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Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care hospital.

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Abstract:

Background: *Staphylococcus aureus* stands as a prominent cause for both nosocominal and community acquired infections across the globe. Clindamycin, a member of MLS_B family, has emerged as the preferred choice for treating *Staphylococcus aureus* infections. However, one critical consideration in administering Clindamycin is the potential development of inducible Clindamycin resistance, posing a significant risk of clinical treatment failure.

Aim: The study was aimed to determine the occurence of inducible Clindamycin resistance among *Staphylococcus aureus* isolates in our geographic region.

Method: The hospital based cross sectional study was conducted over a period of one year from January 2023 to December 2023. A total of 672 Staphylococcus aureus isolates were identified by conventional methods and subjected to antimicrobial susceptibility test by Kirby Bauer disk diffusion method and Erythromycin resistant isolates were tested for D test.

Result: Out of 672 *Staphylococcus aureus* isolates, 517 were MRSA and 155 were MSSA. Erythromycin resistance was seen in 395 (58.77%) isolates. D test revealed inducible clindamycin resistance in 45 (6.69%) isolates, constitutive clindamycin resistance in 91 (13.54%) isolates and MS phenotype in 259 (38.54%) isolates. Inducible clindamycin resistance was more in MRSA (7.73%) isolates compared to MSSA (3.22%) isolates.

Conclusion: D test is a simple and cost-effective test that should be performed as routine laboratory test and will help in guiding the clinicians regarding judicious use of Clindamycin.

Keywords: Inducible Clindamycin resistance, D test, inducible MSL_B phenotype, constitutive MSL_B phenotype, MS phenotype, MRSA, *Staphylococcus aureus*.

Introduction: Staphylococcus aureus is a major human pathogen responsible for diverse array of illness, ranging from minor skin infections, chronic bone infection to septicemia and endocarditis. Infections are common both in community and hospital settings and the treatment remains challenging due to emergence of multidrug resistant strains such as Methicillin resistant *Staphylococcus aureus* (MRSA). Antibiotics like Vancomycin, Linezolid, Quinupristin-Dalfopristin are prefered for therapy of MRSA isolates. However widespread utilization of these antibiotics has made the current usage of these therapeutic options often unsuccessful[1].

Macrolide (eg-Erythromycin) -lincosamide (eg- Clindamycin) -streptogramin B (MSL_B) a group of antibiotic serves as an alternative for the treatment of Staphylococcal infections .

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Clindamycin, a member of MLS_B family is preferred, because of its proven efficacy, safety, convenience of administration (parenteral and oral), and excellent pharmacokinetic properties. One important issue in Clindamycin treatment is the risk of clinical failure during therapy.

Therapeutic failure caused by MLS_B inducible resistance, are being more commonly reported[2].

The MSL_B resistance can occur through various mechanisms, one of which involves an efflux mechanism that is mediated by the *msr*A (Macrolide streptogramin resistance A) gene leading to manifestation of MS phenotype. These strains appear Clindamycin sensitive and Erythromycin resistant *in vitro* test and do not become Clindamycin resistant during therapy [3] . Another mechanism is -methylation of the ribosomal target site is mediated by *erm* (Erythromycin ribosome methylase) gene mediated by an rRNA methylase enzyme that inhibits protein synthesis by binding to the 50S ribosomal subunit. This mechanism can be either constitutive ($cMSL_B$) where this enzyme is always produced or inducible ($iMSL_B$) where an inducing agent (eg- Erythromycin) is required for its production [4].

In routine antimicrobial susceptibility testing of *Staphylococcus aureus* isolates with a constitutive phenotype exhibit resistance to both Erythromycin and Clindamycin. On the other hand, isolates with an inducible phenotype are resistant to Erythromycin but appear susceptible to Clindamycin if not placed adjacent to each other in the *in vitro* test. As a result, treating patients with Clindamycin in such circumstances leads to the emergence of constitutive *erm* mutants causing therapeutic failure [5].

Owing to this, the Clinical and Laboratory Standards Institute (CLSI) recommends using the double-disk diffusion method (*D*-test) for detecting inducible resistance to clindamycin among *Staphylococcus aureus* isolates [6] .

D-test involves the placement of an Erythromycin disk in proximity to the disk containing Clindamycin. As the Erythromycin diffuses through the agar, the resistance to the Clindamycin is induced, resulting in a flattening or blunting of the Clindamycin zone (D shaped zone) of inhibition adjacent to Erythromycin disk.

