Abstract:
ABSTRACT-Objective -To demonstrate inducible clindamycin resistance in the Staphylococcal isolates from various clinical samples by D- test .Materials and methods- A total of 125 Staphylococcus aureus and COagulase Negative Staphylococcus (CONS) isolates were collected. Of the isolates, 82 strains were erythromycin resistant. They were tested for inducible clindamycin resistance by using the D -test (at 15mm disc separation) as per CLSI guidelines.Results- In this study, among the 125 isolates, 11 percentage showed inducible clindamycin resistance (iMLSB) .Inducible clindamycin resistance observed among MRSA, MSSA, and MRCONS were 16 percent, 5 percent and 11 percent respectively. There was no inducible clindamycin resistance seen in MSCONS. Constitutive resistance (cMLSB) was seen in 9 percentof the total isolates. The MS phenotype strains were 46 percent and S phenotype were 34 percent.Conclusion- The inducible clindamycin resistance in this study was 11 percent and it was higher among MRSA (16 percent). D-test is a very convenient test to perform. It can be done along with the routine antibiotic susceptibility testing in all the staphylococcal isolates. Accurate reporting of clindamycin resistance is mandatory for a successful treatment outcome.
Keyword : Staphylococcus aureus, inducible clindamycin resistance, Coagulase Negative Staphylococcus, D-Test.

INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCI IN HOSPITAL ABSTRACT-:
Objective
To demonstrate inducible clindamycin resistance in the Staphylococcal isolates from various clinical samples by D- test.
Materials and methods:-
A total of 125 Staphylococcus aureus and COagulase Negative Staphylococcus (CONS) isolates were collected. Of the isolates, 82 strains were erythromycin resistant. They were tested for inducible clindamycin resistance
by using the D-test (at 15mm disc separation) as per CLSI guidelines.

Results:
In this study, among the 125 isolates, 11 percent showed inducible clindamycin resistance (iMLS\textsubscript{B}). Inducible clindamycin resistance observed among MRSA, MSSA, and MRCONS were 16 percent, 5 percent and 11 percent respectively. There was no inducible clindamycin resistance seen in MSCONS. Constitutive resistance (cMLS\textsubscript{B}) was seen in 9 percent of the total isolates. The MS phenotype strains were 46 percent and S phenotype were 34 percent.

Conclusion:
The inducible clindamycin resistance in this study was 11 percent and it was higher among MRSA (16 percent). D-test is a very convenient test to perform. It can be done along with the routine antibiotic susceptibility testing in all the staphylococcal isolates. Accurate reporting of clindamycin resistance is mandatory for a successful treatment outcome. Keywords-Staphylococcus aureus, inducible clindamycin resistance, Coagulase Negative Staphylococcus, D-Test.

Materials and Methods
This cross-sectional study was conducted in Tirunelveli medical college hospital at Tirunelveli, from December 2010 to April 2011. A total of 125 isolates of Staphylococcus species from various clinical samples like pus, sputum, urine, blood, and body fluids were collected and tested. The specimens were cultured on Lincosamide – Streptogramin B (MLS\textsubscript{B}) antibiotics. Clindamycin is preferred for treating the infections because of its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production. Clindamycin furthermore, is a useful choice in cases of penicillin allergy. (Kasten) Macrolide resistance may be due to the enzymes encoded by erm genes. They are MLS\textsubscript{B} phenotype, which may be constitutive (cMLS\textsubscript{B}) or inducible (iMLS\textsubscript{B}). A second mechanism of resistance is due to active efflux pump encoded by msrA gene. They are the MS phenotype. (Betran.G. Katzag) Strains with inducible clindamycin resistance are not detectable by the routine antimicrobial susceptibility tests. They appear to be erythromycin resistant and clindamycin susceptible in vitro. In these cases, management with clindamycin will not be effective. To overcome this, the Clinical and Laboratory Standards Institute (CLSI) has recommended the erythromycin-clindamycin disc approximation test (D-test). The present study was done to find out the prevalence of erythromycin induced clindamycin resistance in Staphylococcus aureus and Coagulase Negative Staphylococci (CONS) isolates of our institution using this simple, reliable test.
Nutrient agar, Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24 hours. The isolates were identified by using conventional laboratory methods like colony morphology, gram staining, catalase test, coagulase test and standard biochemical tests. (Patrick R Murray Manual) Antibiotic sensitivity testing was performed by Kirby – Bauer disc diffusion method on Mueller - Hinton agar plates using the following antibiotics, ciprofloxacin (5gm), clindamycin (2 gm), gentamicin (10gm), erythromycin (15gm), vancomycin (30gm), cefoxitin (30g), doxycycline (30gm), co-trimoxazole (1.25/23.75 gm). Methicillin resistance was detected by taking cefoxitin as a surrogate marker. Results were interpreted according to the CLSI guidelines. Antibiotic discs were procured from HiMedia India, Private Limited. Staphylococcus aureus ATCC 25923 was used for quality control. Positive strain (ATCC BAA 977 ermA) and negative strain (ATCC BAA 976 efflux pump msrA) (Pfizer) were used as quality control for D-test. (Angel et al)

