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Full Length Research Paper

Phenotypical examination of the macrolide-lincosamide-streptogramin B resistance in *Staphylococcus* isolates

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The aim of the present study was to evaluate the phenotypic characteristics of the macrolide-lincosamide-streptogramin B (MLS_B) resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) strains isolated from various clinical samples in our hospital. The study was conducted on 516 *Staphylococcus* isolates isolated from various clinical samples in Microbiology Laboratory of Diyarbakir State Hospital between January, 2009 and December, 2009. After the identification of microorganisms via conventional methods and the evaluation of their methicillin resistance profile, disk approximation test was performed using erythromycin (15 µg) and clindamycin (2 µg) disks in order to determine MLS_B resistance phenotypes. Of 516 *Staphylococcus* isolates, 208 were determined to be *S. aureus* and 308 were CNS. The MLS_B resistance of isolates was 56.2%, whereas the resistance due to the efflux pump was determined to be 3.5%. The MLS_B resistance phenotype was determined in 38% of *S. aureus* strains and 68.5% of CNS strains. The presence of MLS_B resistance was determined to be higher in methicillin-resistant group (74.7%) compared to the methicillin-susceptible group (23.9%). While constitutive MLS_B resistance ($cMLS_B$) and inducible MLS_B resistance ($iMLS_B$) were determined in 48.9 and 19.1% of methicillin-resistant *S. aureus* strains, respectively, these rates were 2.6 and 10.5% for methicillin-susceptible strains, respectively. The rate of constitutive resistance was determined to be 41.5% in methicillin resistant CNS, whereas the rate of inducible resistance was determined to be 35.9%. In methicillin-susceptible CNS group, $cMLS_B$ and $iMLS_B$ resistances were determined to be 17.6 and 23%, respectively. The $cMLS_B$ phenotype was more common among methicillin-resistant *S. aureus* and CNS group, whereas $iMLS_B$ phenotype was more common among methicillin-susceptible *S. aureus* strains. In conclusion, we suggest that the determination and reporting of the presence of inducible resistance is of great importance regarding the success of therapy; therefore, it would be beneficial to use D test in routine antibiogram studies.

Key words: *Staphylococcus aureus*, coagulase-negative staphylococci, macrolide-lincosamide-streptogramin B.

INTRODUCTION

The change in Gram-positive bacteria that cause infections and the increase in their antimicrobial resistances accompany the problems related to the treatment options (Hancock, 2005). *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) are the most important causes of hospital-acquired and community-acquired infections among Gram-positive bacteria.

Today, increased prevalence of methicillin resistance to staphylococci is a significant problem, so alternative antibiotics should be investigated (Patel et al., 2006). Macrolides and streptogramins are considered among

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these alternative treatment options. Although the macrolide, lincosamide, and streptogramin B antibiotics are chemically different, they have similar effects on the inhibition of bacterial protein synthesis (Patel et al., 2006; Cetin et al., 2008). Therefore, the genes causing resistance to one of the macrolide-lincosamide-streptogramin B (MLSB) antibiotics may develop a resistance to others.

The resistance to these antibiotics usually develops via modification by methylation (23S ribosomal RNA methylase-mediated ribosomal modification coded by erm gene) of the ribosomal target or via the active efflux pump encoded by macrolide streptogramin resistance (msrA) gene. When resistance to erythromycin develops due to active efflux pump system, the isolates that are resistant to erythromycin are susceptible to clindamycin, whereas these isolates may be resistant to clindamycin in the case that macrolide resistance develops due to ribosomal methylation (Roberts et al., 1999).

The ribosomal resistance that commonly affects the MLS_B group antibiotics may be either constitutive ($cMLS_B$) or inducible ($iMLS_B$). While the isolates with constitutive resistance are resistant to all MLS_B group antibiotics, the inducible resistance develops due to the presence of strong inducers of methylase synthesis, such as erythromycin and azithromycin (Lim et al., 2002; Fiebelkorn et al., 2003).

Owing to the fact that cross resistance can develop in the microorganisms that are resistant to one of the MLS_B group antibiotics, investigating the resistance phenotypes is of great importance for the success of antibiotic treatment. Therefore, in the present study, it was aimed to evaluate the phenotypic characteristics of the MLS_B resistance in *S. aureus* and CNS strains isolated from various clinical samples in Diyarbakir State Hospital.

MATERIALS AND METHODS

The present study was conducted on 516 *staphylococcus* isolates isolated from different samples obtained from either hospitalized or ambulatory patients in Diyarbakir State Hospital between January, 2009 and December, 2009. The isolates that had been re-isolated from the same patient were excluded from the study. Microorganisms were identified via conventional methods such as colony morphology, gram staining, catalase test, coagulase test and DNAse test.

