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Study of Inducible Clindamycin Resistance in *Staphylococcus aureus* in a Tertiary Care Hospital

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A B S T R A C T

Very few therapeutic options are available from the time of emergence of MRSA. Macrolide lincosamide streptogramin B (MSL_B) antibiotics can be used in such scenarios with clindamycin being the preferred agent due to its excellent pharmacokinetic properties. Staphylococcal resistance to clindamycin may be inducible (iMLSB - inducible Macrolide-Lincosamide Streptogramin B resistance) or constitutive. The treatment of patients harbouring $iMLS_B$ Staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure. If inducible Clindamycin resistance can be reliably detected by placing relevant disc adjacent to each other at proper distance as a routine basis in clinically significant isolates, Clindamycin can be safely and effectively used in patients with true Clindamycin susceptible strains. Objective of the study is to determine prevalence of inducible clindamycin resistance in S.*aureus*. 100 Staphylococcus *aureus* isolates collected from various clinical samples were subjected to routine antibiotic susceptibility testing and screening for Methicillin Resistance was done as per CLSI guidelines. Detection of inducible clindamycin resistance was done using Double disk diffusion test or D test. Out of 100 *S. aureus* (88 MRSA, 12 MSSA) isolates, prevalence of inducible clindamycin resistance was found to be 39% (39 isolates). Inducible Clindamycin resistance was found to be higher in MRSA 40.9% (36 MRSA isolates) as compared to MSSA 25% (3 MSSA isolates). We conclude that whenever clindamycin is intended for *S. aureus* infection, the microbiology lab should tests the isolated organism for $iMLS_B$ by D test.

Introduction

In genus Staphylococci, the most virulent species is *Staphylococcus aureus*[1]. Methicillin resistance to *S. aureus* was first reported in 1961. At present MRSA is a major nosocomial pathogen worldwide[2]. Very few therapeutic options are available from the time of emergence of MRSA. Macrolide lincosamide streptogramin B MSL_B antibiotics can be used in such scenarios[3] with clindamycin being the preferred agent due to its excellent pharmacokinetic properties[4]. Clindamycin can be given orally or parenterally. Food doesn't interfere with its absorption. It has wide distribution in inflamed tissues except for the CNS as it does not cross the blood-brain barrier even in the

presence of inflamed meninges. Dosage adjustments will not be required even in severe hepatic or renal dysfunctions[5].

Three unrelated groups of antimicrobial agents share the same ribosomal binding site in the bacterial cell- macrolides (erythromycin), lincosamides (clindamycin), and type b streptogramins (MLS_B). Therefore, it is possible that resistance to one group of antibiotics (macrolides) might predict resistance to the other groups. Resistance to erythromycin is used as an indicator of possible resistance to clindamycin[6]. *Staphylococcus aureus* resistance to macrolide can be mediated by

A) *msrA* gene coding for efflux mechanism
B) *erm* gene encoding for enzymes that confer inducible or constitutive resistance to MLS_B antibiotics.

The most mechanism for acquiring resistance is through erm gene. In constitutive resistance erm gene will always produce r-RNA methylase. It provides resistance to both erythromycin and clindamycin in vivo as well as in vitro. It can be easily identified by using routine disk diffusion test. In inducible resistance r-RNA methylase is produced only in the presence of an inducing agent[8]. In such cases inducible resistance to clindamycin are difficult to detect in vitro by using routine laboratory as they appear resistant to erythromycin and sensitive to clindamycin. The treatment of patients harbouring $iMLS_B$ Staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure[9].

Erythromycin is an effective inducer whereas clindamycin is a weak inducer. If inducible Clindamycin resistance in S. *aureus* can be reliably detected by D-test on a routine basis, Clindamycin can be safely and effectively used in patients with true Clindamycin susceptible strains[10]. The laboratories and clinicians must be aware of local prevalence of $iMLS_B$. From hospital to hospital prevalence of inducible clindamycin resistance may vary[11]. The present study is therefore undertaken to know the prevalence of inducible clindamycin resistance in our hospital as well as aware and aid our clinicians in using appropriate antibiotics to treat the infections of patients caused by *Staphylococcus aureus* in Khaja Banda Nawaz Teaching & General Hospital, Kalaburagi.