**Interpretation of the diameters of zones of inhibition was done according to CLSI guidelines as follows**

ER S > 23mm; ER intermediate sensitive - 14-22 mm; ER –R ≤13 mm
CL-- S > 21mm; CL intermediate sensitive -15-20 mm; CL - R ≤ 14mm.

Cefoxitin – S ≥ 22mm; Cefoxitin- R ≤ 2 mm .

[ER-Erythromycin, CL-Clindamycin, S – Sensitive, R-Resistant.]

**D- Test**

The susceptibility testing was performed among the staphylococcal isolates by Kirby –Bauer disc diffusion method. A 0.5 Mac Far lands standard bacterial suspension was inoculated on Mueller-Hinton agar plates. Erythromycin (15g) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2g) disc on the same plate and were incubated at 37°C overnight.

**INTERPRETATION OF D- TEST:**

Four different phenotypes were interpreted as follows

1. **Inducible iMLS$_B$ phenotype** – isolates showing resistance to erythromycin (zone size 13mm) and sensitive to clindamycin (zone size 21mm) with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc. (Fig. 1)

2. **Constitutive cMLS$_B$ phenotype** – isolates showing resistance to both erythromycin (zone size13mm) and clindamycin (zone size 14mm) with circular shape of zone of inhibition around clindamycin.

3. **MS phenotype** – isolates showing resistance to erythromycin (zone size 13mm) while being sensitive to clindamycin (zone size 21 mm) with a circular zone of inhibition around clindamycin.

4. **S phenotype** – isolates showing sensitivity to both erythromycin zone size 23mm and clindamycin 21mm. (Surrerat et al)
Figure 1
Figure 1. Double-disc diffusion test (D-test) showing E (Erythromycin disc) induction of C (Clindamycin disc) resistance - blunting of the zone of inhibition around the C disc is produced that forms a D shape.

Figure 2
Figure 2-Constitutive cMLS\textsubscript{B} phenotype – isolates showing resistance to both erythromycin (zone size 13mm) and clindamycin (zone size 14mm) with circular shape of zone of inhibition around clindamycin.

Figure 3
Figure 3- Positive control isolate showing resistance to erythromycin (zone size 13mm) and sensitive to clindamycin (zone size 21mm) with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.

Figure 4
Figure 4- Negative control isolate showing resistance to erythromycin (zone size 13mm) while being sensitive to clindamycin (zone size 21 mm) with a circular zone of inhibition around clindamycin.

Statistical Analysis
The data were analyzed with the help of the statistical software namely PASW statistics 18.0. The P values <0.05 were considered as significant (P<0.05)

Results
Among the 125 isolates studied, 74 were Staphylococcus aureus and 51 were CO-agulase Negative Staphylococci (CONS). Inducible clindamycin resistance in this study was 14 out of the total isolates 11%. (14/125=11%). Out of the 14, there were eight iMLS\textsubscript{B} among MRSA, five iMLS\textsubscript{B} isolates among MRCONS and one iMLS\textsubscript{B} isolates among MSSA. There was no inducible clindamycin resistance seen among MSCONS. There were 51 MRSA strains out of the total 125 isolates, among the 51 MRSA strains iMLS\textsubscript{B} were 8 which is 16%. (8/51=16%). The constitutive resistance (cMLS\textsubscript{B}) observed among the total isolates was 11(9%).Out of 11 isolates, 6 were MRSA and 5 were MRCONS.There was no constitutive resistance observed among MSSA and MSCONS. The MS phenotype seen among the total isolates was 57 (46%).Out of them, 22, 10, 23, and 2 were MRSA,
MSSA, MRCONS and MSCONS respectively. The S phenotype were observed to be 43 (34%) among the total isolates. Out of them, 15 were MRSA and 12 were MSSA, 13 were MRCONS and 3 were MSCONS. Macrolide resistance patterns of the isolates are given in Table 1.

