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Original article

Determination of Inducible Clindamycin Resistance in Staphylococci Strains Isolated from Clinical Samples

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Clindamycin has been an alternative to methicillin as a result of incrase the prevelance of methicillin resistant staphylococci strains. However, inducible Macrolide-Lincosamide-Streptogramin B ($iMLS_B$) resistance to clindamycin could limit the use of this drug. The aim of this study was to determine the prevalance of iMLSB resistance in staphylococci strains, isolated from various clinical samples. 79 (21%) methicillin resistant *Staphylococcus aureus* (MRSA) and 60 (16%) methicillin sensitive *S. aureus* (MSSA), 154 (41.1%) methicillin resistant and 82 (21.9%) methicillin sensitive coagulase negative stapylococci for a total of 375 isolates were included in this study. $iMLS_B$ resistance was investigated by D-test using clindamycin and erytromycin disk on the basis of guidelines by the Clinical and Laboratory Standards Institute. 223 of total 375 staphylococci isolates were found to be resistant to erythromycin (ER-R). 55 (24.6%) of total 223 (59.5%) ER-R isolates showed $iMLS_B$ phenotype. 40 of 55 $iMLS_B$ resistant isolates were also methicillin resistant. Since $iMLS_B$ resistance is not detected by classical susceptibility tests, using of D-test on a routine laboratory application will help safety usage of clindamycin in treatment of especially methicillin resistant staphylococci infections.

Key words: Coagulase negative staphylococci, D-test, Inducible clindamycin resistance, *Staphylococcus aureus.*

Klinik Örneklerden İzole Edilen Stafilokoklarda İndüklenebilir Klindamisin Direncinin Belirlenmesi

Metisilin dirençli stafilokok suşlarının prevalansındaki artış neticesinde klindamisin metisiline alternatif olmuştur. Ancak klindamisine karşı saptanan indüklenebilir Makrolid-Linkozamid-Streptogramin B ($iMLS_B$) direnci bu antibiyotiğin kullanımını sınırlamaktadır. Bu çalışmanın amacı çeşitli klinik örneklerden izole edilen stafilokok suşlarında indüklenebilir klindamisin direncinin belirlenmesidir. 79 (%21) metisilin dirençli *Staphylococcus aureus* (MRSA), 60 (%16) metisilin duyarlı *S. aureus*(MSSA), 154 (%41.1) metisilin dirençli koagülaz negatif Stafilokok (MRCNS) ve 82 (%21.9) metisilin duyarlı koagülaz negatif stafilokok (MSCNS) olmak üzere 375 suş çalışmaya dahil edilmiştir. $iMLS_B$ direnci klindamisin ve eritromisin diskleri kullanılarak "Clinical and Laboratory Standards Institute (CLSI)" tarafından önerilen D test yöntemi ile belirlenmiştir. 223 (%59.5) suş Eritromisine dirençli (ER-R) bulunmuş ve ER-R suşların 55 (%24.6)' i $iMLS_B$ fenotipi göstermiştir. Bu izolatların 40'ının aynı zamanda metisilin dirençli olduğu belirlenmiştir. $iMLS_B$ direncinin klasik duyarlılık testleri ile belirlenememesi nedeniyle, D-test yönteminin rutin laboratuvar uygulamaları arasında yer alması özellikle metisilin dirençli stafilokok infeksiyonlarının tedavisinde klindamisinin güvenilir kullanımını sağlayacaktır.

Anahtar kelimeler: Koagülaz negatif stafilokok, D-test, İndüklenebilir klindamisin direnci, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcal infections, especially methicilline resistant *Staphylococcus aureus* (MRSA) are increasing and treatment of those infections pose diffuculties and clindamycin is an effective antibiotics specially in community associated MRSA infections (1,2).

The Macrolide – Lincosamide – Streprogramin B (MLS_B) family of antibiotics is commonly used in treatment of staphylococcal infections particularly skin and soft-tissue infections. This family is chemically distinct but has similar inhibitory effects on bacterial protein synthesis. Therefore the genes, cause resistance against one of the MLS_B antibiotics, can lead to the development of cross-resistance to the other members of the group as well (3,4).