The main objectives of this study includes to determine the prevalence of inducible clindamycin resistance in *Staphylococcus aureus*

Materials and Methods

This study was conducted at Microbiology laboratory of Khaja Banda Nawaz Teaching and General Hospital, attached to KBN institute of Medical Sciences, Kalaburagi for a period of one year i.e., from January 2018 to December 2018. Observational Cross Sectional Study was performed. Written informed consent was taken from the subject after explaining the nature of the study. This study was approved by ethical committee.

A total of 100 isolates of *S. aureus* were collected from various clinical specimens. Identification of *Staphylococcus aureus* was done as per standard guidelines[12]. Each isolate was subjected to the disk diffusion test for detection of MRSA as recommended by the CLSI [10].

Detection of inducible clindamycin resistance[10]

The isolates which were resistant to erythromycin were further studied for inducible clindamycin resistance by doing D

test. Lawn culture of *S. aureus* isolates was prepared on Mueller-Hinton agar plate and standard discs of erythromycin (15 µg) and clindamycin (2 µg) were placed 15-26mm apart and incubated at 37^0 C for 18-24 hours.

Four different phenotypes were detected in D-test which are as follows:

1. D test positive or Inducible MLS_B phenotype: S. *aureus* isolates which were sensitive to clindamycin (zone size ≥21 mm) and resistant to erythromycin (zone size ≤13 mm) and giving D shaped zone of inhibition around clindamycin disc with flattening towards erythromycin disc were taken as D test positive (Figure 1).
2. D test negative or MS Phenotype: S. *aureus* isolates which are sensitive to clindamycin (zone size ≥21 mm) and resistant to erythromycin (zone size ≤13 mm) and giving circular zone of inhibition around clindamycin disc were taken as D test negative (Figure 2).
3. Constitutive MLS_B phenotype: S. *aureus* isolates which showed resistance to both clindamycin (zone size ≤14 mm) and erythromycin (zone size ≤13 mm) (Figure 3).
4. S. *aureus* isolates which were sensitive to both erythromycin (zone size ≥23 mm) and clindamycin (zone size ≥21 mm). (Figure 4).

Results and Discussion

Cefoxitin disc sensitivity done on Mueller-Hinton agar revealed that 88 isolates were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 12 were Methicillin Sensitive *Staphylococcus aureus* (MSSA) (Table 1).

Out of the 100 S. *aureus* (88 MRSA; 12 MSSA) isolates, 30 (24 MRSA; 6 MSSA) isolates were susceptible to both erythromycin and clindamycin, 27 (24 MRSA; 3 MSSA) isolates showed constitutive MLSB resistance i.e., resistant to both erythromycin and clindamycin, 39 (36 MRSA; 3 MSSA) isolates showed inducible clindamycin resistance i.e., resistant to erythromycin and sensitive to clindamycin and showing D test positive and 4 (all from MRSA) isolates showed MS phenotype i.e., resistant to erythromycin and sensitive to clindamycin and showing D test negative (Table 2).

Table.1 Distribution of S. *aureus* based on Methicillin Sensitivity

Methicillin Sensitivity	Frequency
MRSA	88
MSSA	12
Total	**100**

Table.2 Erythromycin & Clindamycin susceptibility pattern of *S. aureus* isolates

Susceptibility pattern	MRSA	MSSA	Total
E-S, CD-S	24	06	30
E-R, CD-R (Constitutive MLS_B)	24	03	27
E-R, CD-S; D test positive (Inducible MLS_B)	36	03	39
E-R, CD-S; D test negative (MS Phenotype)	04	0	04
Total	**88**	**12**	**100**

