

## Antimicrobial profile of inducible clindamycin resistant strains of staphylococcus species

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### Abstract

**Introduction:** The appearance of continuous resistant to multiple drugs among Staphylococci is a global burden due to its ability to cause severe infections. The selective use of drugs is necessary to overwhelm the situation. Taking in account present study was carried out to rule out true susceptibility of clindamycin towards staphylococcus species and its antimicrobial profile for judicial use of the drugs.

**Material and Methods:** All the clinical samples received in the Department of microbiology were screened for Staphylococci as per standard guidelines which were further subjected to Antimicrobial susceptibility testing and D-test to detect MLSb phenotypes.

**Results:** A total of 421 *Staphylococcus* species, 359(85.3%) were *Staphylococcus aureus* and 62(14.8%) were Coagulase negative Staphylococci; among them 42(10%) were *Staphylococcus epidermidis* & 20(4.8%) were *Staphylococcus saprophyticus*. D-test for *S.aureus* shows that 173(48.2%) inducible Clindamycin resistant, 113(31.5%) strains were constitutive MLSb phenotypes and 58(16.2%) strains shown to have MSb phenotypes. Among CoNS; among *Staphylococcus epidermidis* and *S.saprophyticus* 9.5% & 5% were Inducible Clindamycin resistance, 38.1% & 85% were constitutive MLSb phenotypes and 28.6% & 10% were MSb phenotypes respectively. All the isolates were sensitive to Linezolid, Vancomycin and Ceftaroline.

**Conclusion:** Inducible clindamycin resistant strains of Staphylococci found to be among half of the strains, indicating that true susceptibility of clindamycin should be rule out on routine basis for proper institution of the therapy.

**Keywords:** Staphylococci, MLSb phenotypes, AST.

### Introduction

A notorious pathogen, which is gram positive cocci arranged in clusters, is making the condition worsen day by day with acquisition of multidrug resistance. Specifically, *Staphylococcus aureus* have left use very few therapeutic alternatives to treat such infection.<sup>1,2,3</sup> *Staphylococcus aureus* causes variety of infection range from minor skin and soft tissue infection to life threatening condition like endocarditis, septicemia, toxic shock syndrome, Osteomyelitis etc.<sup>4,5</sup>

Emergence of methicillin resistant strains of *Staphylococcus aureus* is one of the major concerns. First resistance to this organism was noted in year of 1930 for sulfonamide, which was tackled by benzyl penicillin in 1941. The continuous uses of penicillin cause selection of resistant strain by the production of beta-lactamases enzyme. Introduction of synthetic penicillin like methicillin and cloxacillin were seems to be control of, but in the year of 1962, methicillin resistant *Staphylococcus* species has started to emerge, that have evolved resistance to all penicillin group of drugs, newer synthetic penicillins and cephalosporins. Methicillin resistant strains of *Staphylococcus aureus* is mediated by the production of low affinity Penicillin binding protein 2a encoded by *mecA* genes.<sup>6-8</sup>

The macrolide-lincosamide-streptogramin B (MLSb) group of antibiotics found to be good

therapeutic alternative to treat MRSA, with Clindamycin being preferred agent due to its excellent pharmacokinetic properties. In recent year, resistant to MLSb antibiotics has started to emerge, additionally inducible resistance to Clindamycin under the influence of *erm* genes lead to therapeutic failure.<sup>9,10</sup>

The MLSb group of drugs interact with 30s ribosomal subunit to inhibit protein synthesis of bacterial genes. The erythromycin (Macrolide) resistant strains of staphylococci enhance the production of methylase enzyme encoded by *erm* genes which modifies the target site and might predict the resistance to other group of drugs (Clindamycin). The target site modification either expressed inducible or constitutively, and these strains are difficult to detect as they appear as erythromycin resistant and Clindamycin sensitive in-vitro. In such case, in-vivo therapy with Clindamycin, *erm* genes mutant may be express constitutively resulting in therapeutic failure. Second mechanism of resistance to MLSb group antibiotics is presence of efflux pump encoded by *msrA* genes which leads to resistance to Macrolide and Streptogramin-B but not to Lincosamide known as MSb phenotypes. The genotypic detection of *erm* genes can be done, but it is costly and inconvenient in resource constraint settings, CLSI recommend phenotypic D-test which is simple, reliable, inexpensive, can be perform on routine basis.<sup>11,12</sup>

The present study was carried out to detect inducible Clindamycin resistant strains of staphylococcus species by using simple D-test and their antimicrobial susceptibility profile to find out the resistance pattern at our area.

**Material and Methods**

Present study was carried out in the Department of microbiology. After the clearance of RAC and IEC, all the clinical samples received from in-patients and out-patients were analysed for the isolation of pathogen. A total of 421 Staphylococcus species were isolated as per standard guidelines. All the isolates were further antimicrobial susceptibility testing by using Kirby-Bauer disc diffusion method as per CLSI guidelines. Those isolates which were resistant to erythromycin and sensitive to Clindamycin were evaluated to detect MLSb phenotypes by using D-test.<sup>13,14</sup> Briefly; Erythromycin (15µg) disc was placed at a distance of 15mm (edge to edge) from Clindamycin (2µg) disc on a MHA plate previously inoculated with 0.5 McFarland bacterial suspensions, incubated at 37°C for 24 hours and result was interpreted as per CLSI guidelines as follows:<sup>15</sup>

**1. MSb phenotype:** Staphylococcus species exhibiting resistance to Erythromycin (Zone size ≤ 13 mm) and sensitive to Clindamycin (Zone size ≥

21 mm) and giving circular zone of inhibition around Clindamycin disc.