The local resistance data holds immense importance in optimizing antibiotics usage, guiding empirical treatment decisions and effectively managing infection. Thus, the present study aimed to determine the occurrence of inducible Clindamycin resistance among *Staphylococcus aureus* isolate from different clinical samples in a tertiary care hospital.

Objectives:

1.Phenotypic detection of inducible Clindamycin resistance in *Staphylococcus aureus* isolated from different clinical samples.

2. Antimicrobial susceptibility pattern among *Staphylococcus aureus*.

Materials and methods:

This hospital based cross sectional study was conducted in the Department of Microbiology at Jorhat Medical College and Hospital for a period of one year duration from January 2023 to December 2023. A total of 672 non duplicate consecutive isolates of *Staphylococcus aureus* were recovered from various clinical samples like pus, wound swab, body fluid,

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sputum, blood. The isolates were identified as *Staphylococcus aureus* by colony morphology, Gram staining, motility and standard biochemical tests. All *S. aureus* isolates were then subjected to antimicrobial susceptibility testing using Kirby–Bauer disk diffusion method on Mueller-Hinton agar (MHA) plates according to CLSI guideline and the different antibiotics tested include Penicillin (10 units), Azithromycin (15μg), Erythromycin (15μg), Clindamycin (2 μg), Doxycycline (30μg), Levofloxacin (5μg), Ciprofloxacin (5μg), Co-trimoxazole (1.25/23.75 μg), Linezolid (30μg), Tetracycline (30μg), Gentamycin (10μg). Staphylococcal isolates were screened for MRSA (Methicillin resistant Staphylococcus aureus) by using 30 μg Cefoxitin disc as per CLSI guideline.

All Erythromycin resistant *Staphylococcus aureus* strains were then subjected to D test as per CLSI guideline to detect inducible clindamycin resistance.

D test: Erythromycin (15 μg) disc is placed at a distance 15 mm (edge to edge) from Clindamycin (2 μg) disc on a Mueller Hinton agar plate previously inoculated with 0. 5 McFarnald standard bacterial suspension. Following overnight incubation at 37 °C, a D-shaped zone of inhibition zone around Clindamycin indicates that the isolate is positive for inducible Clindamycin resistance.

Three different phenotypes were appreciated after testing and then interpreted:

1. **MS phenotype** - *Staphylococcal* isolate exhibiting resistance to Erythromycin (zone size ≤13 mm) while sensitive to Clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around Clindamycin was labelled as having this phenotype.

2. **Inducible MLS_B phenotype** - *Staphylococcal* isolate showing 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 was labelled as having this phenotype.
3. **Constitutive MLS_B phenotype** - This phenotype was labelled for those *Staphylococcal* isolates, which showed resistance to both Erythromycin (zone size ≤13 mm) and Clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around Clindamycin.

Results: Out of 672 *Staphylococcus aureus* isolates, 517 of them were Methicillin resistant *Staphylococcus aureus* (MRSA) and 155 were Methicillin sensitive *Staphylococcus aureus* (MSSA) as shown in the figure -1. The antimicrobial susceptibility pattern of among *Staphylococcus aureus* isolates showed that all isolates (both MRSA and MSSA) were resistant to Penicillin and highly sensitive to Linezolid. The results of antimicrobial susceptibility pattern for different antibiotics is depicted in the table - 1

From 672 *Staphylococcus aureus* isolates 395 of them were Erythromycin resistant. These isolates were then subjected to D test, 91 (MRSA - 83 and MSSA -8) showed resistance to Erythromycin and Clindamycin indicating constitutive MSL_B phenotype, 45 (MRSA -40 AND MSSA -5) showed D test positive indicating inducible MSL_B phenotype while 259 (MRSA -232 and MSSA -27) showed true sensitivity to Clindamycin (D test negative indicating MS phenotype), 277 were susceptible phenotype (susceptible to both Erythromycin and Clindamycin) as depicted in the table -2.

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The age and gender wise distribution of patients with D test positive isolates of *Staphylococcus aureus*, revealed that the highest number of isolates were found in the age group of 21-30 years and it was predominant in females (figure-3).