Table.3 Erythromycin and Clindamycin susceptibility pattern of MRSA isolates

Susceptibility pattern	MRSA
E-S, CD-S	24 (27.3%)
E-R, CD-R (Constitutive MLS_B)	24 (27.3%)
E-R, CD-S; D test positive (Inducible MLS_B)	36 (40.9%)
E-R, CD-S; D test negative (MS Phenotype)	04 (4.5%)
Total	**88(100%)**

Table.4 Various studies across India reporting the prevalence of Inducible Clindamycin Resistance in S. *aureus*

Sl. No	Study Series	Inducible Clindamycin Resistance	
		MRSA(%)	MSSA(%)
1.	Ciraj *et al.,*[17]	38.4	12.9
2.	Gadepalli *et al.,*[18]	30	10
3.	Shantala *et al.,*[7]	32.5	15.53
4.	Saikia *et al.,*[19]	9.3	3.3
5.	Deepak juyal *et al.,*[20]	13.3	28.9
6.	Dhanalakshmi *et al.,*[21]	13.1	6
7.	Amruth KU *et al.,*[6]	35.33	11.74
8.	Ajantha *et al.,*[22]	74	45
9.	Lall *et al.,*[23]	37.1	6
10.	Schreckenberger *et al.,*.[24]	7	20
11.	Levin *et al.,*[25]	12.5	68.5
12.	**Present study**	**40.9**	**25**

Figure.1 D-test Positive (E-R, CD-S; Inducible MLS_B)

Figure.2 D-test Negative (E-R, CD-S; MS Phenotype)

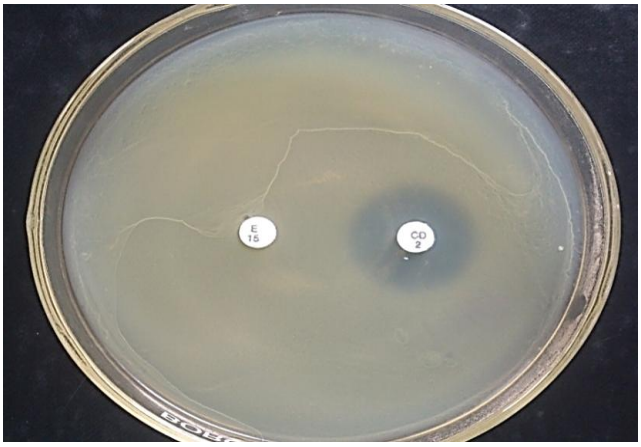
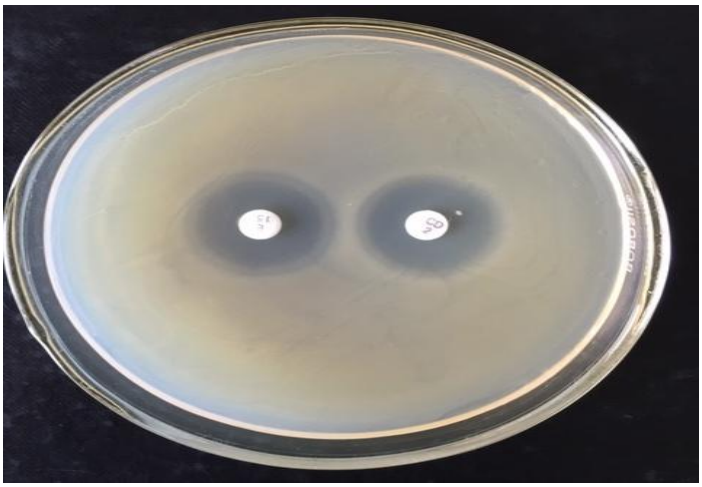


Figure.3 Constitutive MLS_B (E-R, CD-R)

Figure.4 S. *aureus* sensitive to both Erythromycin and Clindamycin (E-S, CD-S)



Out of the 88 MRSA isolates, 24(27.3%) were susceptible to both erythromycin and clindamycin, 24 (27.3%) showed constitutive MLS_B resistance, 36% (40.9%) showed inducible clindamycin resistance and 4 (4.5%) showed MS phenotype (Table 3).

