



Research Article

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**Detection of inducible clindamycin (iMLSB) resistance in *Staphylococcus aureus* in tertiary care centre of South India.**

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**ABSTRACT**

Macrolides have been known for >5 decades. Macrolide-lincosamide-streptogramin (MLS) antibiotics are commonly used in treatment of staphylococcal infections. Clindamycin is also used as an alternative for patients who are allergic to penicillin. In the present study *Staphylococcus aureus* isolates were done to find out the percentage of *Staphylococcus aureus* having inducible clindamycin resistance in our geographic area by using D-test. Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients attending Shri B M Patil Medical College and Hospital were selected for study from January 2013 to June 2013. Samples which yielded *Staphylococcus aureus* were included in the study. *S. aureus* was identified by conventional techniques. Isolates were screened for erythromycin resistance. The isolates that were found to be erythromycin resistant were further tested for inducible clindamycin resistance by using D-Test. A total of 51 consecutive, non duplicate isolates of *Staphylococcus aureus* were recovered from various clinical specimens in the Department of Microbiology during the study period. The present study showed higher rates of iMLSB(20%) when compared with cMLSB (10%) phenotypes. The present study showed higher rates of iMLSB(20%) when compared with cMLSB (10%) phenotypes. Therefore we recommend the use of D test for detecting the inducible clindamycin resistance, so that the misuse of clindamycin is prevented and also limiting the treatment failure in many patients suffering from serious infections.

**Key words:** Inducible clindamycin resistance, iMLSB, MRSA, antibiotic resistance, antibiotic susceptibility testing, Drug resistance. *Staphylococcus aureus*

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**INTRODUCTION**

*Staphylococcus aureus* and coagulase-negative staphylococci are recognized as causing nosocomial and community-acquired infections in every region of the world. *Staphylococcus aureus* was first described by Sir Alexander Ogston in 1882. The resistance to antimicrobial agents among staphylococci is an increasing problem. [1,2]

Macrolides have been known for >5 decades. Macrolide-lincosamide-streptogramin (MLS) antibiotics are commonly used in treatment of staphylococcal infections. Clindamycin is also used as an alternative for patients who are allergic to penicillin. [3,4,5]

Erythromycin (a macrolide, ERY) and clindamycin (a lincosamide, CLI) represent two distinct classes of antimicrobial agents that act by binding to the 50s ribosomal subunit of bacteria to inhibit its protein synthesis. Macrolide resistance in *Staphylococcus aureus* is by diverse mechanisms. The resistance to macrolide can arise by

efflux mechanism, classically mediated by *msrA* gene. Another mechanism is via *erm* gene, which encodes enzymes that confer inducible or constitutive resistance to macrolide, lincosamide and Type B streptogramin (MLS<sub>B</sub> resistance). This resistance mechanism can be constitutive, where rRNA methylase is always produced (cMLS<sub>B</sub>) or can be inducible where methylase is produced only in the presence of an inducing agent (iMLS<sub>B</sub>). ERY is an effective inducer whereas CLI is a weak inducer. [6]

In the present study *Staphylococcus aureus* isolates were tested with ERY and CLI separately and by disc approximation test using D-test to find out the percentage of *Staphylococcus aureus* having inducible clindamycin resistance in our geographic area.

## EXPERIMENTAL SECTION

### Source of data:

The study was carried out in the Department of Microbiology, Shri B.M Patil Medical College Hospital, Bijapur. *Staphylococcus aureus* isolated from various clinical samples that were sent to the microbiology department formed the material for the study.

### Method of collection of data: (including sampling procedure)

Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients attending Shri B M Patil Medical College and Hospital were selected for study from January 2013 to June 2013.

### Statistical analysis :

Data was analyzed by

- 1) Diagrammatic representation
- 2) Proper statistical tests like chi square test etc.

**Inclusion criterion:** Samples which yielded *Staphylococcus aureus* were included in the study.

**Exclusion criterion:** Samples which did not yield *Staphylococcus aureus* were excluded from the study.

Specimens were screened by preliminary Gram's stain and then inoculated on 10% sheep blood agar and MacConkey's agar. *S. aureus* was identified by conventional techniques. [7]

Detection of the MRSA were done by cefoxitin disc diffusion method.[8-10] All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using cefoxitin discs. (30µg) The isolates were considered methicillin resistant if the zone of inhibition was 21 mm or less as per recommendations of the CLSI. Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC 43300 - were used as negative and positive controls, respectively.[8]

All the isolates were screened for erythromycin resistance using the Kirby Bauer disc diffusion method. The isolates that were found to be erythromycin resistant were further tested for inducible clindamycin resistance by using D- Test.[4] An erythromycin disk was placed 15 mm (edge to edge) from a clindamycin disk in a standard disk diffusion test and incubated for 18- 24 hours at 37<sup>0</sup> C. Three different phenotypes were interpreted[11] as follows:

