Original Research Article

Prevalence and Detection of Inducible Clindamycin Resistance among Community and Hospital Associated Staphylococcus aureus Isolates from Pus Samples

K. H. Harshan* and Sunil Kumar D Chavan

1Sree Gokulam Medical College and Research foundation, Venjaramoodu, Trivandrum, India  
2Raja Rajeswari Medical College, Bangalore-560074, India  
*Corresponding author

ABSTRACT

The resistance to antimicrobial agents among Staphylococcus aureus is a major concern worldwide. The resistance to macrolide can be mediated by msr A gene or erm gene that confer inducible or constitutive resistance to macrolide, lincosamide and type B streptogramin (i MLSB). To find out the percentage of inducible clindamycin resistance among hospital and community associated Staphylococcus aureus isolates in our hospital. A detailed history regarding hospitalisation and prior antibiotic intake was obtained from each patient to differentiate hospital acquired and community associated isolate. Staphylococcus aureus were identified by conventional methods. Susceptibility to routine antimicrobial agents was carried out using Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by “D test” as per CLSI guidelines. A total of 205 Staphylococcus aureus from pus samples were studied. Of these 160(78%) were hospital associated and 45(22%) were community associated. The overall prevalence of i MLSB was 43(20.9%). Community associated Staphylococcus aureus revealed lower prevalence of 7(15.5%) of i MLSB compared to hospital associated 36(22.5%). Majority of i MLSB isolates were sensitive to amikacin (85%) and resistant to ampicillin (97%). The high frequency of i MLSB resistance in hospital as well as community set up raises concern of clindamycin treatment failure. It is essential to include “D test” for accurate identification of inducible clindamycin resistance in routine antimicrobial susceptibility testing.

Keywords
Clindamycin, Dtest, Staphylococcus aureus, i MLSB

Introduction

Staphylococcus aureus is recognised to be causing nosocomial and community acquired infections in every region of the world. Skin and soft tissue infections (SSTI) are a common manifestation of Staphylococcal disease in many community outbreaks (Patel et al., 2006). Emergence of methicillin resistance in Staphylococcus aureus has left us with very few therapeutic alternatives available to treat Staphylococcal infections. The Macrolide-Lincosamide-Streptogramin M (MLSB) family of
antibiotics serves as one such alternative. Erythromycin, a macrolide and clindamycin, a lincosamide re represent two distinct classes of antimicrobial agents that act by binding to the 50s ribosomal subunit of bacteria to inhibit protein synthesis. The good oral absorption of clindamycin makes it an attractive option for use in outpatients or as follow up treatment. Expression of inducible resistance to clindamycin could limit the effectiveness of this drug (Angel et al., 2008).

Present study was aimed to find out the percentage of inducible clindamycin resistance among hospital and community associated *Staphylococcus aureus* in our hospital. Also to know the difference in the antibiotic resistance pattern among these isolates.

**Materials and Methods**

Study period was from January 2015 to June 2015. All the *Staphylococcus aureus* strains isolated from pus samples received at department of Microbiology during the study period were included.

Isolates were designated as hospital acquired if the source patient had following risk factors:

72 hour or more duration of hospital stay, residence in a long term care facility, post operative wound, history of hospitalisation, dialysis or surgery within one year. If none of the above factors were present, the isolate was considered community acquired.

All the specimens were subjected to preliminary tests like gram stain from direct sample and streak culture on 5% sheep blood agar, MacConkey agar, mannitol salt agar. *Staphylococcus aureus* was identified by colony morphology, gram stain, catalase test, tube coagulase test, DNAse test.

Anti microbial susceptibility testing was done on Mueller Hinton agar using the disc diffusion test as outlined by CLSI

Inducible clindamycin resistance was detected by disk diffusion induction test-“D” test (Fiebelkron et al., 2003). Mueller Hinton agar plate was inoculated with Staphylococcal bacterial suspension with 0.5 McFarland turbidity. Erythromycin (15mg) disk was placed at a distance of 15 mm (edge to edge) from clindamycin (2mg) disk (Ajantha et al., 2008). Following overnight incubation at 37°C, flattening of zone –D shaped, around clindamycin in the area between the two discs indicates inducible clindamycin resistance.

Staphylococcal isolates exhibiting resistance to erythromycin (< 13mm zone size) while sensitive to clindamycin (>21 mm zone size) were labelled as having MS phenotypes. Isolates showing resistance to erythromycin while being sensitive to clindamycin and giving D shaped zone of inhibition around clindamycin with flattening towards Erythromycin disc were labelled as having inducible iMLSB phenotype. Further isolates showing small colonies growing near clindamycin disc in otherwise clear zone were labelled as D+ inducible iMLSB phenotypes. Isolates showing resistance to both erythromycin and clindamycin (< 14 mm zone size) were labelled as having constitutive cMLSB phenotype.

ATCC *Staphylococcus* strains 25923 and 29213 were used as quality control. Statistical analysis of data was done using SPSS software.

**Results and Discussion**

*Staphylococcus aureus* strains were isolated from 205 samples. Of these 160(78%) were
considered as hospital acquired and 45(22%) as community associated.