The methicillin resistance of *staphylococcus* isolates, as well as the MLS_B resistance phenotypes, was investigated in accordance with the criteria of the Clinical Laboratory Standards Institute (CLSI) (2009), via disk diffusion method using Sensi-Disc (Becton-Dickinson Microbiology Systems, Franklin Lakes, NJ, USA). For this purpose, the bacterial suspension equivalent to the 0.5 McFarland turbidity standard was spread over the surface of Mueller-Hinton agar (Oxoid Ltd., London, England). Cefoxitin (30 µg) and oxacillin (1 µg) disks were used for the investigation of methicillin resistance, whereas erythromycin (15 µg) and clindamycin (2 µg) disks were used for the investigation of MLS_B resistance. The plates were evaluated after being incubated in aerobic conditions at 35°C for 18 to 24 h.

Double disk approximation test was used to determine the MLS_B resistance phenotypes. For this purpose, two disks containing 15 µg erythromycin were placed at a distance of 15 and 26 mm from the margin of 2 µg clindamycin disk (Clinical and Laboratory Standards Institute, 2009). Erythromycin and clindamycin resistant isolates were considered as $cMLS_B$. Flattening of the growth inhibition zone of clindamycin disk adjacent to the erythromycin disk in the shape of the letter D was referred to as D-zone. The isolates resistant to erythromycin and susceptible to clindamycin and showing the presence of D-zone (D-test positive) around the clindamycin disk were considered as $iMLS_B$. The isolates showing the absence of D-zone (D-test negative), and resistant to erythromycin and susceptible to clindamycin were considered as efflux pump phenotype (Leclercq, 2002). *S. aureus* ATCC 25923 was used as control strain.

The statistical analysis of the data was performed using SPSS version 15.0 for windows (SPSS Inc., Chicago, IL, USA). For the comparison of data Chi square test was used. The level of significance was accepted at $p \leq 0.05$.

RESULTS

Of 516 *staphylococcus* isolates, 208 (40.3%) were *S. aureus* and 308 (59.7%) were CNS. The MLS_B resistance was determined in 290 (56.2%) isolates; whereas efflux pump phenotype was determined in 18 (3.5%) isolates. The distribution of resistance phenotypes for *S. aureus* and CNS is presented in Table 1.

In the present study, the data concerning CNS and *S. aureus* strains were grouped according to their resistance status against methicillin, and these groups were statistically analyzed according to their MLS_B resistance status. The results of the analyses are presented in Table 2.

There was no significant difference between methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCNS) groups regarding the presence of MLS_B resistance ($\chi^2 = 3.046$, $p = 0.081$). However, when the MLS_B resistant isolates were divided into two groups according to their resistance types as $iMLS_B$ [*S. aureus*: n = 18 (19.1%), CNS n = 84 (35.9%)] and $cMLS_B$ [*S. aureus*: n = 46 (48.9%), CNS n = 97 (41.5%)], a significant difference was noted between MRSA and MRCNS strains in $iMLS_B$ group ($\chi^2 = 9.186$, $p = 0.01$).

$iMLS_B$ resistance was detected in 21 S. aureus and 78 CNS strains when the distance between of the erythromycin and clindamycin disks were 26 mm. However, it was detected in 30 *S. aureus* and 101 CNS strains when the distance was shortened to 15 mm.

DISCUSSION

Macrolides and lincosamides are the antibiotics commonly used in the treatment of staphylococcal infections (Patel et al., 2006; Maravic, 2004). Streptogramins have similar effects with these two antibiotic groups. This similarity between the antibiotics may lead to microorganisms to gain resistance to the antibiotics in the

Table 1. The distribution of the MLS_B resistance phenotypes.

	***S. aureus* (n = 208)**			**CNS (n = 308)**			
	Total n (%)	**MR n (%)**	**MS n (%)**	**Total n (%)**	**MR n (%)**	**MS n (%)**	**Total n (%)**
MLS_B (+)	290 (56.2)	64 (68)	15 (13.1)	79 (38)	181 (77.3)	30 (40.6)	211 (68.5)
$cMLS_B$	159 (30.8)	46 (48.9)	3 (2.6)	49 (23.6)	97 (41.5)	13 (17.6)	110 (35.7)
$iMLS_B$	131 (25.4)	18 (19.1)	12 (10.5)	30 (14.4)	84 (35.9)	17 (23)	101 (32.8)
MLS_B (-)	226 (43.8)	30 (32)	99 (89.9)	129 (62)	53 (22.7)	44 (59.4)	97 (31.5)
Efflux Pump	18 (3.5)	6 (6.4)	1 (0.9)	7 (3.4)	6 (2.6)	5 (6.8)	11 (3.6)
Non-resistant	208 (40.3)	24 (25.5)	98 (86)	122 (58.7)	47 (20.1)	39 (52.7)	86 (27.9)
Total	516 (100)	94 (100)	114 (100)	208 (100)	234 (100)	74 (100)	308 (100)

S. aureus: *Staphylococcus aureus;* CNS: coagulase-negative staphylococci; MR: methicillin-resistant; MS: methicillin-susceptible; MLS_B: macrolide-lincosamide-streptogramin B; $cMLS_B$: constitutive MLS_B resistance; $iMLS_B$: inducible MLS_B resistance.

