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Abstract: The incidence of inducible clindamycin resistance were studied in Milad Hospital of Tehran, Iran. Of 175 isolates of S. aureus 17 (9.7%) isolates showed inducible clindamycin resistance. Of 17 inducible clindamycin isolates of S. aureus, 11 strains were methicillin resistant S. aureus (MRSA) and 6 isolates were methicillin susceptible S. aureus (MSSA). All isolates were susceptible to vancomycin and lincosamide. We conclude that it is necessary to perform D-test for detection of inducible clindamycin in staphylococci in routine laboratory practices.

Key words: Staphylococcus aureus, inducible clindamycin resistant, D-test

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) infections have become common among hospitalized and nonhospitalized patients. Optimal therapy for MRSA infections has yet to be determined, but this matter is complicated by the possibility of inducible macrolide-lincosamide-streptogramin B resistance (Goyal et al., 2004; Patel et al., 2006).

Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50S ribosomal subunits of susceptible organism. Target site modification is the most common mechanism of acquired resistance to macrolides, lincosamide and streptogramin B (MLSb) antibiotics in Staphylococcus aureus and confer cross-resistance to the MLS antibiotics (the so-called MLSb phenotype). MLSb resistance can be either constitutive (MLSb+) or inducible (MLSb-). When it is inducible, bacteria often test resistant to erythromycin but susceptible to clindamycin (Schreckenberger et al., 2004; Deliaioglu et al., 2005; Steward et al., 2005; Zelazny et al., 2005).

Fiebelkorn et al. (2003) have recently described a practical disk diffusion method for detection of inducible clindamycin resistance in S. aureus in clinical specimens. This test involve the placement of an erythromycin and clindamycin disk in close proximity 15-26 mm apart on a Mueller-Hinton agar plate. This method has been standardized recently by Clinical Laboratories Standard Institute (CLSI M100-S15 2005) and known as D-test.

Because the rate of inducible clindamycin resistance (MLSb) in our country is unknown, the objective of this study was (i) to determine the rate of inducible clindamycin in both methicillin resistant and susceptible strains of S. aureus in Milad Hospital of Tehran; (ii) to introduce a simple and practical double disk diffusion agar inhibitory assay or double disk diffusion method (D-test) for detection inducible clindamycin resistance in clinical microbiology laboratories in our country.

MATERIALS AND METHODS

One hundred and seventy five isolates of S. aureus comprising, methicillin-resistant S. aureus (MRSA) and methicillin susceptible S. aureus (MSSA) isolates were obtained from microbiology laboratory of Milad hospital of Tehran, Iran. Isolated microorganisms were identified by the conventional microbiological methods including colony morphology, Gram stain, catalase, slide and tube coagulase tests and DNase (Kloos and Barnerman, 1995). Methicillin resistance was detected according to the recommendation of national committee for clinical laboratory standards (NCCLS). Briefly by using 1 μg of oxacillin disk (Most diagnostic group) on a swab inoculated Muller-Hinton agar plate supplemented with 2% NaCl and incubating at 35°C for 24 h. Susceptibility testing to the other antibiotics performed by disk diffusion method as recommended by NCCLS (2004). The antibiotics used were penicillin (10IU), lincoside (30 μg) muopiromcin (5 μg) vancomycin (30 μg) and trimethoprim-sulfamethoxazole (1.25-23.75 μg).

To detect inducible clindamycin resistance, 15 μg erythromycin and 2 μg clindamycin disks were placed on a Mueller-Hinton agar plate at a distance 15-20 mm for
Table 1: MLSB resistance phenotype of S. aureus

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Constitutive MLSB resistance (%)</th>
<th>Inducible MLSB resistance (%)</th>
<th>MS phenotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (53)</td>
<td>25 (47.6)</td>
<td>12 (22.6)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>MSSA (122)</td>
<td>2 (1.6%)</td>
<td>5 (4.0)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>S. aureus (175)</td>
<td>27 (17.1)</td>
<td>17 (10.8%)</td>
<td>9 (5.7%)</td>
</tr>
</tbody>
</table>

Fig. 1: Double-disk diffusion test (D-test) demonstrating erythromycin disk (E) induction of clindamycin resistance; a blunting of the zone inhibition around the clindamycin (CL) disk is produced a D shape double-disk diffusion test. If there is inducible clindamycin resistance, the erythromycin will diffuse through the agar and resistance to clindamycin will be induced, resulting flattening of clindamycin inhibition adjacent to erythromycin disk and giving D-shape to the zone. Strains were resistant to both erythromycin and clindamycin were defined as showing constitutive MLSB resistance, those showing flattening of the clindamycin zone adjacent to the erythromycin disk were defined as having inducible MLSB and those were resistant to erythromycin and sensitive to clindamycin were defined as showing the MLSB phenotype (Fiebelkorn et al., 2003; CLSI, 2006). An example of inducible clindamycin is shown in Fig. 1.