MLS_B resistance is the most common and important resistance mechanism detected in Gram-positive organisms. Resistance to MLS_B antibiotics occurs either through target site modification, by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, through efflux mechanism encoded by msr A genes and by drug inactivation (5). Erm genes encode enzymes that confer inducible or constitutive resistance to MLS_B agents via methylation of the 23S ribosomal RNA, thereby reducing binding by MLS_B agents to the ribosome (6).

The inducible resistance can not detectable by routine susceptibility test methods but can be distinguished by erythromycin-clindamycin disk approximation test (D-test) according to the recommendation of the Clinical and Laboratory Standards Institute (6-8). In vitro staphylococci isolates with constitutive resistance are resistant to erythromycin (ER) and clindamycin (CL), while isolates with inducible resistance are resistant to ER but appear susceptible to CL (5,9). Treatment of an infection using clindamycin or any non-inducer macrolide, caused by a strain carrying inducible erm gene, can lead to clinical failure (3,10). In this study, we aimed to determine the presence of inducible clindamycin resistance among the clinical isolates of staphylococci.

EXPERIMENTAL

Strains

Three hundred and seventy-five strains of stapyhlococci isolated from various clinical samples period between January 2011 and June 2012 at the Clinical Microbiology Laboratory of three different hospitals of Ankara, Turkey. The strains from the same patient were excluded. The isolates were identified by conventional bacteriological methods including colony morphology, Gram strain, catalase, coagulase production. *S.aureus* ATCC 25923 was used as quality control strain.

Methicillin susceptibility

Oxacillin (1 µg) disk was used for the investigation of methicillin resistance.

Inducible clindamycin resistance

The erythromycin resistant isolates were examined for inducible clindamycin resistance ($iMLS_B$) by using double disk approximation test (D-test). Briefly, 0.5 McFarland-equivalent suspension of organisms was inoculated onto a Mueller-Hinton agar plate as described in the CLSI recommendations (7). An erythromycin (15 µg) disk was placed 15 to 26 mm (edge to edge) from a clindamycin (2 µg) disk in a standard disk diffusion test. Erythromycin and clindamycin disks were procured from Bioanalyse Limited in Turkey. Plates were analyzed after 18 to 24 hours incubation at 35 °C. Interpretation of the inhibition zone diameters was as follows: If an isolate was ER-R and CL susceptible with a flattening or blunting of the clindamycin zone in the area between two disks (D-shaped zone), it was considered to be positive for inducible resistance (D test positive). If the isolate was ER-R and CL susceptible, with both zones inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D test negative), but to have an active efflux pump (M/MS_B). The isolate was resistance to both ER and CL indicated constitutive ($cMLS_B$) phenotype(6).

Statistical analysis

The data were analyzed using the statistical program SPSS version 17.0 with chi-square test ($p<0.05$ was considered statitically significant).

RESULTS

A total of 223 (59.5%) out of 375 clinical isolates were determined erythromycin resistant. Among these ER-R isolates 130 (58.3%) were found MRCNS and 34 (15.3%) were found MSCNS. In ER-R *S.aureus* isolates, 50 (22.4%) and 9 (4%) were found as MRSA and MSSA respectively.

The rates of cMLSB, M/MS_B, $iMLS_B$ phenotype were determined 98 (44%), 70 (31.4%), 55(24.6%) in all ER-R strains respectively. 40 (72.7%) of 55 $iMLS_B$ resistant isolates were also determined methicillin resistant. Fifteen of 40 isolates were found MRSA and 25 of 40 isolates were found MRCNS. Resistance phenotypes of *S.aureus* and CNS strains are shown in Tables 1 and 2 respectively.

DISCUSSION

The increasing of methicillin resistance among staphylococci isolates is an important problem, and clindamycin is considered to be one of the alternative agents to methicillin. This study was conducted to investigate of MLS_B resistance in 375 staphylococci isolates. Some studies have indicated a higher prevalence of $iMLS_B$ phenotype (10,11) while others have reported lower incidence (12-14). In this present study 223 isolates were found ER-R and 55 (24.6%) of these isolates showed $iMLS_B$ similar to studies that reported by Gadepalli et al., Fiebelkorn et al. (6,15). The different patterns of resistance observed in various studies in the world because MLS_B resistance varies by geographical region, methicillin susceptibility and from hospital to hospital.