**2. Inducible MLSb phenotype:** Staphylococcus species exhibiting resistance to Erythromycin (Zone size ≤ 13 mm) and sensitive to Clindamycin (Zone size ≥ 21 mm) and giving D shaped zone of inhibition around Clindamycin disc.

**3. Constitutive MLSb phenotype:** Staphylococcus species exhibiting resistance to both Erythromycin (Zone size ≤ 13 mm) and Clindamycin (Zone size ≤ 14 mm) with giving circular shape of zone of inhibition if any around Clindamycin disc.

**Statistical Analysis**

It was done by using **IBM SPSS (20 version)** software. Frequencies & percentages were calculated for all the parameters. Non Parametric test was run by selecting one sample, in which automatically compares observed data to hypothesized using the Chi-Square test.

**Observation and Result**

A total of 421 Staphylococcus species isolated from various clinical samples, out of which 359 were *Staphylococcus aureus* and 62 were CoNS. All the isolates were subjected to detection of methicillin resistance and MLSb phenotypes.

**Table 1: Methicillin resistant strains of Staphylococcus species**

Methicillin resistance		Frequency	Percent	Valid Percent	Cumulative Percent
S.aureus	MRSA	280	78.0	78.0	78.0
	MSSA	79	22.0	22.0	100.0
	Total	359	100.0	100.0	
CoNS	MRCNSS	44	71.0	71.0	71.0
	MSCNSS	18	29.0	29.0	100.0
	Total	62	100.0	100.0	

**Table 2: Frequency of MLSb phenotype among Staphylococcus aureus**

Type of resistance	Frequency	Percent	Valid Percent	Cumulative Percent
ER- Sensitive	15	4.1	4.1	4.1
ER-R, CD-S (iMLSb)	113	31.5	31.5	35.6
ER-R, CD-R (cMLSb)	173	48.2	48.2	83.8
ER-R, CD-S (MSb)	58	16.2	16.2	100.0
Total	359	100.0	100.0	
One-sample Chi-square test	Test statistics: 156.733 Degree of freedom: 3 Asymptomatic p-value(2-sided test): 0.000			

**Table 3: Distribution of MLSb phenotype among MRSA and MSSA isolates**

Type of resistance	MRSA (n=280)		MSSA (n=79)	
	Frequency	Percent	Frequency	Percent
ER-sensitive	0	0	15	19
iMLSb phenotype	101	36.1	12	15.2
cMLSb phenotype	162	57.9	11	13.9
MSb phenotype	17	6	41	51.9
Total	280	100	79	100

**Table 4: Frequency of MLSb phenotypes among CoNS**

CoNS	Phenotypes	Frequency	Percent	Valid Percent	Cumulative Percent
<i>S.epidermidis</i>	ER-Sensitive	10	23.8	23.8	23.8
	cMLSb	16	38.1	38.1	61.9
	iMLSb	4	9.5	9.5	71.4
	MSb	12	28.6	28.6	100.0
	Total	42	100.0	100.0	
<i>S.saprophyticus</i>	ER-Sensitive	0	0	0	
	iMLSb	1	5.0	5.0	5.0
	cMLBb	17	85.0	85.0	90.0
	MSb	2	10.0	10.0	100.0
	Total	20	100.0	100.0	
One-sample Chi-square test		Test statistics: 7.143 Degree of freedom: 3 Asymptomatic p-value(2-sided test): 0.067			

**Table 5: Distribution of MLSbphenotype among MRCNSS and MSCNSS**

Type of strains	Type of resistance			
	iMLSb	cMLSb	MSb	Total
MRCNSS (n= 44)	04	31	03	38
MSCNSS (n=18)	02	00	11	13
Total	06	31	14	51

**Table 6: Antibiotic sensitivity pattern of MLSbphenotypes *Staphylococcal aureus***

Antibiotics	iMLSb(n=113)		cMLSb(n=173)		MSb(n=58)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Erythromycin	00	113(100)	00	173(100)	00	58(100)
Clindamycin	113(100)	00	00	173(100)	58(100)	00
Cefoxitin	12(10.6)	101(89.4)	11(6.4)	162(93.6)	41(70.7)	17(29.3)
Penicillin	00	113(100)	00	173(100)	00	58(100)
Trimetho-sulfa	05(4.5)	108(95.5)	67(38.8)	106(61.2)	39(67.2)	19(32.8)
Ceftaroline	113(100)	00	173(100)	00	58(100)	00
Linezolid	113(100)	00	173(100)	00	58(100)	00
Tetracycline	16(14.2)	97(85.8)	00	173(100)	41(70.7)	17(29.3)
Vancomycin	113(100)	00	173(100)	00	58(100)	00
Rifampin	113(100)	00	173(100)	00	55(94.8)	03(5.2)
Chloramphenicol	16(14.2)	97(85.8)	27(15.6)	146(84.4)	06(10.4)	52(89.6)
Ofloxacin	00	113(100)	16(9.3)	157(90.7)	45(77.6)	13(22.4)
Gentamycin	15(13.3)	98(86.7)	16(9.3)	157(90.7)	38(65.5)	20(34.5)

