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INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATES FROM VARIOUS CLINICAL SAMPLES

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Abstract :
BACKGROUND Increasing frequency of methicillin resistant Staphylococcus aureus (MRSA) infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide - lincosamide- streptogramin (MLS) antibiotics to treat such infections. Widespread use of macrolide- lincosamide- streptogramin (MLS) antibiotics, has led to an increase in a number of staphylococci acquiring cross-resistance to MLS antibiotics. The most common mechanism for such resistance is target site modification mediated by erm genes which can be expressed either constitutively (constitutive MLSB phenotype) or inducibely (inducible MLSB phenotype). Another mechanism of resistance mediated through msr A genes i.e. efflux of antibiotic. This study aims at detecting inducible clindamycin resistance among Staphylococcus aureus isolates collected from various clinical samples by using D test. AIM AND OBJECTIVE To detect the methicillin resistant Staphylococcus aureus. To detect the inducible clindamycin resistance among Staphylococcus aureus isolates by using D test. MATERIALS AND METHODS Staphylococcus aureus were identified by colony characteristics and standard biochemical techniques. Methicillin resistant Staphylococcus aureus was detected using cefoxitin (30micrograms) disc diffusion method as per CLSI 2013 guidelines. Inducible Clindamycin resistance was detected by D test according to CLSI 2013 guidelines. RESULT Out of 489 samples, 200(40.89 percent) were found to be erythromycin resistant. inducible clindamycin resistance (iMLSB phenotype) was observed in 76(15.54 percent), Constitutive resistance (cMLSB phenotype) was seen in 64(13.08 percent), clindamycin sensitive and erythromycin resistance (MS Phenotype) was observed in 60(12.26 percent). Inducible clindamycin resistance (iMLSB phenotype) was higher in MRSA samples 49(18.70 percent) than in MSSA 27(11.89 percent). CONCLUSION It is imperative to detect inducible clindamycin resistance by using D test which should be done routinely in laboratory to guide the clinician for the judicious use of clindamycin and for effective treatment with clindamycin.
Keyword : Staphylococcus aureus, iMLSB phenotype, MRSA, D test.

INTRODUCTION:
Staphylococcus aureus is one of the most common pyogenic bacteria infecting man. Staphylococcus aureus is known for acquiring antimicrobial resistance promptly after the introduction of new antibiotics1. The increasing prevalence of resistance to most antimicrobial agents in Staphylococci, enabled the spread of resistant strains in the community, signifying the need for new effective agents to treat Staphylococcal infections2. Increasing frequency of methicillin resistant *Staphylococcus aureus* (MRSA) infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide - lincosamide- streptogramin (MLS) antibiotics to treat such infections 3, 4. Clindamycin belongs to lincosamide class and acts by protein synthesis inhibition. It is often used in the treatment of skin and soft-tissue infections including osteomyelitis, caused by the Staphylococcal species as well as anaerobes. It has excellent tissue penetration (except for the CNS) and accumulates in abscesses and no renal dosing adjustments are needed. Good oral absorption makes this drug an important option in outpatient therapy or as a follow up after intravenous therapy. Clindamycin is also used as an alternative for patients who are allergic to penicillin3, 4, 5, 6, 7. Clindamycin has long been an option for the treatment of both methicillin susceptible Staphylococcus aureus (MSSA) and Methicillin resistant Staphylococcus aureus (MRSA) infections3, 8.

The macrolide lincosamide-streptogramin B (MLSB) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due its excellent pharmacokinetic properties9, 10. Widespread use of macrolide–lincosamide–streptogramin (MLS) antibiotics, has led to an increase in a number of staphylococci acquiring cross-resistance to MLS antibiotics. This cross-resistance to MLS antibiotic (MLS resistance) in Staphylococci is generally attributable to one of two mechanisms 2, 4.