Table -1 : Antimicrobial susceptibility pattern of ***Staphylococcus aureus*** isolates

Isolates	Sensitivity Pattern	P	AZ	DO	LE	CIP	E	CD	GEN	LZ	COT	TE
MRSA	S	0	252	477	189	300	164	423	339	488	379	446
	R	517	265	40	328	217	353	94	178	29	138	71
MSSA	S	0	117	151	91	108	113	148	126	153	149	148
	R	155	38	4	64	47	42	7	29	2	6	7
Total	S	0	369	628	280	408	277	571	465	641	528	594
	R	672	303	44	392	264	395	101	207	31	144	78

S= sensitive ,R=resistant ,P=penicillin ,AZ=Azithromycin ,CD=Clindamycin , E=Erythromycin , DO=Doxycycline , LE=Levofloxacin ,CIP=Ciprofloxacin ,TE=Tetracycline , COT=Co-trimoxazole ,GEN=Gentamycin ,LZ=Linezolid

Table -2 : Erythromycin and Clindamycin susceptibility pattern among the isolates

Susceptibility pattern (phenotype)	Number(percentage) MRSA(N=517)	Number(percentage) MSSA (N=155)	Number(percentage) Total (N=672)
E=S , CD=S	170 (32.88%)	107 (69.03%)	277 (41.22%)
E=R ,CD=R cMSL$_B$	83 (16.05%)	8 (5.16%)	91 (13.54%)
E=R ,CD=S iMSL$_B$ (D test +ve)	40 (7.73%)	5 (3.22%)	45 (6.69%)
E=R ,CD=S MS (D test -ve)	232 (44.87%)	27 (17.41%)	259 (38.54%)

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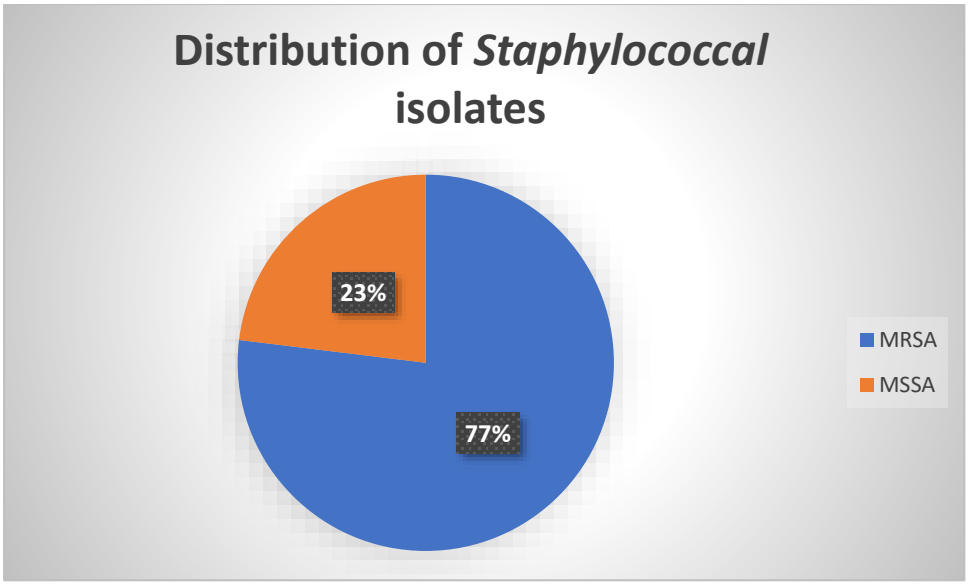

