription for the patients infected with macrolid-resistant isolates that are actually sensitive to Clindamycin [6]. Trace level of Erythromycin is the most effective inducer of iMLSB resistance [7].

To detect iMLSB isolates, there is a particular method using adjacent Erythromycin and Clindamycin disks with an approximate distance of 15-20 mm. In this method, Erythro-

mycin is released among agar medium and induces Clindamycin resistance consequently creating a flat area in the growth inhibition zone near Clindamycin, which makes the shape of letter D (D-test) [5,8].

The aim of this study was to determine the prevalence of Clindamycin-resistance phenotypes in *S. aureus* isolated from clinical samples using D-test method from Shiraz, south west of Iran.

Materials and Methods

This cross-sectional study was performed during 2012 on a total of 302 *S. aureus* isolates collected from two university hospitals; Namazi and Faghihi in Shiraz, south west of Iran. The isolates were obtained from different wards from blood, wound, sputum, urine and other clinical specimens. The study was in accordance with Declaration of Helsinki; however, because we only used leftovers from clinical specimens, the local ethics committee waived the need for informed consent.

The isolates were identified as *S. aureus* at the microbiology laboratory using standard procedures (colony morphology, Gram stain, and catalase activity, growth on mannitol salt agar, DNase test, and tube coagulase test). MRSA were screened based on resistance to Cefoxitin (30µg) disk (Neo-Sensitabs, Rosco, Denmark) by Clinical and Laboratory Standards Institute (CLSI) recommended disk diffusion method [9]. Outpatient and duplicate isolates were not included in the study.

Isolates were tested for susceptibility to Clindamycin (2 µg) and Erythromycin (15 µg) (Neo-Sensitabs, Rosco, Denmark). *S. aureus* ATCC 25923 which is a MSSA, was used in this study as the control strain. Interpretation of the diameters of zones of inhibition was according to CLSI guidelines [9].

Clindamycin-susceptible and Erythromycin-resistant isolates were tested for the detection of inducible resistance using D-test method [9].

This method was performed on 0.5 McFarland equivalent suspensions of organisms which was spread and incubated on Muller-Hinton agar (Oxoid, UK) plate as described in CLSI recommendations. Erythromycin and Clinda-

mycin disks were placed 15-20 mm apart from each other on the plates. After 18h incubation at 37°C, plates were checked for flattening of the inhibition zone (D-shaped) around Clindamycin which was considered as inducible Clindamycin resistance (Figure. 1).

Statistical analysis was performed using SPSS 19.0 software. Chi-square test was conducted to analyzing data. Values of $p < 0.05$ were regarded as statistical significance.

Results

Of 302 collected *S. aureus* isolates, 134 (44.4%) were MRSA and 168 (55.6%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Distribution of MRSA and MSSA isolates in different clinical specimens is presented in Table 1.

Of all MRSA isolates, 14 isolates showed Erythromycin-resistance and Clindamycin-susceptibility (MSB phenotype). D-zone phenotype, a blunted edge facing Erythromycin but an otherwise clear zone of inhibition around Clindamycin disk (iMLSB resistance)

Figure 1. D-zone of inhibition around Clindamycin disk (iMLSB Phenotype)

Table 1. Distribution of Clinical Sources of Staphylococcal Isolates

Samples	MRSA	MSSA
Sputum	48	29
Blood	20	16
Wound	18	42
Urine	14	31
Pus	8	14
ETT	5	3
Throat	4	3
Body fluid	3	2
Nose	3	14
Eye	3	5
CSF	2	2
Axillary	2	0
Plural fluid	1	0
TIPS	0	1
Other	3	6
Total	168	134

CSF: Cerebrospinal Fluid.

ETT: Endotracheal Tube.

TIPS: Transjugular Intrahepatic Portosystemic.

was observed for all of these 14 isolates comprising 10.4% of all recovered MRSA. Among all tested MSSA isolates, 9 were MSB phenotype, and 5 of them (3.0%) showed iMLSB resistance.

77.6% (n=104) of MRSA isolates showed constitutive Erythromycin and Clindamycin resistance (cMLSB phenotype), and confluent growth was noted around both disks with no inner zone of inhibition; this phenotype was observed in 4.1% (n=7) of the tested MSSA isolates.

Finally, 12.0% (n=16) of MRSA and 89.9% (n=151) of MSSA isolates showed sensitivity to both Erythromycin and Clindamycin disks with large zones of inhibition around them (Table 2).

Distribution of resistant phenotypes among different specimens for MRSA and MSSA isolates is shown in Table 3. Most isolates ($P < 0.05$) with iMLSB resistance were recovered from pus and urine samples presenting 7 and 4 positive D-tests, respectively.

Moreover, there was significant difference ($P < 0.001$) between iMLSB resistant isolates recovered from two studied hospitals; the majority of which were iMLSB resistant isolates (13 MRSA and 3 MSSA isolates) belonging to Faghihi hospital rather than Namazi hospital (1 MRSA and 2 MSSA isolates).

Table 2. Distribution of Resistance Phenotypes Among Studied MRSA and MSSA Isolates

Phenotype	MRSA N (%)	MSSA N(%)	Significant level
Constitutive MLSB	104 (77.6%)	7 (4.1%)	P < 0.0001
Inducible MLSB	14 (10.4%)	5 (3.0%)	P < 0.05
No resistance	16 (12.0%)	151 (89.9%)	P < 0.0001
ERY-R / CLI-S	0	5 (3.0%)	NS
Total	134 (100%)	168 (100%)	

CLI-R: Clindamycin-Resistance.