A total of 142(69.2%) isolates were sensitive to both erythromycin and clindamycin. Inducible MLSB detected by using D test revealed 43(20.9%) isolates were iMLSB, 12(5.8%) cMLSB and 8(3.9%) MS phenotype. Distribution of MLSB phenotypes and comparison of MLSB phenotypes among hospital acquired and community associated isolates shown in table 1&2 respectively. Antibiotic resistance pattern of MLSB phenotypes shown in table 3. All the 205 isolates were sensitive to vancomycin and linezolid.

*Staphylococcus aureus* strains have shown a disconcerting propensity to develop resistance to antimicrobial agents and have become a challenge for infection control programme and the clinicians. Resistance to antimicrobial agents is a major concern worldwide and is exemplified by the global spread of MRSA and development of resistance to Macrolide, Lincosamide, Streptogramin B (MLSB) group of antibiotics (Laclerq, 2002).

A total of 205 *Staphylococcus aureus* strains were isolated from 620 samples received during study period of 6 months. In our study 160 strains were hospital acquired indicating staphylococcal infection was more pronounced among hospitalised patients.

D test performed by placing erythromycin and clindamycin discs at a distance of 15 mm from edge to edge has been found satisfactory by several studies. The overall incidence of iMLSB in the present study 20.9% is in agreement with Yilmaz et al. (2007) report. Different investigators have reported incidence ranging from 11.8% to 29.8%. Favourable factor is incidence of cMLSB resistance is very low 5.8%

compared to other studies. We did not observe any D+ isolate in our study. *Staphylococcus aureus* isolates showed higher drug resistance rate towards most of the antibiotics tested. More than 50 % of iMLSB were found to be among MRSA.

Among the 43 iMLSB majority were sensitive to amikacin (85%). iMLSB strains showed a resistance of 97% to ampicillin, 83% to amoxyclav, 91% to gentamicin, 78% to cotrimoxazole which are routinely used drugs for empirical treatment of skin and soft tissue infection. Isolates showing constitutive MLSB strains were 72% sensitive to amikacin, 57 % sensitive to tetracycline. All cMLSB strains were 100% resistant to ampicillin and amoxyclav.

All the isolates were 100 % sensitive to vancomycin, linezolid, teicoplanin, netilmicin and rifampicin.

Overall 77% of erythromycin resistant and 24% of clindamycin sensitive isolates were shown to have inducible iMLSB by D test. Strains with iMLSB demonstrate in vitro resistance to erythromycin while appearing susceptible to lincosamide and type B streptogramin. These observations suggest that without D test all these isolates 43 with iMLSB resistance would have been misidentified as clindamycin susceptible resulting in underestimated clindamycin resistance rate.

Labelling all erythromycin resistance as clindamycin resistance will prevent the use of clindamycin in treating infection that would likely to respond to therapy. In the present study only 8 erythromycin resistance isolates showed true clindamycin susceptibility. Patients with infection caused by such isolates can be treated with clindamycin without emergence of resistance during therapy.
**Table 1** Frequency of MLSB phenotypes

<table>
<thead>
<tr>
<th>MLSB phenotypes</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMLSB</td>
<td>43</td>
</tr>
<tr>
<td>cMLSB</td>
<td>12</td>
</tr>
<tr>
<td>MS</td>
<td>08</td>
</tr>
</tbody>
</table>

**Table 2** Comparison of incidence of MLSB phenotypes among Hospital associated (HA) and Community associated (CA) isolates

<table>
<thead>
<tr>
<th>MLSB type</th>
<th>HA (n=160)</th>
<th>CA(n=45)</th>
<th>TOTAL(n=205)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMLSB</td>
<td>36(22.5%)</td>
<td>07(15.5%)</td>
<td>43(20.9%)</td>
</tr>
<tr>
<td>cMLSB</td>
<td>09(5.6%)</td>
<td>03(6.6%)</td>
<td>12(5.8%)</td>
</tr>
<tr>
<td>MS</td>
<td>06(3.75%)</td>
<td>02(4.4%)</td>
<td>08(3.9%)</td>
</tr>
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</table>

**Table 3** Antibiotic resistance pattern of different MLSB phenotypes

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>iMLSB(n=43)</th>
<th>cMLSB(n=12)</th>
<th>MS(n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>97%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Amoxyclov</td>
<td>83%</td>
<td>100%</td>
<td>20%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>37%</td>
<td>43%</td>
<td>27%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>91%</td>
<td>94%</td>
<td>69%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>15%</td>
<td>28%</td>
<td>20%</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>78%</td>
<td>79%</td>
<td>27%</td>
</tr>
</tbody>
</table>

In conclusion, invitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution and disk diffusion testing with Erythromycin and clindamycin discs in nonadjacent positions. Erythromycin and clindamycin disc approximation test (Dtest) is simple, reliable method to detect iMLS resistant to clindamycin. D test is necessary to correctly discriminate between iMLSB resistance and true susceptibility.

The different patterns of resistance phenotypes observed in various studies are because iMLSB resistance varies by geographical region, age group, methicillin susceptibility and even from hospital to hospital. Hence it should be determined in individual settings. Periodic surveillance of the prevalence of iMLSB isolates in the community and effective policy for the control of antimicrobial usage is required to monitor and to prevent the spread of these strains.

**Reference**


