



## INDUCIBLE CLINDAMYCIN RESISTANCE AMONG COMMUNITY AND HOSPITAL ACQUIRED ISOLATES OF STAPHYLOCOCCUS SPECIES

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### ABSTRACT

The rate of methicillin resistant *Staphylococcus aureus* (MRSA) infections has increased in recent years and clindamycin is considered to be one of the alternatives in these infections. In our study, a total of 337 *Staphylococcus* isolates from exudate samples in a tertiary care hospital were tested for inducible clindamycin resistance by D test according to the clinical laboratory standards institute (CLSI) guidelines. The inducible clindamycin resistance was higher 22% in hospital acquired MRSA isolates when compared to 9.8% in community acquired MRSA isolates. All methicillin resistant coagulase negative *Staphylococci* (MRCoNS) isolates were hospital acquired and they showed 20% inducible clindamycin resistance. Our study shows the increasing prevalence of inducible clindamycin resistance among Methicillin resistant *Staphylococcal* isolates and denotes that D test should be carried out routinely in all laboratories.

**Key words:** Clindamycin resistance, D test, *Staphylococcus*



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## INTRODUCTION

Staphylococci are important causative agents of nosocomial and community acquired infections in skin and soft tissues. Emergence of methicillin resistance in Staphylococci has left us with very few therapeutic alternatives available to treat. The macrolide – lincosamide - streptogramin B (MLS<sub>B</sub>) family of antibiotics serves as one such alternative with clindamycin being preferred agent due to its excellent pharmacokinetic properties<sup>1</sup>. Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory test, as they appear erythromycin resistant and clindamycin sensitive in vitro, when not placed adjacent to each other. In such cases, in-vivo therapy with clindamycin may select therapeutic failure<sup>2,3</sup>.

Macrolide resistant isolates of *Staphylococcus aureus* and coagulase negative *Staphylococcus* species may express constitutive or inducible resistance to clindamycin encoded by the *erm* gene or may be resistant only to macrolides, by efflux mechanism encoded by *msrA* gene (conferring resistance to macrolides, lincosamides, and type B streptogramins only)<sup>4,5</sup>. This study denotes that the detection of inducible clindamycin resistance by simple D test as described in CLSI guidelines is mandatory for all clinical laboratories.

## MATERIALS AND METHODS

Consecutive 337 Staphylococcal isolates from exudate samples from a tertiary care hospital were taken for the study. The study period was 17 months from January 2009 to May 2010. Detailed history of the patient was collected to differentiate hospital acquired (HA) and community acquired (CA) isolates. By routine culture and biochemical tests *Staphylococcus aureus* (*S.aureus*) and coagulase negative Staphylococci (CoNS) were identified.

The Staphylococci isolates were subjected to routine antibiotic susceptibility testing according to the CLSI guidelines. The

following antibiotic discs were used: penicillin 10 µg, cefazolin 30 µg, cefoxitin 30 µg, cotrimoxazole 23.75/1.25 mg, gentamicin 10 µg, vancomycin 30 µg, linezolid 30 µg, clindamycin 2 µg, erythromycin 15 µg, rifampicin 5 µg, chloramphenicol 30 µg, teicoplanin 30 µg, and tetracycline 30 µg. The isolates which were resistant to 30 µg cefoxitin disc with zone size less than 22 mm were taken as methicillin resistant Staphylococci. Methicillin resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin sensitive coagulase negative Staphylococci (MScoNS) and methicillin resistant coagulase negative Staphylococci (MRCoNS) were identified. All erythromycin resistant Staphylococcal isolates were subjected to D test to detect the inducible clindamycin resistance.