The most common mechanism for such resistance is target site modification mediated by *erm* genes which results in rRNA methylase production that can be either constitutive (constitutive MLSB) or inducible (iMLSB phenotypes) where methylase is produced only in the presence of an inducer like erythromycin. Strains with *erm* mediated erythromycin resistance may possess inducible clindamycin resistance but

may appear susceptible to clindamycin by disc diffusion. Another mechanism is efflux mechanism encoded by *mrs (A)* gene and confers resistance to macrolides and type B streptogramins, but clindamycin remains active (MSB phenotype) 2,4,5,6,9,11.There is a third mechanism which appears to be rare is by inactivation of lincosamides by chemical alteration mediated by the *inu A* gene11. If inducible clindamycin resistance can be reliably detected on a routine basis by D test in clinically significant isolates, clindamycin can be safely and effectively used only in those patients with truly Clindamycin-susceptible strains6. This study aims at detecting inducible clindamycin resistance among staphylococcus aureus isolates collected from various clinical samples by using D test.

AIM AND OBJECTIVE:

1. To detect the methicillin resistant Staphylococcus aureus.
2. To detect the inducible clindamycin resistance among Staphylococcus aureus isolates by using D test.

MATERIALS AND METHODS:

Type of study: Cross sectional study. 489 Staphylococcus aureus isolates from various clinical samples like pus, urine, sputum, blood and body fluids were studied from May 2014 to September 2014. Bacteria other than Staphylococcus aureus were excluded. The isolates were identified as Staphylococcus aureus by colony characteristics and standard biochemical techniques12. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller Hinton agar plates. Methicillin resistant Staphylococcus aureus was detected by disc diffusion method using cefoxitin (30g) as per CLSI 2013 guidelines13. Inducible Clindamycin resistance was detected by D test according to CLSI 2013 guidelines13.

Cefoxitin disc diffusion test for detection of MRSA13, 14

The test is performed by placing 30g of cefoxitin disc in the Mueller Hinton Agar plate inoculated with bacterial suspension equivalent to 0.5 McFarland standard and the plate is incubated at 37°C . The zone of inhibition is determined after 18 hrs and the zone size is interpreted as susceptible 21mm, resistant 21 mm as per CLSI 2013 guidelines.

D-test - Erythromycin and clindamycin disc approximation test 5, 9, 13

Staphylococcus aureus isolates which were resistant to erythromycin were subjected to “D test” as per CLSI 2013 guidelines. The test was done by placing clindamycin disc(2 g) and erythromycin disc (15 g) at a distance of 20 mm (edge to edge) on Mueller Hinton agar plate inoculated with 0.5 McFarland bacterial suspension. Following incubation at 37°C for16-18hrs, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicates inducible clindamycin resistance. Three different phenotypes were appreciated and interpreted as follows:

1. **Sensitive phenotype (S Phenotype):** Staphylococcus aureus isolates which was sensitive to both erythromycin (zone size 23 mm) and clindamycin (zone size 21 mm).
2. **Constitutive MLSB phenotype (cMLSB):** Staphylococcus aureus isolates which showed resistance to both erythromycin (zone size 13 mm) and clindamycin (zone size 14mm) with circular shape zone of inhibition around clindamycin.
3. **Inducible MLSB phenotype (iMLSB):** Staphylococcus aureus isolates which showed resistance to erythromycin (zone size 13 mm) while being sensitive to clindamycin (zone size 21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc **(D test positive)**.
4. **MS phenotype:** Staphylococcus aureus isolates exhibiting resistance to erythromycin (zone size 13 mm), while sensitive to clindamycin (zone size 21 mm) and giving circular zone of inhibition around clindamycin **(D test negative).**

RESULT:

489 Staphylococcus aureus isolates collected from various clinical samples were studied. 200(40.89%) were found to be erythromycin resistant and subjected to D test.

TABLE NO. 1 – DISTRIBUTION OF STAPHYLOCOCCUS AUREUS (n=489).

Samples	Total no of Staphylococcus aureus isolates(n=489)
Pus	341(69.73%)
Urine	84(17.17%)
Blood	53(10.83%)
Sputum	7(1.43%)
Fluid	4(0.82%)

CHART -1
DISTRIBUTION OF STAPHYLOCOCCUS AUREUS (n=489).