RESULTS

Of the 175 isolates S. aureus, there was 85 (48.5%) urine, 37(21.4%) wound, 22(12.5%), tracheal tube aspirates, 9(5.1%), blood and 22(12.5) isolates from other biological samples. We found that 17(9.7%) isolates showing inducible clindamycin, resistance. Of the 17 inducible clindamycin resistant isolates of S. aureus there were 11 isolates demonstrating methicillin resistant (MRSA) while 6 isolates methicillin susceptible (MSSA). It was also found that there were 27 isolates showing resistant to both erythromycin and clindamycin (constitutive resistant) and finally 9 isolates were resistant to erythromycin and susceptible to clindamycin (Table 1).

Of 175 isolates of S. aureus 53(35%) isolates were resistant to methicillin (MRSA). All isolates were susceptible to linezolid and vancomycin, 1.6% were resistant to mupirocin and 28.5% of isolates were resistant to trimethoprim-sulfamethoxazole.

DISCUSSION

Accurate results of antibiotic susceptibility tests are important for deciding appropriate and effective therapy of infections caused by S. aureus. Applying proper disk placement on routine disk diffusion method can detect inducible clindamycin resistance. However clinical microbiology laboratories should report in vitro inducible clindamycin resistance in S. aureus isolates and that clinician should be aware of the potential for clinical failure when clindamycin is used to treatment of such infections due to S. aureus (MRSA or MSSA) with in vitro inducible clindamycin resistance (Sibery et al., 2003; Levin et al., 2005).

In this study we have found that resistance to erythromycin and clindamycin were higher in MRSA in comparison of MSSA strains (Table 1). The proportion of inducible clindamycin resistance in MRSA was double in comparison to MSSA Lim et al. (2002) reported that inducible clindamycin resistance was 14.6% in erythromycin resistant S. aureus isolates and 9.6% in erythromycin resistant coagulase-negative staphylococci isolates by employing double-disk diffusion methods in a Korean hospital population. In other study, Hamilton-Miller and Shah (2000) have reported that in S. aureus isolates, 12% had inducible and 2% had constituitive MLS resistance and 1% had the MS phenotype. In their study 31% of coagulase-negative staphylococci strains had inducible, 11% had constitutive MLS resistance and 13% had the MS phenotype. In study from Turkey it was found that 7.6% strains of S. aureus had inducible clindamycin resistance whilst 24.7% had constitutive MLS. By the contrary, there was not MS phenotype (Delioglu et al., 2005). In a study by Kader et al. (2005) from Saudi Arabia of the 291 erythromycin resistant Staphylococci 82 (28%) isolates demonstrated
constitutive clindamycin resistance [2 (2.9%) S. aureus, 43 (53%) MRSA and 37 (26%) coagulase-negative staphylococci]. Inducible clindamycin resistance was demonstrated in 113 (38.8%) of Staphylococcal isolates. In study from United State America Schreckenberger et al. (2004) have shown that, the incidence of inducible clindamycin resistance at two hospital (an inner-city hospital and a suburban community hospital) were 7 and 12% for MRSA. The corresponding figures MSSA were, 20 and 19%, respectively. In the other studies (Bueno-Chavez et al., 2005) the rate of inducible (MLS) β have ranged from 8 to 95% in different times in the United States. In our study 10.8% isolates of were inducible MLS resistance, 17.1% constitutive and 5.7% MS phenotype. The reasons for the differences between the present study and that Schreckenberger et al. (2004) are, inducible clindamycin resistance may vary by region, age group and methicillin susceptibility. (MRSA or MSSA) with in vitro inducible clindamycin resistance.

Clindamycin is a useful drug in the treatment of skin and soft-tissue infections and serious infections caused by staphylococcal species as well as anaerobes. Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, in vitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution method and by disk diffusion testing with erythromycin and clindamycin disks in nonadjacent position however if inducible resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in those patients with true clindamycin-susceptible strains (Fiebelkorn et al., 2003). This study detected the rate of inducible clindamycin in S. aureus in our hospital, we also introduced a simple reliable method to detect inducible clindamycin resistance to clindamycin in erythromycin-resistant isolates of S. aureus.

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REFERENCES


