

Original Research Article

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A Retrospective Study for Detection of Inducible Resistance to Clindamycin in *Staphylococcus aureus* Isolates Using D-Test at RIMS Teaching Hospital, Raichur, India

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ABSTRACT

Staphylococcus aureus is recognized as causing nosocomial and community-acquired infections in every region of the world. Clindamycin is considered as useful alternate drug in penicillin-allergic patients in the treatment of skin & soft tissue infections caused by *Staphylococcus aureus*. *Staphylococcus spp.* can be resistant to erythromycin through either *erm* or *msr* A genes. Strains with *erm*-mediated erythromycin resistance may possess inducible Clindamycin resistance but may appear susceptible to Clindamycin by disc diffusion test. The objective of the present study was to know the prevalence of erythromycin-induced Clindamycin resistance among clinical isolates of *S. aureus*. A total of 160 *S. aureus* isolates from various clinical samples submitted in the Dept. of Microbiology, RIMS teaching hospital, Raichur were studied. Methicillin resistant *S. aureus* strains were identified by Cefoxitin disc diffusion method. Inducible Clindamycin resistance was detected by erythromycin and Clindamycin disc approximation test (D-zone test) as per CLSI guidelines. Among the 160 *S. aureus* isolates, 75 strains (46.8%) were detected as MRSA of which 32(42.6%) strains showed inducible Clindamycin resistance (D-test positive) and 12(14.1%) isolates out of 85 strains MSSA were D-test positive. 73(45.6%) isolates of *S. aureus* were sensitive to both erythromycin and Clindamycin. High prevalence of strains with inducible Clindamycin resistance particularly among MRSA indicates that inducible Clindamycin resistance testing (D-test) should be included as a part of routine antibiotic susceptibility. These isolates may be missed in routine antibiotic testing by disk diffusion method.

Keywords

Clindamycin resistance, MRSA, MSSA, D test.

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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 (MLSB) family of antibiotics serves as one such alternative with Clindamycin being preferred agent due to its excellent pharmacokinetic properties.^[1]

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.^[2, 3] However widespread use of Macrolides-lincosamides-Streptogramin B (MLSB) antibiotics has led to an increased resistance

to these antibiotics by *Staphylococcal* strains.^[4]

The Macrolide-Lincosamide-Streptogramin B (MLSB) family of antibiotics is commonly used in the treatment of *Staphylococcal* infections. Clindamycin a protein synthesis inhibitor is an attractive option for use in the scenario of increasing drug resistance among the *Staphylococci* especially for skin and soft tissue infections and as an alternative in Penicillin allergic patients.

This drug has excellent tissue penetration, requires no renal dosing adjustments and has a good oral absorption. All these factors make it convenient for outpatient prescription or as follow-up after intravenous therapy.^[5] However, one important issue with Clindamycin treatment is the risk of clinical failure during therapy.

The MLS family of antibiotics has three different mechanisms of resistance: target site modification, enzyme antibiotic inactivation and Macrolide efflux pumps.^[6] Macrolide antibiotic resistance in *Staphylococcus aureus* and *Coagulase negative Staphylococci* (CONS) may be due to an active efflux mechanism encoded by *msr A* (macrolides Streptogramin resistance) genes, conferring resistance to macrolides and type B Streptogramin only^[7] or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B Streptogramin (MLSB resistance). *Erm* (erythromycin resistant methylene) genes are responsible for encoding enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA and reducing binding by MLS agents to the ribosome.^[8] Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if isolates are not tested for inducible Clindamycin resistance. This

resistance missed by using standard susceptibility test methods such as standard broth-based or agar dilution susceptibility tests. The inducible MLSB resistance can be detected by a simple test known as Disk approximation test or D test. Low level of Erythromycin is an inducer of MLSBi phenotype and this is the basis of performing D test.^[9] Failure to identify inducible MLSB resistance may lead to clinical failure of Clindamycin therapy. Conversely, labelling all erythromycin-resistant *staphylococci* as Clindamycin resistant prevents the use of Clindamycin in infections caused by truly Clindamycin-susceptible *Staphylococcal* isolates.

The present study was conducted for detection of inducible resistance to Clindamycin in *Staphylococcus aureus* isolates using D – test at Raichur Institute of Medical Sciences-teaching hospital, RAICHUR, Karnataka, India.

Materials and Methods

The study was conducted from September 2014 to April 2016 in the Department of Microbiology, Raichur Institute of Medical Sciences-teaching hospital, RAICHUR, Karnataka, India. A total of 160 *S. aureus* strains were isolated from various clinical specimens like pus, wound swabs, aspirates, blood, sterile fluids etc. Only one isolate per patient was included in the study. All the isolates were tested for their susceptibility to penicillin (10 units), Gentamicin (10 µg), tetracycline (30 µg), Cotrimoxazole (25 µg), erythromycin (15 µg), ciprofloxacin (5 µg), Vancomycin (30 µg) and linezolid (30µg) by Kirby Bauer disc diffusion method using criteria of standard zone of inhibition. Methicillin resistance was detected by Cefoxitin disk diffusion method using a 30 µg disk (Hi-media laboratories Pvt. Ltd., Mumbai).

D-test

D-test Negative (MS Phenotype): Isolates showing resistance to Erythromycin ($\leq 13\text{mm}$) but susceptible to Clindamycin ($\geq 21\text{mm}$) and showing circular zone of inhibition around Clindamycin.

Constitutive Resistance (cMLSB Phenotype): Isolates showing resistance to both Erythromycin ($\leq 13\text{mm}$) and Clindamycin ($\leq 14\text{mm}$) with circular zone of inhibition if any around Clindamycin.