Figure -1 : Distribution of staphylococcal isolates

Figure 2 : D test showing D shaped zone of inhibition around Clindamycin disk

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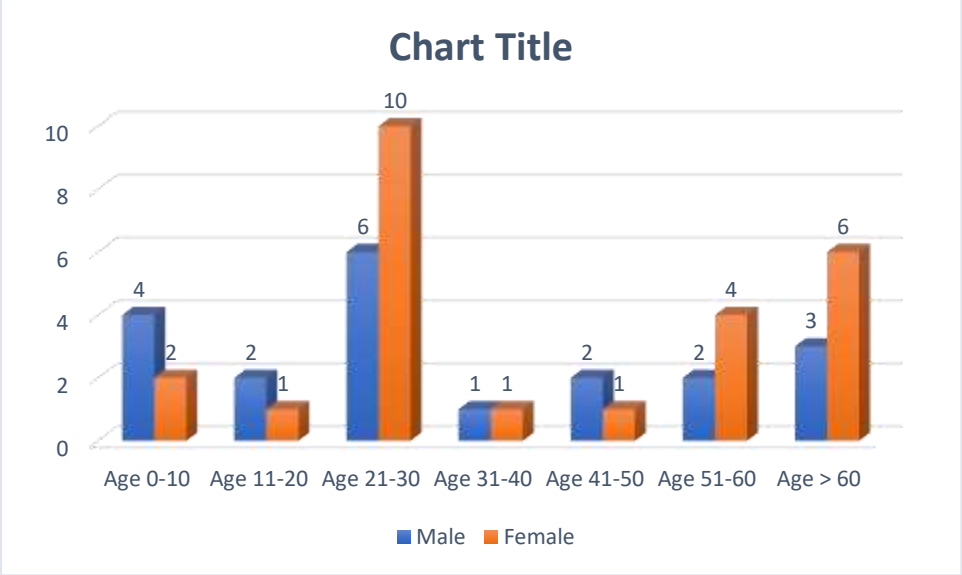

Figure 3 :Age and gender wise distribution among D test positive cases

Discussion :

In recent times, clindamycin has become an excellent drug for some *Staphylococcal* infections, particularly skin and soft tissue infections and as an alternative in penicillin allergic patients[7]. However, resistance to clindamycin is highly variable, and the incidence of constitutive and inducible MLS_B-resistant phenotypes varies by geographic region and even between different health care centres[8] .

In our study ,out of 672 *Staphylococcus aureus* isolates , 77 % were MRSA .Almost similar result was reported by Tiwari et al. (77.6%)[9] from Uttar Pradesh and Phukan et al. (74.42%)[10] from Assam . Lyall et al. had reported a much higher percentage of MRSA (91.5%) in their study [11] , on the other hand Singh et al.[12] had reported only 37.8% of MRSA .The variation in result could be due to variation in study population ,sample size , infection control policies.

In the present study , Erythromycin resistance was observed in 58.77% *Staphylococcus aureus* isolates . In different studies , Erythromycin resistance ranged from 41.17% to 70.25%[3,13,14] . All Erythromycin resistant isolates were subjected to D test. Our study revealed 6.69% of *Staphylococcus aureus* isolates were D test positive which is consistent with Phukan et al. (7 %)[10] whereas higher percentages were reported by Panwala et al. (37.5%) [14], Tiwari et al. (20.08%)[9] and Kumar et al. (40%) [15]. It was observed that inducible Clindamycin resistant isolates were higher among MRSA (7.73%) compared to MSSA (3.22%) in our study. Most authors have reported a higher percentage of inducible Clindamycin resistant isolates in MRSA compared to MSSA[16,9,15,13] . On the other hand Sasirekha et al. had shown a higher percentage in MSSA (8.49 %) compared to MRSA (.65%) [3].

In the present study $cMSL_B$ was observed in 13.54 % isolates and our finding is similar with Panwala et al (15%)[14] and Banik et al (16.88%)[8]. A Higher percentage of cMSLB phenotype was reported by Sigh et al (64.8%)[12], Nikam et al (47.52%)[13] and Gupta et al (31.67%) whereas lower percentage was reported by Regmi et al (3.5%)[17] .The constitutive resistant

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isolates were found to be higher among MRSA (16.05%) compared to MSSA (5.16%) in our study as reported by different studies [1615,13,9] .

In this present study 38.54% isolates showed true Clindamycin susceptibility (MS phenotype) which is higher among MRSA (44.87%) compared to MSSA (17.41 %). This result is similar to a study done by Patel et al (38.5%)[18]. This fact implies that Clindamycin can be safely and effectively used as a therapeutic option in this group of patients without emergence of resistance during therapy .

Conclusion: The inducible Clindamycin resistant *Staphylococcus aureus* isolates obtained in our study is 6.69%. Although the percentage is quite low in our study, its crucial to recognise the potential for misidentification as Clindamycin sensitive if we had not been performed D test . Moreover , Clindamycin can be safely used in patients with true clindamycin sensitive (MS phenotype) Staphylococcal strains, which is confirmed only after performing the D test . Therefore implementation of D test routinely for Staphylococcal isolates will help us guiding the clinicians regarding the judicious use of Clindamycin .

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