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

Banu KASKATEPE*, Sulhiye YILDIZ

1 Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06100 Ankara, TURKEY

Clindamycin has been an alternative to methicillin as a result of increase the prevalence of methicillin resistant staphylococci strains. However, inducible Macrolide-Lincosamide-Streptogramin B (iMLSB) resistance to clindamycin could limit the use of this drug. The aim of this study was to determine the prevalence 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 staphylococci 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.

*Correspondence: E-mail: bkaskatepe@ankara.edu.tr
INTRODUCTION

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

The Macrolide – Lincosamide – Streptogramin 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 staphylococci 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/MSB). 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 statistically 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/MSb, iMLSb phenotype were determined 98 (44%), 70 (31.4%), 55(24.6%) in all ER-R strains respectively. 40 (72.7%) of 55 iMLSb 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.

Table 1. Resistance phenotypes of S. aureus strains

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>S.aureus n(%)</th>
<th>MRSA n(%)</th>
<th>MSSA n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-S/ CL-S</td>
<td>80 (57.5)</td>
<td>29 (36.7)</td>
<td>51 (85)</td>
</tr>
<tr>
<td>ER-R/ CL-R</td>
<td>25 (18)</td>
<td>22 (27.9)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>ER-R/ CL-S D⁺</td>
<td>18 (13)</td>
<td>15 (18.9)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>ER-R/ CL-S D⁻</td>
<td>16 (11.5)</td>
<td>13 (16.5)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>139</td>
<td>79</td>
<td>60</td>
</tr>
</tbody>
</table>

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

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 MLSb resistance in 375 staphylococci isolates. Some studies have indicated a higher prevalence of iMLSb 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 iMLSb 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 MLSb 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 cMLSb rate was observed in MRCNS, the highest iMLSb rate was observed in CNS strains.

Table 2. Resistance phenotypes of CNS strains

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

MRCNS: Methicillin-resistant coagulase- negative staphylococci, MSCNS: Methicillin-sensitive coagulase- negative staphylococci
MRSA. 40 of 55 iMLS\textsubscript{B} resistant isolates were methicillin resistant according to our results.

Among \textit{S. aureus} strains MRSA showed higher MLS\textsubscript{B} resistance rates than MSSA and the incidence of cMLS\textsubscript{B} predominated and iMLS\textsubscript{B} was followed by M/MS\textsubscript{B} in MRSA. There was a statistically significant higher iMLS\textsubscript{B} resistance in MRSA when compared with MSSA strains (p=0.038) and also there was a statistically significant difference of cMLS\textsubscript{B} between MRSA and MSSA strains (p=0.001).

As observed in our study in Gadepalli et al.’s study conducted with 200 \textit{S. aureus} isolates, they found higher MLS\textsubscript{B} resistance rates in MRSA strains. In MRSA isolates, 38% had the constitutive, 30% had the inducible MLS\textsubscript{B} resistance and 12% had the MS phenotype (15). In MSSA, 15 and 10% isolates were found to have the constitutive and inducible MLS\textsubscript{B} resistance phenotypes respectively while 12% exhibited the MS phenotype. In Turkey, Adaleti et al. found cMLS\textsubscript{B} resistance rate 69.6% in MRSA and 28.9% in MSSA in a total of 516 \textit{S. aureus} strains (17), however differently from our study iMLS\textsubscript{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\textsubscript{B} resistance in MRSA compared to MSSA but no statistically significant difference of iMLS\textsubscript{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 \textit{S. aureus} (18).

The distribution of MLS\textsubscript{B} among CNS isolates was found a higher incidence of constitutive phenotype, followed by M/MS\textsubscript{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\textsubscript{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\textsubscript{B} positive and 72.2% of these iMLS\textsubscript{B} positive isolates were methicillin resistant. So determination of iMLS\textsubscript{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\textsubscript{B} resistance.

REFERENCES


Received: 14.11.2013
Accepted: 19.12.2013