1. A flattening of the zone of inhibition in the area between the disks was considered to be inducible clindamycin resistance. (iMLS<sub>B</sub> phenotype)
2. Growth up to CLI and ERY discs indicates resistance to both ERY and CLI (cMLS<sub>B</sub> phenotype)
3. MS Phenotype *Staphylococcus* isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin (MS Phenotype)

## RESULTS AND DISCUSSION

Bacterial resistance to antimicrobial agents generally involves drug inactivation, target site modification, impermeability, or efflux mechanisms. Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) may be due to an active efflux mechanism encoded by *msrA* (conferring resistance to

macrolides and type B streptogramins only) or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins (MLS<sub>B</sub> resistance). *erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, reducing binding by MLS agents to the ribosome. Resistance is induced by the binding of a macrolide to upstream translational attenuator sequences, leading to changes in mRNA secondary structure, exposure of the ribosomal binding site, and translation of the *erm* methylase. Alterations in these 5' upstream sequences, including deletions, duplications, and other mutations, lead to constitutive expression of the methylase gene and constitutive MLS<sub>B</sub> resistance. Strains with inducible MLS<sub>B</sub> resistance (MLS<sub>B</sub><sup>i</sup>) strains demonstrate in vitro resistance to 14- and 15-member macrolides (e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins; strains with constitutive MLS<sub>B</sub> resistance (MLS<sub>B</sub><sup>c</sup> strains) show in vitro resistance to all of these agents [4]

A total of 51 consecutive, non duplicate isolates of *Staphylococcus aureus* were recovered from various clinical specimens in the Department of Microbiology during the study period. Fifty one isolates *Staphylococcus aureus* of were screened for erythromycin resistance using the Kirby Bauer disc diffusion method. The isolates that were found to be erythromycin resistant were further tested for inducible clindamycin resistance by using D- Test. An erythromycin disk was placed 15 mm (edge to edge) from a clindamycin disk in a standard disk diffusion test and incubated for 18- 24 hours at 37<sup>0</sup> C. Three different phenotypes were observed as shown in Table 1.

**Table 1: Distribution of clindamycin resistance in *Staphylococcus aureus* isolates**

Clindamycin Phenotypes	Number (n=51)	Percentage
Inducible MSLB Phenotype	10	19.6
Constitutive MLSB Phenotype	5	9.8
MS phenotype	3	5.88
Sensitive to Erythromycin and clindamycin	33	64.7

There have been varied reports on the MLSB resistance among the Staphylococci across the country and around the world; some have reported high prevalence of the iMLS<sub>B</sub> phenotype, while the others have reported high frequency of the cMLS<sub>B</sub> phenotype. [12-15] The present study showed higher rates of iMLS<sub>B</sub>(20%) when compared with cMLS<sub>B</sub> (10%) phenotypes. The present study showed iMLS<sub>B</sub> prevalence of 20% this is similar to study done by Velvezhi et al.,[13] and Angle et al., [14] who reported 19% iMLS<sub>B</sub>, contrary to our studies Fibelkorn et al.,[4] reported slightly higher rates (30%) of MLS<sub>B</sub><sup>i</sup>. The reason for this lower incidence may be the geographical and the environmental factors which were entirely different in the different clinical set ups. Moreover, our study was done in a remote place and a majority of the population belongs to the rural areas and hence is less exposed to the antimicrobial agents.[12]

Clindamycin is a useful drug in the treatment of skin and soft-tissue infections and serious infections caused by staphylococcal species, as well as anaerobes. It has excellent tissue penetration (except for the central nervous system) and accumulates in abscesses, and no renal dosing adjustments are needed. Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. The emergence of the resistance to multiple antibiotics among the gram positive organisms has left limited options for the clinicians and an appropriate therapeutic decision is not possible without the relevant antibiotic susceptibility data. This is where the D-test becomes significant.[4,12]

**Table 2: MRSA among different phenotypes of *Staphylococcus aureus***

Clindamycin Phenotypes	Number	MRSA	Percentage
Inducible MSLB Phenotype	10	9	90
Constitutive MLSB Phenotype	5	5	100
MS phenotype	3	3	100
Sensitive to Erythromycin and clindamycin	33	16	48
Total	51	33	65

In the current study, the prevalence of the MRSA strains were 65%.(table 2) which is consistent with study carried by Mallick et al.[16], and Anuprabha et al.,[17] Our hospital is a tertiary care hospital and primarily caters to the rural population of north karnataka Lack of awareness and the indiscriminate and the improper use of antibiotics before coming to the hospital might be the contributory factors for the high prevalence of MRSA in our study.[16]

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**CONCLUSION**

The present study showed higher rates of iMLSb(20%) when compared with cMLSb (10%) phenotypes. Therefore we recommend the use of D test for detecting the inducible clindamycin resistance, so that the misuse of clindamycin is prevented and also it helps in limiting the treatment failure in many patients suffering from serious infections.

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