ERY-R: Erythromycin-resistance

NS: Not Significant.

Table 3. Distribution of IMLSB Phenotypes Among Sources of MRSA and MSSA Isolates

Sample Isolated	Pus	Urine	Sputum	Wound	TIPS	Nose	CSF	Total
MSSA	2	1	0	1	1	0	0	5
MRSA	5	3	3	1	0	1	1	14

CSF: Cerebrospinal Fluid.

TIPS: Transjugular Intrahepatic Portosystemic Shunt.

Discussion

Antimicrobial surveillance studies in health-care settings are of prime importance allowing for identification of trends in pathogen incidence and antimicrobial resistance including identification of emerging resistant pathogens at national and global levels [10]. Clindamycin is an excellent drug of choice for outpatient therapy or as a continued treatment after an intravenous antibiotic therapy. It proves good oral absorption, good tissue penetration and accumulates in abscesses [11]. Although, emerging resistant isolates to macrolids has become a problem in some clinical settings. Failure in Clindamycin therapy in some Staphylococcal infections is not uncommon since many Erythromycin-resistant isolates have iMLSB resistance. This has led to an avoidance of Clindamycin prescriptions against any Erythromycin-resistant Staphylococci [12-14].

However, this problem is manageable if inducible resistance is excluded by reliable detection methods like D-test on a routine basis in clinical isolates. Clindamycin, though, can be safely and effectively used for true susceptible strains causing an infection. In our study,

all of our iMLSB isolates of *S. aureus* showed a false sensitivity to Clindamycin by the routine disk diffusion test.

The majority of our MRSA isolates were cMLSB resistant (77.6%). Some published reports in Iran have indicated that around 52-84% of Erythromycin-resistant MRSA isolates have cMLSB phenotype [15, 16]. Same studies in Turkey and India reported 8.1% and 8.6% cMLSB phenotype prevalence rates respectively, comparing lower rates than Iran; in contrast with a study from Japan reporting this rate to be 61.3% among MRSA isolates [17-19]. cMLSB phenotype was seen in 4.1% of our MSSA isolates. Similar finding was reported from Turkey and Libya [20,21]. Previous studies in Iran demonstrated up to 31.3% rate [15,16]. In our MSSA study, 89.9% isolates were susceptible to both Erythromycin and Clindamycin. It was significantly higher than MRSA isolates with 12.0% susceptibility. Previous reports from Iran and some other countries show higher susceptibility rates in MSSA isolates [15-18].

In early screening, we detected 10.4% and 6.0% Erythromycin resistance and Clindamycin sensitivity among MRSA and MSSA isolates, respectively. All of these MRSA

isolates were found to be inducible Clindamycin resistant (D-test positive), which was significantly higher than MSSA isolates. Of all MSSA isolates, 3.0% were iMLSB resistant and 3.0% were MSB phenotype. A previous study performed in Iran reported iMLSB resistance ranging 2.3% – 33.3 % for MRSA and 4.8%-9.3% among MSSA isolates [15,16, 22]. Various rates from other countries are reported; in almost all of them these rates were higher in MRSA isolates [11,12,17,18]. However, in a study conducted in the US and Japan, the percentages of iMLSB resistance rate in MSSA were seen to be double from MRSA isolates [14,19].

Based on our data, Erythromycin and Clindamycin resistance rates in MRSA isolates were 88% for each of them. In MSSA isolates, Erythromycin resistance rate was calculated 10.1% and 7.1% for Clindamycin resistance (intermediate isolates not included in resistance rate). A report from Mashhad, north east of Iran, in 2012, indicates 88.6% Erythromycin resistance and 52.3% Clindamycin resistance among MRSA isolates. Anyhow, Erythromycin and Clindamycin resistance in MSSA strains was 22% and 11.4%, respectively [15]. Also, results from Tehran, north of Iran, in 2009 showed that resistance to Erythromycin and Clindamycin was 37.4% and 31.3% for MSSA isolates, compared to 93.2% and 83.9% for MRSA isolates, respectively [16]. In both aforementioned studies, similar to our finding, higher rates of resistance were seen in MRSA isolates comparing to MSSA isolates. Regarding clinical specimens in the present study, the highest rate of inducible Clindamycin

resistant *S. aureus* isolates was originated from pus and urine samples. Some published articles from Turkey and India have reported similar results to our finding [17, 23]

According to our study, high rates of iMLSB and cMLSB resistance among MRSA isolates and a low prevalence of MSB phenotype in MSSA isolates were observed. It seems that doing a routine D-test could be a fine indicator for predicting Clindamycin resistant among clinical isolates. Similarly, for cost-benefit reasons, it is recommended to perform this test on Erythromycin resistant isolates.

Conclusion

Accurate susceptibility data are important for appropriate therapy decisions. Previously, iMLSB resistance rate has been shown to vary from different geographical regions, but in our study this point becomes more prominent when we observed different resistance patterns from our aimed hospitals which are less than 2km distant from each other. This finding suggested that routine performing of D-test for *S. aureus* infections is recommended for each medical center individually. However, more studies with larger sample size are suggested.

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