ABSTRACT

The resistance to antimicrobial agents among Methicillin resistant Staphylococcus aureus (MRSA) is an increasing problem. Clindamycin is commonly used for the treatment of serious soft tissue infections produced by Staphylococcus aureus and treatment failures are reported during therapy. Routine antibiotic sensitivity test for Clindamycin susceptibility may fail to identify iMLSB (inducible MLSB) strains of Staphylococcus aureus due to erm gene. Present study was carried out to find out the percentage of inducible Clindamycin resistance in MRSA in our hospital using D-test. 250 isolates of Staphylococcus aureus from various clinical samples were confirmed by standard protocol and included in this study. MRSA were detected by using Oxacillin (1ug) and Cephoxitin (30ug) disc diffusion method. Results were interpreted according to CLSI criteria. Antibiotic sensitivity to routine antimicrobial agents was carried out using Kirby Bauers disc diffusion method. D-test was performed on all Erythromycin resistant Staphylococcus aureus to detect inducible Clindamycin resistance and constitutive Clindamycin resistance. Out of 250 isolates of Staphylococcus aureus, 90(36%) were found to be MRSA. Among these, 46(51.11%) were Erythromycin resistant. 17(36.95%) isolates showed inducible Clindamycin resistance, 4(8.69%) showed constitutive Clindamycin resistance and 25(54.34%) showed MS phenotype in MRSA.

Conclusion – We therefore conclude that it is necessary to perform D-test for detection of inducible Clindamycin resistance among Staphylococcus aureus in routine antibiotic sensitivity test. Therapeutic failures may thus be avoided.

Keywords – Staphylococcus aureus, inducible MLSB, constitutive Clindamycin resistance, D-test, MRSA.

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens which cause wide range of infections. The emergence of resistance to antimicrobial agents among Staphylococci is an increasing problem. Emergence of methicillin resistance in Staphylococcus aureus has resulted in therapeutic alternatives to treat Staphylococcal infections. The Macrolides (Erythromycin, Clarithromycin) and lincosamides (Clindamycin, Lincomycin) serve as one such alternative. Macrolides, Lincosamides and Streptogramin B are structurally unrelated but microbiologically
having same mode of action (1). Clindamycin is commonly used for the treatment of serious Staphylococcal infection due to its excellent pharmacokinetic properties but sometimes treatment failures were reported during therapy (2, 3).

However widespread use of Macrolides-lincosamides-streptogramin B (MLSB) antibiotics has led to an increased resistance to these antibiotics by Staphylococcal strains (4). The well established mechanism for such resistance is target site modification mediated by \textit{erm} gene which can be expressed either constitutively or inducibly. Erythromycin & Clindamycin discs are commonly used while doing antibiotic sensitivity test for \textit{Staphylococcus aureus} strains. Inducible resistance to Clindamycin is difficult to detect in routine antibiotic sensitivity test as organisms are resistant to Erythromycin and sensitive to Clindamycin in vitro when not placed adjacent to each other. In such cases, in vivo therapy with Clindamycin may select constitutive \textit{erm} mutants leading to clinical therapeutic failure.

Another mechanism of resistance to Macrolides by MRSA is efflux of antibiotic. Staphylococcal isolates appear Erythromycin resistant and Clindamycin sensitive in both in vivo and in vitro and the strain does not typically become Clindamycin resistant during therapy (5).

The present study was conducted from November-2010 to November-2011. A total 250 isolates of \textit{Staphylococcus aureus} were isolated from various clinical specimens like pus, wound swabs, blood and various aspirations received in the department of Microbiology, B.V.D.U.M.C & H. Sangli. All the Staphylococcus species were identified by conventional microbiological methods including colony morphology, gram stain, slide coagulation test, tube coagulation test and mannitol fermentation test, and then subjected to susceptibility testing by Kirby – Bauer’s disc diffusion method on Muller Hinton agar plate using routine antibiotic discs as per CLSI guidelines. Methicillin resistance was detected by using Oxacillin (1-ug) and Cephoxitin (30-ug) on Muller-Hington agar supplemented with 2% NaCl followed by incubation at 35°C. (6)

