Original Research Article  

MRSA and Inducible Clindamycin resistance in *Staphylococcus aureus* from Various Samples in A Tertiary Care Hospital

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The study was done to note the presence of MRSA and Inducible resistance to Clindamycin in various samples of the *Staphylococcus aureus* from the Hospital. A total of 1607 of Staphylococci were isolated from various clinical samples like Pus, Wound swab, Body fluids, Urine, Vaginal swab, Throat swab and other samples coming from hospital. Among the 1607 clinical isolates of *Staphylococcus aureus* MRSA (Methicillin resistance *Staphylococcus aureus*) were seen in 695 (43.2%). Among this 188 (97MRSA, 91MSSA) isolates showed Inducible Clindamycin resistance that is resistance to Erthromycin and sensitive to Clindamycin sensitive showing D test positive. 524 (294MRSA; 230MSSA) showed Constitutive MLsB resistance that is resistance to both Erythromycin and Clindamycin. The resistance of inducible Clindamycin and Constitutive MLsB was more in MRSA than in Methicillin Sensitive *Staphylococcus aureus*. In addition to MRSA and inducible Clindamycin resistance other antibiotics were also noted.

**A B S T R A C T**

**Keywords**
Clindamycin resistance, Patient, *Staphylococcus*, Methicillin resistance, Outbreaks

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**Introduction**

Among the genus *Staphylococcus* the most pathogenic is *Staphylococcus aureus*. This organism is responsible for causing suppurative types of infections. They have ability to spread and cause outbreaks in hospitals. Most *Staphylococcus aureus* infections resolve spontaneously or in response to antibiotic treatment, but in recent years there have been increasing in developing resistance to multiple antibiotics (Srinivasan et al., 2002).

Among the commonest resistance is Methicillin resistance *Staphylococcus aureus* (MRSA). MRSA is responsible for causing outbreaks in hospital. MRSA resistance was first noted in 1961. Methicillin resistance is usually confirmed by altered penicillin
binding protein that causes resistance to all β-lactam antimicrobial agents (Fishovitz et al., 2014). Although a predominantly a hospital pathogen, MRSA is becoming more common in community infections. Hospital personnel harboring MRSA act as chief source of nosocomial infections. The other predisposing factors that increase the chance of emergence and spread of MRSA are prolonged and repeated hospitalization, indiscriminate use of antibiotics, and lack of awareness, intravenous drug abuse, and presence of indwelling medical devices (Weisblum et al., 1985).

The resistance in Staphylococcus is also seen with Erythromycin (ERY) (a Macrolide) and Clindamycin (CLI) (a Lincosamide). These two drugs represent two distinct classes of antimicrobial agents that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells. Resistance to both of these antimicrobial agents can occur through methylation of their ribosomal target site. Such resistance is typically mediated by erm genes. Resistance to macrolides also can occur by efflux, typically mediated by the msrA gene. An in vitro induction test can distinguish Staphylococci that have inducible erm-mediated resistance from those with msrA mediated resistance. Methicillin-resistant Staphylococcus aureus (MRSA) are increasingly being reported as multidrug resistant with high resistance to Macrolides (Erythromycin, Clarithromycin) and Lincosamides (Clindamycin, Lincomycin) leaving very few therapeutic options.

Main aim of the study was to see the presence of MRSA and inducible Clindamycin resistance in Staphylococcus aureus.

Materials and Methods

The study was conducted in the Bacteriology section of Microbiology Department of one year from June 2017 to June 2018 in Government Medical College Aurangabad. During this period 1607 Staphylococci aureus were isolated from various clinical samples like blood culture, urine, pus, vaginal swab, throat swabs, body fluids and others (sputum, aspirates, respiratory, central line/neck line/umbilical catheter tips) were included in the study.

Isolation of Staphylococcus aureus were done by conventional bacteriological methods such as colony morphology, Gram staining, Catalase, Coagulase test. All samples were processed as per standard procedures.

Antibiotic sensitivity testing of Staphylococcus aureus was done using Modified Kirby Bauer disc diffusion method (CLSI guidelines Jan 2017).

The isolates were tested for Cefoxitin (30μg), Amoxillin, Azithromycin (15 μg), Erythromycin (15 μg), Cotrimaxazole (1.25/23.75 μg), Ciprofloxacillin (5 μg), Clindamycin, Vancomycin and Linezolid (30 μg).

The Staphylococcus aureus isolates were assessed for Methicillin and Clindamycin resistance. Methicillin resistance was determined by using Cefoxitin antibiotic (30μg) disc. In our study MRSA were 695 (43.2%) and MSSA were 912 (56.7%) as shown in Table 1.

Maximum number of MRSA were found in Pus 340 (48.9%), Blood 194 (27.9%), body fluids 50 (7.1%), Urine 20 (2.8%), throat swabs 20 (2.8%), Vaginal 9 (1.2%) and others 62 (8.9%) as shown in Table 2.