**Table 1. Distribution of phenotypes.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA-Methicillin Resistant Staphylococcus aureus</td>
<td>15</td>
</tr>
<tr>
<td>MRCONS-Methicillin Resistant COagulase-Negative Staphylococci</td>
<td>13</td>
</tr>
<tr>
<td>MSSA-Methicillin-Sensitive Staphylococcus aureus</td>
<td>12</td>
</tr>
<tr>
<td>MS phenotype - Erythromycin resistant and susceptible to clindamycin</td>
<td>13</td>
</tr>
<tr>
<td>S phenotype - Susceptible to both erythromycin and clindamycin</td>
<td>3</td>
</tr>
<tr>
<td>iMLSB - inducible clindamycin resistance</td>
<td>9</td>
</tr>
<tr>
<td>cMLSB - constitutive erythromycin and clindamycin resistance</td>
<td>4</td>
</tr>
<tr>
<td>ER - Erythromycin resistant isolates</td>
<td>1</td>
</tr>
</tbody>
</table>

The comparative interpretations given in table 2 revealed that the iMLS_B among MRSA (15.7%) was greater than the iMLS_B of MSSA (4.3%). The difference between them was statistically significant (P<0.05). Similarly, the cMLS_B (11.88%) of MRSA was statistically highly significant. The MS phenotypes among the MRSA (43.1%) and MSSA (39.1%) was not significant statistically (P>0.05). The S phenotypes in MRSA (29.4%) were lesser than that of MSSA (56.5%). The difference was statistically highly significant. (P<0.01).

**Discussion:**
The highlight among staphylococcal infections is their emerging multidrug resistant patterns. Increase in methicillin resistance is observed among staphylococcal isolates. Treatment of these infections is a difficult task for the clinicians, unless detected appropriately. Clindamycin can be the drug of choice in these situations. Cross resistance among the MLS_B group is another challenging factor.

A precise antibiotic susceptibility testing is of utmost importance, in treating patients successfully with clindamycin. The D-test is a convenient test to detect inducible clindamycin resistance along with the routine antibiotic susceptibility testing. It helps to overcome misinterpretation and treatment failures. It also serves as a useful tool for MLS_B phenotyping.

The prevalence of inducible clindamycin (iMLS_B) resistance varies from area to area and also from hospital to hospital. In this study, among the 125 isolates, 11% inducible clindamycin resistant strains were detected. Inducible resistance pattern in this study was in concordance with that reported by Sureerat et al with 9.9%. Fiebelkorn et al had 10% of iMLS_B phenotype and Ciraj et al from Karnataka reported 13.1% iMLS_B. A north Indian study showed higher incidence of inducible resistance (67%) than that of the present study. (Gupta et al) In this study there was (19%) iMLS_B among Staphylococcus aureus isolates. This was in close agreement with that of Kyung –Hee Kim et al who had 21.1% inducible resistance among Staphylococcus aureus. Deotale et al have reported 14.5% iMLS_B among Staphylococcus aureus in their study. Inducible clindamycin resistance was higher in MRSA (16%) when compared to MSSA (5%) and MRCONS (11%). There was no inducible resistance observed in MSCONS. A study from Turkey had inducible clindamycin resistance of 10.7% in MSSA. (Delioglu et al). Shailesh et al reported an incidence of 18.8% of iMLS_B in MRCONS which is in range with the results of this study. The low constitutive clindamycin resistance in this study (9%) was in range with the study of Deotale et al.
it may be due to the minimal usage of clindamycin in the hospital.

### Conclusion
This study will help in documentation and understanding of inducible clindamycin resistance in this area. This is not only important in the clinicians point of view but also for epidemiological purposes. Hence, this simple, easy to perform D-test is recommended routinely for the detection of inducible clindamycin resistant (iMLS$_B$) isolates. Blind prescription of clindamycin without antibiotic susceptibility testing can lead on to treatment failures. Therefore, in any hospital set up, regular surveillance of resistance pattern will be useful in formulating institutional antibiotic policies and for a better antibiotic approach.

### References


Singh, P. Charles & S. Stephen: Detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci - a study from South India. The Internet

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name Of the Drugs</th>
<th>Name of the Organisms</th>
<th>Total no of isolates (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus(n=69)</td>
<td>CONS(n=4)</td>
</tr>
<tr>
<td>1</td>
<td>Ampicillin (A)</td>
<td>29 (28.9%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>2</td>
<td>Erythromycin (E)</td>
<td>21 (30.4%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>Cotrimoxazole (CO)</td>
<td>25 (35.2%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>4</td>
<td>Gentamicin (G)</td>
<td>50 (72.4%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>5</td>
<td>Ciprofloxacin(CF)</td>
<td>49 (71%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>6</td>
<td>Vancomycin (V)</td>
<td>69 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>7</td>
<td>Cefoxitin(CX)</td>
<td>37 (53.6%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>8</td>
<td>Oxacillin (OX)</td>
<td>37 (53.6%)</td>
<td>3 (75%)</td>
</tr>
</tbody>
</table>
Sureerat Ce-lae, Varaporn, Manthana, Urait, Sineenart.