The isolates which were found to be Erythromycin resistant were examined for inducible Clindamycin resistance [iMLSB] by using double disc approximation test (D test) as per CLSI guidelines. Erythromycin (15-ug) disc was placed at a distance of 15 mm (edge to edge) from Clindamycin (2-ug) disc on Muller-Hington agar plate. After overnight incubation at 37°C, flattening of zone (D shaped) around Clindamycin in the area between the two discs indicated inducible Clindamycin resistance. Strains that do not form inhibition zone around Clindamycin and Erythromycin discs are accepted as constitutive to MLSB phenotype (cMLSB). (6, 7) Figure No. 1, 2, and 3 show iMLSB, cMLSB and MS phenotype

RESULT

Specimen wise distribution of 250 samples is shown in table No.1. Out of the 250 \textit{Staphylococcus aureus} isolates, 90 were found to be MRSA. Among these, 46 (51.11%) were Erythromycin resistant. D-test was performed for these isolates, 17 (36.95%) isolates showed inducible MLSB phenotype (D-test positive), 4 (8.69%) showed constitutive MLSB phenotype and 25 (54. 34%) showed D- test negative suggesting MS phenotype. (Table No. 2)
Table 1: Specimen wise distribution of MRSA and Erythromycin resistance.

<table>
<thead>
<tr>
<th>Clinical specimen</th>
<th>Staphylococcus aureus (n=250)</th>
<th>MRSA (n=90)</th>
<th>ERY resistance (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus and wound swab</td>
<td>197</td>
<td>66</td>
<td>32</td>
</tr>
<tr>
<td>Urine</td>
<td>30</td>
<td>13</td>
<td>08</td>
</tr>
<tr>
<td>Blood</td>
<td>22</td>
<td>11</td>
<td>06</td>
</tr>
<tr>
<td>Body fluids</td>
<td>01</td>
<td>00</td>
<td>00</td>
</tr>
</tbody>
</table>

Table 2: Phenotypic pattern of inducible Clindamycin resistance.

<table>
<thead>
<tr>
<th>Phenotype pattern</th>
<th>MRSA</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERY-R, CL-R (Constitutive MSLB)</td>
<td>4</td>
<td>8.69%</td>
</tr>
<tr>
<td>ERY-R, CL-S, D test positive (iMLSB)</td>
<td>17</td>
<td>36.95%</td>
</tr>
<tr>
<td>ERY-R, CL-S, D test negative MS</td>
<td>25</td>
<td>54.34%</td>
</tr>
</tbody>
</table>

Total ERY resistant MRSA = 46

ERY-Erythromycin, CL –Clindamycin, R-Resistant, S- Sensitive.

DISCUSSION
MRSA strains which cause serious infections may give serious problems in treatment. Prevalence of MRSA has been reported to vary between 9 and 54.6% in hospital based infections (8, 9, 10). Characteristically, reports from different regions have shown different patterns of resistance. Some reports show high prevalence while others show low prevalence. Prevalence of MRSA in
our study was 36%. A study from south Maharashtra had reported that more than 90% isolates are resistant to Penicillin, Ampicillin, Erythromycin and Gentamycin where as only 39.1% were resistant to Methicillin (14). Kandle et al showed 32.8% MRSA (15) and study from Nepal had shown 31.60% MRSA (16). Our results correlate with all of them.

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. Resistance to macrolide, lincosamides, and streptogramin B [MLSB] antibiotics most only results from acquisition of Erythromycin resistant methylase gene (erm gene) which encodes enzymes that methylate the 23S rRNA. In our study iMLSB was found in 36.95% MRSA which is less than P. Sreenivasulu Reddy et al who observed it in 46.2% cases in MRSA (11) but is more than Yilmaz et al who found it in 24.4% and Gadepalli et al who found in 30% of MRSA cases (12) (4). Constitutive resistance in our study is seen in 8.69% of MRSA isolates which is in accordance with V. Deotale-2010 study (7.3%) (17). 54.34% of our isolates of MRSA were sensitive to Clindamycin, against which it would be safe & appropriate to use Clindamycin or other Macrolides. It correlates with previous studies who have reported 57% of susceptibility towards Clindamycin among MRSA stains (13). Above studies showed that prevalence of inducible resistant isolates may differ from place to place.

CONCLUSION
Our observations suggest that, D-test should be mandatory for all microbiological laboratories before reporting Clindamycin susceptibility as Clindamycin is not a suitable drug for D-test positive isolates while it can definitely prove to be a drug of choice in case of D-test negative isolates. Therefore, regular surveillance of antimicrobial susceptibility pattern of MRSA, determination of phenotypic pattern of inducible Clindamycin resistance and formulation of a definite antibiotic policy may be helpful in reducing the burden of MRSA infections and failures in Clindamycin treatment in the hospital.

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