Detection of Inducible Clindamycin test is done on same plate by placing Clindamycin (2μg) discs is placed 15mm from an Erythromycin(15μg) adjacent to each other on Mueller-Hinton agar. Flattening of the
Clindamycin zone suggests inducible Clindamycin resistance (Watanakunakorn et al., 1976). According to studies, D test should be used as a mandatory method in routine disc diffusion testing to detect inducible Clindamycin resistance in Staphylococci for the optimum treatment of patients.

Out of 1607 staphylococcus aureus (695 MRSA; 912 MSSA) were isolates. Out of this 355 (64 MRSA; 291MSSA) were susceptible to Erthryomycin and Clindamycin. 524 (294MRSA; 230MSSA) showed Constitutive MLSB resistance that is resistance to both Erythromycin and Clindamycin.

188 (97 MRSA, 91 MSSA) isolates showed Inducible Clindamycin resistance that is resistance to Erthromycin and sensitive to Clindamycin sensitive showing D test positive as shown in Table 3. The resistance of inducible Clindamycin was more in MRSA that is 97 (13.9%) than in MSSA.

And those showing MS Phenotype 540(240 MRSA; 300 MSSA) showed Erythromycin resistance and Clindamycin sensitive and D test negative.

In addition to MRSA and inducible Clindamycin resistance other antibiotics also shows resistance. These drugs were Amoxclav (79%), Co-trimoxazole (56%) and Gentamycin (40.2%). However, all (100%) isolates were susceptible to Vancomycin and Linezolid.

Results and Discussion

Staphylococcus aureus is a common human pathogen that causes a wide variety of infections. MRSA is a major Nosocomial pathogen causing significant morbidity and mortality (Sachdev et al., 2003). In our study MRSA was found to be 43.4% of the total Staphylococcus aureus isolates, similar type of study was done by Mittal S et al., had reported the same percentage of MRSA (Mittal et al., 2019).

In our study among the various samples maximum number of MRSA were found in Pus 340 (48.9%) samples. Similar observation was made by Rupali Mantri et al., (2014) in Akola and reported high isolation rate of upto 67.85% of MRSA strains from pus specimens.

188 (97MRSA, 91MSSA) isolates showed Inducible Clindamycin resistance that is resistance to Erthromycin and sensitive to Clindamycin showing D test positive. 524 (294MRSA; 230MSSA) showed Constitutive MLSB resistance that is resistance to both Erythromycin and Clindamycin. The resistance of inducible Clindamycin 97 (13.9%) and 294 (56.1%) Constitutive MLSB resistance was more in MRSA than in MSSA. Similar observation was noted in Javeria Firdous and Patil (2019).

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms Fiebelkorn KR et al., (2003)

The increasing incidence of MRSA brought the need for other effective antibiotics. Clindamycin is a drug which fits that role. It can be given orally. It is safe as well as effective. All these lead to the increased usage of Clindamycin and thereby development of resistance to it. This makes the detection of Clindamycin resistance important and useful (Fig. 1 and 2).
Table 1 Distribution of MRSA in *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Staphylococcus isolates</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>695</td>
<td>43.2%</td>
</tr>
<tr>
<td>MSSA</td>
<td>912</td>
<td>56.7%</td>
</tr>
<tr>
<td>Total</td>
<td>1607</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Distribution of *Staphylococcus* and MRSA in various samples

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Samples</th>
<th>Total samples</th>
<th><em>Staphylococcus aureus</em></th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pus</td>
<td>3373</td>
<td>600</td>
<td>340</td>
</tr>
<tr>
<td>2</td>
<td>Blood</td>
<td>2872</td>
<td>543</td>
<td>194</td>
</tr>
<tr>
<td>3</td>
<td>Body fluids</td>
<td>100</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Urine</td>
<td>1057</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Throat swabs</td>
<td>50</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Vaginal swab</td>
<td>70</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Others</td>
<td>981</td>
<td>332</td>
<td>62</td>
</tr>
</tbody>
</table>

Fig.1 MRSA with positive D-test
Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses. Clindamycin is also used as an alternative for patients who are allergic to penicillin. MRSA isolates of our study were multidrug resistant. In addition to MRSA and Erthromycin resistance high antibiotic resistance to Amoxclav (79%), Cotrimoxazole (56%) and Gentamycin (40.2%) were noted. Vancomycin and Linozolid were found to be sensitive in MRSA and MSSA cases.

The degree of resistance or sensitivity of MRSA towards commonly used antimicrobials differs from one place to other. Therefore it is necessary to see the antibiotic sensitivity pattern in MRSA cases. In present study reliable method for detection of MRSA
and inducible resistance to Clindamycin in isolates of Staphylococcus aureus were used.
On this basis reporting of resistance to Cefoxitin and Inducible Clindamycin can be
done on routine basis. We conclude that whenever Clindamycin is intended for
Staphylococcus aureus infection, the microbiology lab should tests the isolated
organism for iMLSB by D test. D test is simple, inexpensive and easy to perform test.
Clindamycin is drug of choice in case of D test negative isolates while it is not suitable for
D test positive isolates.

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