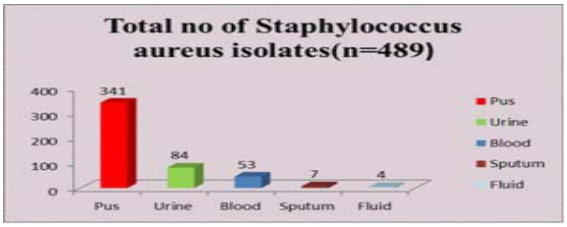


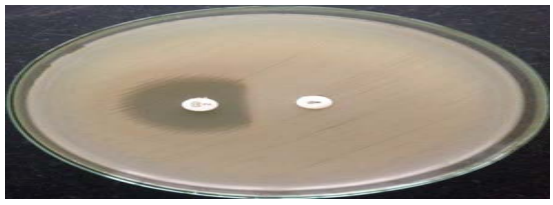
TABLE NO 2

Distribution of the MS_B, $iMLS_B$ and $cMLS_B$ resistance phenotypes among erythromycin resistant Staphylococcus aureus (n=489)

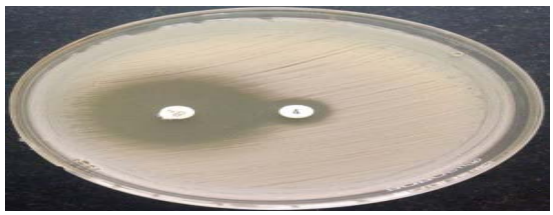
Test	Zone diameter (mm)	No (%)
S phenotype	**E23mm; C21 mm.**	289(59.10%)
Constitutive $cMLS_B$ Phenotype	**E 13 mm; C14 mm.**	64(13.08%)
Inducible $iMLS_B$ phenotype **(D test positive)**	**E 13 mm; C21 mm.** D shaped zone of inhibition around clindamycin and flattening towards erythromycin disc	76(15.54%)
MS Phenotype **(D test negative)**	**E 13 mm; C21 mm.** circular zone of inhibition	60(12.26%)
Total		489

* **E - Erythromycin; * C- Clindamycin.**

Figure - 1



iMLSB phenotype
Figure - 2



MS phenotype

CHART - II
Distribution of the MSB, iMLSB and cMLSB resistance phenotypes among erythromycin resistant Staphylococcus aureus (n=489)

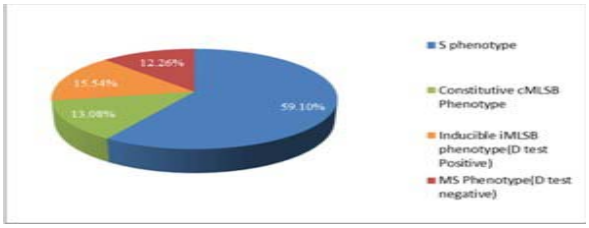


TABLE NO 3 – MSSA VS MRSA (n=489)

Methicillin susceptible Staphylococcus aureus **MSSA**	Methicillin resistant Staphylococcus aureus **MRSA**
227(46.42%)	262(53.57%)

TABLE NO 4

Distribution of the MS_B, $iMLS_B$ and $cMLS_B$ resistance phenotypes among MRSA and MSSA.

Test	MRSA (n=262)	MSSA (n=227)
S phenotype	152(58.01%)	137(60.35%)
Constitutive $cMLS_B$ Phenotype	19(7.25%)	45(19.82%)
Inducible $iMLS_B$ phenotype (D test positive)	49(18.70%)	27(11.89%)
MS Phenotype (D test negative)	42(16.03%)	18(7.92%)
Total	**262**	**227**

CHART- III
Distribution of the MSB, iMLSB and cMLSB resistance phenotypes among MRSA and MSSA.