**Table 7: Antibiotic sensitivity pattern of erythromycin resistant CONS**

Antimicrobial agents	<i>S.epidermidis</i> =42				<i>S.saprophyticus</i> =20			
	S	%	R	%	S	%	R	%
Erythromycin	10	23.8	32	76.2	01	5	19	95
Clindamycin	26	61.9	16	38.1	05	25	15	75
Cefoxitin	18	42.8	24	57.2	00	00	20	100
Penicillin	00	00	42	100	00	00	20	100
Trimethoprim-sulfa	07	16.6	35	83.4	08	40	12	60
Ceftaroline	42	100	00	00	20	100	00	00
Linezolid	42	100	00	00	20	100	00	00
Tetracycline	22	52.4	20	47.6	04	20	16	80
Vancomycin	42	100	00	00	20	100	00	00
Rifampin	40	95.2	02	4.8	19	95	01	5
Chloramphenicol	21	50	21	50	16	80	04	20
Ofloxacin	06	14.3	36	85.7	16	80	04	20
Gentamycin	19	45.2	23	54.8	13	65	07	35

## Discussion

A total of 421 *Staphylococcus* species were isolated from different clinical specimens. Out of which, 359(85.3%) were *Staphylococcus aureus* and 62(14.8%) were Coagulase negative Staphylococci; among them 42(10%) were *Staphylococcus epidermidis* & 20(4.8%) were *Staphylococcus saprophyticus*.

Inducible Clindamycin resistant strains were found out among erythromycin resistant strains of *Staphylococcus species* by using D-test. The test was performed by placing erythromycin and Clindamycin disc at 15 mm distance from edge to edge. The Inter-disc distance of 15 mm has been found to satisfactory by Ajanta G.S. et al.<sup>16</sup> and Fiebelkorn K.R et al.<sup>17</sup> In our study same protocol of keeping inter-disc distance of 15 mm was followed.

In present study, out of 359 *Staphylococcus aureus* isolates, 344(95.8%) were erythromycin resistant, these were further subjected to D-test. The D-test revealed MLSb phenotypes among *Staphylococcus aureus*, 113(31.5%) strains were D-test positive indicating inducible Clindamycin resistant strains of *Staphylococcus aureus* (iMLSb phenotype), 173(48.2%) strains were constitutive MLSb phenotypes and 58(16.2%) strains shown to have MSb phenotypes. Similar studies were conducted by Sunil Hatkaret al.<sup>10</sup> (iMLSb phenotypes 26.13%, cMLSb phenotypes 58.52%, and MSb phenotypes 15.34%), Veena Manjunath et al.<sup>18</sup> (iMLSb phenotypes 33.33%, cMLSb phenotypes 18.75%, and MSb phenotypes 47.9%) which is in concordance with present study.

Prevalence of MLSb phenotypes among MRSA and MSSA were analysed and it was observed that inducible and constitutive Clindamycin resistant strains of *Staphylococcus aureus* were higher amongst MRSA isolates (36.1% & 57.9% respectively) as compared to MSSA isolates (15.2% & 13.9% respectively). In a study of Veena Manjunath et al.,<sup>18</sup> percentages of inducible Clindamycin resistance were higher among MRSA as compared to MSSA (57.63% and 16.22% respectively).

The coagulase negative staphylococci also screened for Inducible Clindamycin resistant strains. Among *Staphylococcus epidermidis* and *S. saprophyticus* 9.5% & 5% were Inducible Clindamycin resistance, 38.1% & 85% were constitutive MLSb phenotypes and 28.6% & 10% were MSb phenotypes.

Antimicrobial susceptibility of inducible Clindamycin resistant strains of *Staphylococcus aureus* (erythromycin resistant & Clindamycin sensitive) were analysed by using Kirby Bauer's disc diffusion method as per CLSI guidelines. It was observed that penicillin, Ofloxacin, were 100% resistant, followed by Trimethoprim-sulfamethoxazole 108(95.5%), Cefoxitin 101(89.4%), Gentamycin 98(86.7%) and 97(85.8%) were resistant to Tetracycline & Chloramphenicol while

Linezolid, Vancomycin, Ceftaroline, Rifampin were 100% sensitive.

## Conclusion

Emergence of multidrug resistant strains of *Staphylococcus species* is an alarming and clinicians should aware about it. Use of Clindamycin without knowing the inducible resistance may lead to therapeutic failure. In present study, prevalence of inducible resistance was significant and we can conclude that the detection of inducible Clindamycin resistance on routine basis is mandatory for judicious use of the drug and proper institution of the therapy.

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