Our study revealed the prevalence of MRSA at Khaja Banda Nawaz Teaching and General Hospital to be 88% which is slightly higher than the findings observed by other workers like by Frazee *et al.,*[13] and Khanal *et al.,*[14] who reported 75% and 68% MRSA isolates respectively. This may be due to the indiscriminate and empirical use of antibacterial agents in our hospital. Our study findings are in contrast to findings observed by Kiran K Mokta *et al.,*[15] and Jyoti kumari *et al.,*[16] who reported only 23.42% and 30.2 % MRSA isolates respectively.

Various workers have reported prevalence of Inducible Clindamycin Resistance among MRSA isolates varying from 9.3% to 74% and among MSSA isolates varying from 6% to 45% as shown in Table 4.

Our study observed that out of 88 MRSA isolates, 36(40.9%) showed inducible clindamycin resistance and out of 12 MSSA isolates, 3(25%) showed inducible clindamycin resistance. In MRSA isolates both constitutive and inducible resistance phenotypes were found to be higher compared to MSSA.

Our study observed inducible clindamycin resistance of 40.9% in MRSA. Ciraj *et al.,* [17] reported 38.4%, Amruth *et al.,*[6] reported 35.33% and Lall *et al.,*[23] reported 37.1% inducible clindamycin resistance in MRSA isolates. However our findings are in contrast with other studies conducted by Saikia *et al.,*[19] reported only 9.3% MRSA isolates with inducible clindamycin resistance and Ajantha *et al.,*[22] who reported a high prevalence of 74% inducible clindamycin resistance in MRSA isolates.

Our study also observed that percentage of inducible clindamycin resistance was higher amongst MRSA (40.9%) as compared to MSSA (25%). Yilmaz *et al.,*[5] (24.4% in MRSA and 14.8% in MSSA), Gadepalli *et al.,*[18] (30% in MRSA and 10% in MSSA) and Mohammed Rahbar *et al.,*[26] (22.6% in MRSA and 4% in MSSA) reported higher percentage of inducible clindamycin resistance in MRSA as compared to MSSA. However our findings are in contrast with Schreckenberger *et al.,*[24] (7% in MRSA and 20% in MSSA) and Levin *et al.,*[25] (12.5% MRSA and 68% MSSA) who reported higher percentage of inducible clindamycin resistance in MSSA as compared to MRSA.

Our study observed constitutive resistance of 27.3% in MRSA isolates, however Angel *et al.,*[27] did not reported any of the strains.

Our study observed that out of 100 *S. aureus* (88 MRSA; 12 MSSA) isolates, 30 {24(27.3%) MRSA; 6(50%) MSSA} were susceptible to both erythromycin and clindamycin, 27 {24 (27.3%) MRSA; 3(25%) MSSA} isolates showed constitutive MLS_B resistance, 39 {36 (40.9%) MRSA; 3(25%) MSSA} isolates showed inducible clindamycin resistance and 4 {4(4.5%) MRSA; 0(0%) MSSA} isolates showed MS phenotype.

This observation suggest that checking for inducible clindamycin resistance is important, otherwise clindamycin therapy therapy would have been started due to misidentification of erythromycin resistant *S. aureus* isolates as clindamycin sensitive, ultimately leading to therapeutic failure. On the other hand, Clindamycin therapy is good therapeutic option in light of the restricted range of antibiotics available in those erythromycin

resistant *S. aureus* isolates showing negative result for inducible clindamycin resistance. Thus in erythromycin resistant *S. aureus* isolates true Clindamycin sensitivity can only be judged after performing D test

We conclude that whenever clindamycin is intended for *S. aureus* infection, the microbiology lab should tests the isolated organism for $iMLS_B$ by D test. D test is simple, inexpensive and easy to perform test. Clindmycin 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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