Constitutive phenotype rates were found higher than inducible phenotype in this study. There are some studies have similar results that indicate higher constitutive phenotype similarly to our results (4,8,15,16). While the highest $cMLS_B$ rate was observed in MRCNS, the highest $iMLS_B$ rate was observed in

Table 1. Resistance phenotypes of *S. aureus* strains

Phenotype	*S.aureus n(%)*	MRSA n(%)	MSSA n(%)
ER-S/ CL-S	80 (57.5)	29 (36.7)	51 (85)
ER-R/ CL-R	25 (18)	22 (27.9)	3 (5)
ER-R/ CL-S D^+	18 (13)	15 (18.9)	3 (5)
ER-R/ CL-S D^-	16 (11.5)	13 (16.5)	3 (5)
Total n (%)	139	79	60

MRSA: Methicillin-resistant *S.aureus*,
MSSA: Methicillin-sensitive *S.aureus*

Table 2. Resistance phenotypes of CNS strains

Phenotype	*CNS n(%)*	*MRCNS n(%)*	*MSCNS n(%)*
ER-S/ CL-S	72 (30.5)	24 (15.6)	48 (58.5)
ER-R/ CL-R	73 (30.9)	64 (41.6)	9 (10.9)
ER-R/ CL-S D^+	37 (15.7)	25 (16.2)	12 (14.7)
ER-R/ CL-S D^-	54 (22.9)	41 (26.6)	13 (15.9)
Total n (%)	236	154	82

MRCNS: Methicillin-resistant coagulase- negative staphylococci,
MSCNS: Methicillin-sensitive coagulase- negative staphylococc

MRSA. 40 of 55 $iMLS_B$ resistant isolates were methicillin resistant according to our results.

Among *S. aureus* strains MRSA showed higher MLS_B resistance rates than MSSA and the incidence of $cMLS_B$ predominated and $iMLS_B$ was followed by M/MS_B in MRSA. There was a statistically significant higher $iMLS_B$ resistance in MRSA when compared with MSSA strains (p=0.038) and also there was a statistically significant difference of cMLSB between MRSA and MSSA strains (p=0.001).

As observed our study in Gadepalli et al.'s study conducted with 200 *S. aureus* isolates, they found higher MLS_B resistance rates in MRSA strains. In MRSA isolates, 38 % had the constitutive, 30 % had the inducible MLS_B resistance and 12 % had the MS phenotype (15). In MSSA, 15 and 10 % isolates were found to have the constitutive and inducible MLS_B resistance phenotypes respectively while 12 % exhibited the MS phenotype. In Turkey, Adaleti et al. found $cMLS_B$ resistance rate 69.6 % in MRSA and 28.9 % in MSSA in a total of 516 *S.aureus* strains (17), however differently from our study $iMLS_B$ resistance rate was higher in MSSA when compared to MRSA in their study. Similarly in Eksi et al.'s study they found a statistically significant difference of $cMLS_B$ resistance in MRSA compared to MSSA but no statistically significant difference of $iMLS_B$ was observed between MRSA and MSSA isolates (8). On the other hand in Shantala et al.'s study among the MRSA isolates, the inducible resistant phenotype (24.89%) predominated over the constitutive phenotype (18.26%) in *S. aureus* (18).

The distribution of MLS_B among CNS isolates was found a higher incidence of constitutive phenotype, followed by M/MS_B and inducible clindamycin resistance. In our study regarding MRCNS isolates as compared to MSCNS isolates there was a statistically significant higher constitutive phenotype in MRCNS (p=0,00). However there was no statistically difference in terms of $iMLS_B$ between MRCNS and MSCNS (p=0,748). Similar results are available in the other study of our country (19).

CONCLUSION

According to our study 24.6 % of ER-R isolates were found $iMLS_B$ positive and 72.2 % of these $iMLS_B$ positive isolates were methicillin resistant. So determination of $iMLS_B$ resistance will be useful for selecting appropriate treatment specially in methicillin resistant isolates infections and for this the D-test is an easy and sensitive test to apply along with the routine susceptibility testing for detecting MLS_B resistance.

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