### ***Phenotypic detection of inducible Clindamycin resistance by D test***

Lawn cultures of the isolates were made on Muller Hinton agar using suspension of each isolate matching 0.5 McFarland's turbidity. Clindamycin and erythromycin disks containing 2 µg and 15 µg of each were placed with 15mm gap between the edges. Inducible resistance is defined as blunting of the clear zone of clindamycin on the side adjacent to erythromycin, producing a D shaped zone denoting inducible clindamycin resistance. It is designated as D test positive (fig.4). Absence of a blunted zone of inhibition (circular zone) is designated as D test negative, which shows that the test strain is truly susceptible to clindamycin as per 2010 CLSI guidelines<sup>6</sup> and Fiebelkorn et al<sup>1</sup>. Interpretation of the diameter zones of inhibition are as follows: Erythromycin sensitive (ER-S) ≥ 23 mm, Erythromycin resistant (ER-R) ≤ 13 mm, Clindamycin sensitive (CL-S) ≥ 21 mm, and Clindamycin resistant (CL-R) ≤ 14 mm. If the ER zone is ≤ 13 mm and the CL zone is ≥ 21 mm and both have circular zones, it indicates that the isolate is resistant to erythromycin and sensitive to clindamycin and

the D test is negative. If the zone around clindamycin is > 21 mm and D shaped, the organism is positive for inducible resistance (D test positive).

Three different phenotypes were appreciated after testing and interpreted as follows:

1. MS phenotype – Staphylococcal isolate exhibiting resistance to erythromycin (zone size < 13mm) while sensitive to clindamycin (zone size > 21mm) and giving circular zone of inhibition around clindamycin disc was MS phenotype. ( fig.3)

2. Inducible clindamycin resistant phenotype – Staphylococcal isolates showing resistance to erythromycin while being sensitive to clindamycin giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc. (D test positive) ( fig.4)

3. Constitutive clindamycin resistant phenotype – this phenotype showed resistance to both erythromycin and clindamycin. (fig.2)



**Figure 1**  
***Erythromycin and Clindamycin sensitive***



**Figure 2**  
***Erythromycin and Clindamycin resistant***



**Figure 3**  
*Erythromycin resistant and Clindamycin sensitive*



**Figure 4**  
*Inducible Clindamycin resistance (D test positive)*

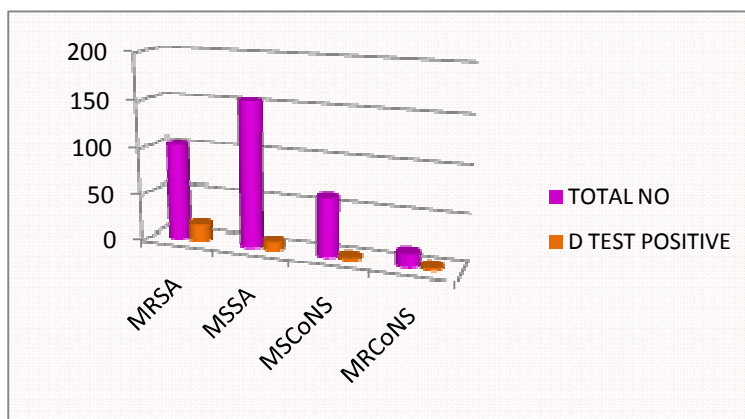
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## RESULTS

**Table1**  
*The split up of Staphylococcal isolates with D test positivity*

Staphylococcus species	Total no of isolates (337)	%	isolates showing positive D test (34)	%
MRSA	104	30.9	18	17
MSSA	155	45.9	10	6.5
MSCoNS	63	18.7	3	4.8
MRCoNS	15	4.5	3	20

*A total of 337 isolates of Staphylococcus species were included, of which 259 (77%) were S.aureus [MSSA (45.9%) were predominant followed by MRSA (30.9%) (table-1,graph1)].*