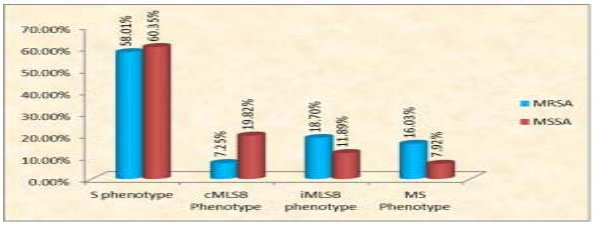
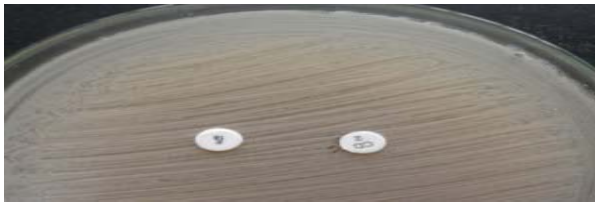
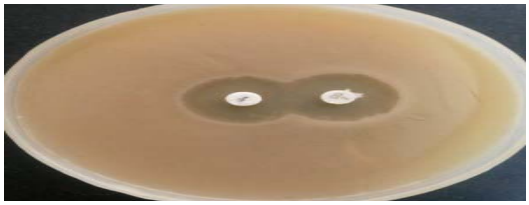


Figure -3



Constitutive cMLSB phenotype

Figure -4



S phenotype

DISCUSSION

As per table 1: Out of 489 samples, 341(69.73%) Staphylococcus aureus were isolated from pus samples, 84 (17.17%) were from urine, 53(10.83%) were isolated from blood culture samples, 7(1.43%) were isolated from sputum samples, 4(0.82%) were from fluid samples. As per table 2: In our study, Inducible clindamycin resistance (iMLSB phenotype) was observed in 76(15.54%), Constitutive resistance (cMLSB phenotype) was seen in 64(13.08%), clindamycin sensitive and erythromycin resistance (MS Phenotype) was observed in 60(12.26%), S phenotype was seen in 289(59.10%).This study is similar to Deotale et al 9 observed that iMLSB phenotypes are 14.5%. Another study by Gadepalli et al3 showed 21% iMLSB phenotype. Velvizhi et al15 showed that 14.2% belong to iMLSB phenotype whereas Ajantha GS et al1 reported 49% of iMLSB phenotype. In our study, Constitutive resistance (cMLSB phenotype) was seen in 64(13.08%), which is in concordance to Deepak Juyal et al10 whose study found that 12.6% were of cMLSB phenotype. Gadepalli et al3 showed that 26.5% belong to cMLSB phenotype. As per table 3: Out of 489 Staphylococcus aureus, 262(53.57%) were methicillin resistant by cefoxitin disc diffusion test whereas 227(46.42%) were methicillin sensitive Staphylococcus aureus which is similar to the results obtained by Gadepalli at al 3(52%) were MRSA, in Mallick SK et al4 (51.6%) were MRSA and in S. Anuprabha et al16 (54.8%) were MRSA. As per table 4 : In our study, inducible clindamycin resistance (iMLSB phenotype) was higher in MRSA samples 49(18.70%) than in MSSA 27(11.89%).This was in concordance to few studies done by Gadepalli et al 3 which showed 30% in MRSA and 10% in MSSA respectively, Deotale et al 9 which showed D test positive isolates higher among MRSA(27.6%). Deepak Juyal et al10also showed that iMLSB phenotype was higher in MRSA (19.4%) than MSSA (6.3%). Angel et al6 observed that iMLSB phenotype was higher in MRSA (64%) than MSSA (5%). Invitro detection of inducible clindamycin resistance by using cost effective D test will be useful for judicious clinical use of clindamycin to effectively treat Staphyloccal infections.

CONCLUSION:

The prevalence of inducible clindamycin resistance varies in geographical region and from hospital to hospital. Data for antibiotic susceptibility should be accurate for effective treatment decision5. Since there is less availability of antibiotics for the treatment of MRSA infections, clindamycin should be considered as a part of the regimen in the management of serious skin and soft tissue infections. Clindamycin can be effective in treatment of D test negative isolates (MS phenotype) which shows erythromycin resistant and true clindamycin sensitive as observed in *invitro* test. Inspite of observing clindamycin sensitivity invitro there may be treatment failures because of inducible clindamycin resistance. Hence, it is imperative to detect inducible clindamycin resistance by D test which should be done

routinely in laboratories to guide the clinician for the judicious use of clindamycin.

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