Table 2. Results of the statistical analysis.

	Resistance Phenotype			
	MLS_B (+), n (%)	**MLS_B (-), n (%)**	**χ^2**	**p**
CNS				
Methicillin-resistant (n = 234)	181 (77.4)	53 (22.6)	46.999	0.000
Methicillin-susceptible (n = 74)	30 (40.5)	44 (59.5)		
S. aureus				
Methicillin-resistant (n = 94)	64 (68.1)	30 (31.9)	63.675	0.000
Methicillin-susceptible (n = 114)	15 (13.2)	99 (96.8)		
Methicillin-resistant				
S. aureus (n = 94)	64 (68.1)	30 (31.9)	3.046	0.081
CNS (n = 234)	181 (77.4)	53 (22.6)		
Methicillin-susceptible				
S. aureus (n = 114)	15 (13.2)	99 (96.8)	17.006	0.000
CNS (n = 74)	30 (40.5)	44 (59.5)		
Total				
S. aureus	79 (38.0)	129 (62.0)	35.308	0.000
CNS	211 (68.5)	97 (31.5)		

MLS_B: macrolide-lincosamide-streptogramin B; CNS: coagulase-negative *staphylococci*; *S. aureus: Staphylococcus aureus.*

same group. Therefore, investigating the resistance phenotypes is of great importance regarding the success of the antibiotic treatment.

MLS_B resistance in *Staphylococcus* has been investigated in many studies. In the studies from various regions, it was observed that the rates of MLS_B resistance changed from 7.2% (10) to 88.9% (Lina et al., 1999) in *S. aureus* strains, whereas it varied between 21.5% (Merino-Díaz et al., 2007) and 82.0% (Fiebelkorn et al., 2003) in CNS strains.

In the previous studies, it has been reported that MLS_B resistance both in *S. aureus* and CNS strains differs by geographic region, hospitals and patient groups. While the rate of MLS_B resistance in CNS strains was reported to be lower than that in *S. aureus* strains (Lim et al., 2002; Lina et al., 1999), Aktas et al. (2007) found the MLS_B resistance to be similar in CNS and *S. aureus* groups. However, in some studies, also in the present study, MLS_B resistance in CNS strains was found to be higher than that in *S. aureus* strains (Patel et al., 2006; Fiebelkorn et al., 2003; Merino-Díaz et al., 2007; Gonullu et al., 2009; Yılmaz et al., 2007; Delialioglu et al., 2005).

In the present study, as well as in the previous studies, it was determined that the rate of inducible resistance phenotype in methicillin-susceptible *S. aureus* (MSSA) strains was higher than the rate of constitutive resistance phenotype (Cetin et al., 2008; Lim et al., 2002; Otsuka et al., 2007; Uyanık et al., 2009; Steward et al., 2005; Schmitz et al., 2000). On the contrary, Shrestha et al. (2009) found the rate of constitutive resistance phenotype higher than the rate of inducible resistance phenotype in MSSA strains. Similar differences have been reported for methicillin-susceptible CNS (MSCNS). In the present study, the rate of constitutive resistance was found 17.6%, whereas the rate of inducible resistance was 23%; in consistent with the results of numerous studies (Cetin et al., 2008; Lim et al., 2002; Lina et al., 1999; YIlmaz et al., 2007). On the other hand, Diaz et al. (Merino-Díaz et al., 2007) found that the rates of constitutive and inducible resistance phenotypes were equal, whereas some other investiga-tors determined the rate of the constitutive resistance phenotype to be higher (Aktas et al., 2007; Gonullu et al., 2009; Delialioglu et al., 2005).

In the present study, the rate of constitutive resistance was higher than the inducible resistance both in MRSA and in MRCNS strains. However, in the some studies, the constitutive resistance has been determined more commonly, but the inducible resistance has been reported to be higher in other studies both in some others are reporting that is higher both in MRSA (Uyanık et al., 2009; Shrestha et al., 2009) and MRCNS strains (Cetin et al., 2008; Lim et al., 2002; Denis et al., 2002; Dogruman et al., 2008).

Based on the results of the present study, it can be suggested that the resistance rates may differ by regions or hospitals. Antibiotic use and the origin (hospital or community) of the isolated strains are important factors for the development of resistance. Nonetheless, the development of resistance to antibiotics may vary according to hospitals, regions and countries due to various factors (Koksal, 2006). Therefore, many factors should be taken into consideration while investigating the differences between the resistance rates.

In this study, the investigation of $iMLS_B$ by D-test showed that; when the distance between the disks was 15 mm, the induction of clindamycin resistance by erythromycin occurred clearly better than 26 mm.

In conclusion, we consider that the determination of the presence of inducible resistance is of great importance for the success of the treatment and that the use of D-test in routine antibiogram analyses would be beneficial.

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