**Graph 1**  
*The split up of Staphylococcal isolates with D test positivity*

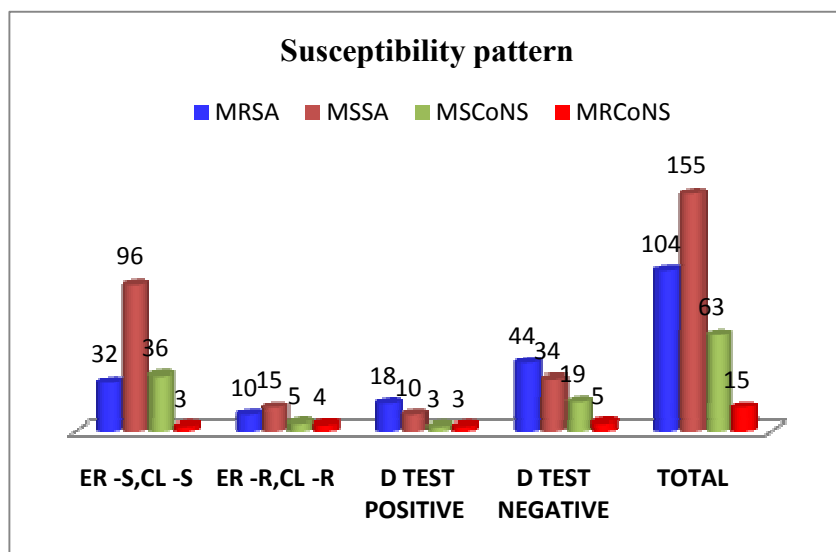
**Table 2.**  
**Susceptibility pattern of Staphylococcal isolates to erythromycin and clindamycin**

Susceptibility pattern	MRSA		MSSA		MSCoNS		MRCoNS	
	no	%	No	%	no	%	no	%
Erythromycin-S Clindamycin-S	32	30.8	96	61.9	36	57.1	3	20
Erythromycin-R Clindamycin-R (Constitutional resistance)	10	9.6	15	9.7	5	7.9	4	26.7
Erythromycin-R Clindamycin-S (D test positive- Inducible resistance)	18	17.3	10	6.5	3	4.8	3	20
Erythromycin-R Clindamycin-S (D test negative)	44	42.3	34	21.9	19	30.2	5	33.3
Total	104		155		63		15	

Out of 337 isolates of Staphylococcus species, erythromycin was resistant in 169 (50.14%) isolates. The split up was 72(69.2%) MRSA, 59 (38.1%) MSSA, 27 (42.8%) MSCoNS, 12 (80%) MRCoNS respectively (table-2,graph-2).

Constitutional Clindamycin resistance among 337 isolates of Staphylococci was 34(10.1%). Among MRSA it was 9.6% (10/104), MSSA 9.7% (15/155), MSCoNS 7.9% (5/63) and MRCoNS 26.7% (4/15) respectively (table-2).

Inducible Clindamycin resistance among 337 isolates of Staphylococci was 10% (34/337). In case of MRSA it was 17.3%(18/104), MSSA 6.5% (10/155), MSCoNS 4.8% (3/63) and in MRCoNS 20% (3/15). Inducible clindamycin resistance was higher (18%) in MRSA than (10%) MSSA. (table-2, graph-2)



**Graph 2.**  
*Susceptibility pattern of Staphylococcal isolates to erythromycin and clindamycin*

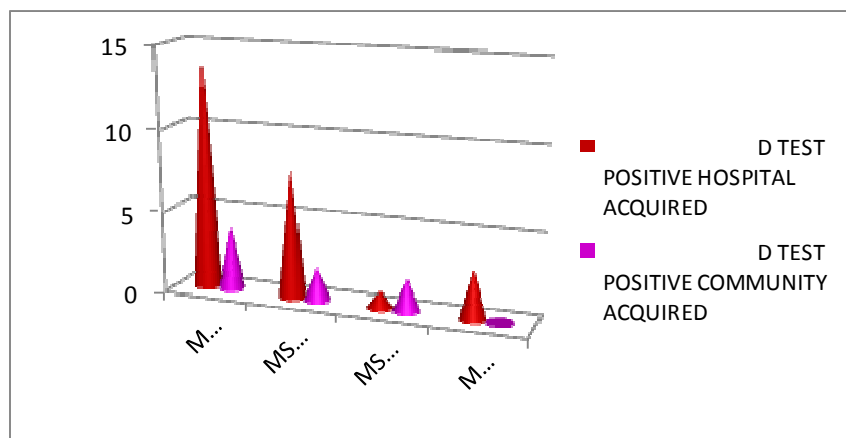
**Table 3.**  
*Comparison of hospital acquired and community acquired Staphylococcal isolates showing positive D test*

Staphylococcus species	Hospital acquired				Community acquired			
	Total		D test positive		Total		D test positive	
	no	%	No	%	no	%	no	%
MRSA (104)	63	60.6	14	22.2	41	39.4	4	9.8
MSSA (155)	101	65.2	8	7.9	54	34.8	2	3.7
MSCoNS (63)	45	71.4	1	2.2	18	28.6	2	11
MRCoNS (15)	15	100	3	20	0	0	0	0

Among MRSA inducible clindamycin resistance was higher (22%) in hospital acquired isolates than (10%) community acquired. In coagulase negative staphylococci 18.7% were Methicillin sensitive and 4.5% were methicillin resistant. The inducible clindamycin resistance was higher (20%) in MRCoNS than (4.8%) MSCoNS. All MRCoNS were hospital acquired (table-3,graph3).

Graph 3.

**Comparison of hospital acquired and community acquired Staphylococcal isolates showing positive D test**



## DISCUSSION

The increasing frequency of Staphylococcal infection among patients and changing pattern in antimicrobial resistance have led to recent interest in the use of clindamycin therapy to treat such infections. Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral and excellent tissue penetration and the fact that it accumulates in abscess and no renal dosing adjustments are needed.<sup>7</sup> Good oral absorption makes it important option in outpatient therapy. Clindamycin is a good alternative for the treatment of both methicillin resistant and susceptible Staphylococcal infections<sup>1</sup>.

Inducible clindamycin resistance cannot be appreciated by routine antibiotic susceptibility testing. Performance of D test becomes essential to avoid therapeutic failure of clindamycin. The clinical failure of clindamycin therapy has been reported before<sup>7,8,9</sup>. Hence, there is a need to identify the mechanisms that confer resistance to MLS antibiotics with regard to clindamycin therapy of staphylococcal infections.

There have been a number of reports on the pattern of macrolide resistance in staphylococci<sup>10,11,12,13,14,15</sup>. Indian reports on

inducible clindamycin resistance are scanty<sup>11,12,13</sup>.

In our study S.aureus isolated were 76.8% (259/337) and CoNS were 23.2% (78/337). Among S.aureus 60.6% were HA MRSA and 39.4% were CA MRSA showing 22%, 9.8% inducible clindamycin resistance respectively. Inducible clindamycin resistance was significantly higher in HA MRSA than CA MRSA.

Among CoNS, 80.7% were methicillin sensitive and 19.2% were methicillin resistant showing 4.8%, 20% inducible clindamycin resistance (ICR) respectively. ICR is significantly higher in MRCoNS than MS CoNS. All MR CoNS were hospital acquired.

In this study 50% (169/337) of Staphylococcal isolates were erythromycin resistant. 25.5% of ER resistant S.aureus isolates showed inducible clindamycin resistance. This is similar to that reported by Gadupalli et al, Fieblkorn et al, and Jorgensen et al<sup>11,1,15</sup>. Some investigators have reported a higher incidence<sup>13,17,18,19,20,21</sup> of inducible clindamycin resistance while others have indicated lower incidence<sup>14,22,23,24</sup>. Different patterns of resistance observed in various studies are because inducible clindamycin

resistance varies by geographical region, age group, methicillin susceptibility and from hospital to hospital.

Erythromycin – clindamycin disc approximation test (or) D test is simple, reliable method to detect inducible clindamycin resistance in erythromycin resistant isolates of Staphylococci<sup>1,2,22</sup>. Sensitivity of D test performed at 15mm disk spacing was 100% correlated with detection of erm and msr genes by polymerase chain reaction(PCR)<sup>1,2,16,18,19</sup>. The double-disc diffusion test is necessary to correctly discriminate between inducible CL-R and susceptibility to CL. However, if inducible resistance can be reliably detected on a routine

basis in clinically significant isolates, CL can be safely and effectively used in those patients with true CL-S strains. ER-R staphylococci should not be assumed to have CL-R.

## CONCLUSION

From the present study it shows that *methicillin resistant Staphylococcus* species has higher percentage of D test positives. False susceptibility results may be obtained if isolates are not tested for inducible clindamycin resistance. This study demonstrates a simple, reliable and significant method for detecting inducible resistance to clindamycin.

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