D-test Positive (iMLSB Phenotype): Isolates showing resistance to Erythromycin ($\leq 13\text{mm}$) and sensitive to Clindamycin ($\geq 21\text{mm}$) and showing D shaped zone of inhibition around Clindamycin with the flattening towards Erythromycin (Fig. 1).

It was observed that many of the isolates were showing resistance to Cefoxitin ($\leq 19\text{mm}$) on routine testing. Suspecting them as MRSA producers, these isolates were further tested for oxacillin ($1\mu\text{g}$) resistance or susceptibility pattern. The results were tabulated and analysed.

Statistical analysis

The data were entered and analysed using Statistical Package for Social Sciences SPSS software version 21.0. P - Value < 0.05 was considered statistically significant.

Results and Discussion

Among the 160 *S. aureus* strains studied, 44 (27.5%) strains were D-test positive i.e. of the inducible MLSB (iMLSB) phenotype as compared to the 25 (15.6%) constitutive MLSB (cMLSB) phenotypic strains (Table 1). High percentage of erythromycin resistance 87 (54.3%) was noted among *S. aureus* strains. In our study, 75 strains (46.8%) were

detected as MRSA of which 32 (42.6%) strains showed inducible Clindamycin resistance. Percentage of both inducible and constitutive resistance was found to be higher amongst MRSA isolates as compared to MSSA ($p < 0.001$) (Table 1).

Clindamycin, a lincosamide, is one of the most efficient antibiotics in treating staphylococcal skin and soft tissue infections, including osteomyelitis because of its excellent tissue penetration except in CNS.^[10] It accumulates in abscesses and no dosage requirements are needed in the presence of renal disease. It also directly inhibits the staphylococcal toxin production and is a useful alternative for patients who are allergic to penicillin.^[11]

Clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and such isolates, can undergo a rapid in vitro and in vivo conversion to a constitutive resistance phenotype.^[12]

Reporting *S. aureus* as susceptible to Clindamycin without checking for inducible resistance may result in institution of inappropriate Clindamycin therapy. On the other hand negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility and provides a very good therapeutic option.^[13] Therefore accurate susceptibility data are important for proper treatment decisions. This is where the D-test becomes significant.

In present study, when *S. aureus* isolates were subjected to D-zone test, it was found that 44 (27.5%) isolates showed inducible Clindamycin resistance (iMLSB phenotype) and 25 (15.6%) showed constitutive resistance (cMLSB phenotype). A study from Bangalore reported that 24.9% of their *S. aureus* strains were of iMLSB phenotype and 18.3% were of cMLSB phenotype.^[14]

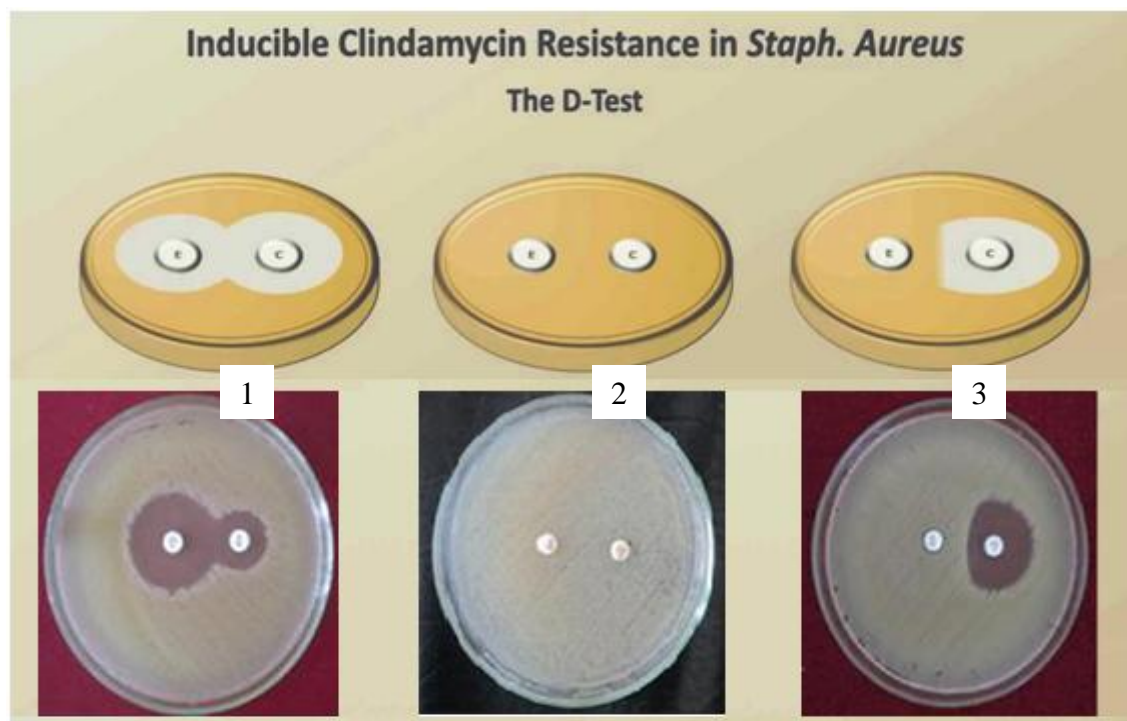
There have been various reports on the pattern of the MLSB resistance among the staphylococci; some reports indicate a high prevalence of the iMLSB phenotype, while the others indicate an increasing frequency of the cMLSB phenotype. The true incidence depends on the patient population studied, the geographical region, the hospital characteristics and methicillin susceptibility.^[10] In this study, it was found that both the inducible and constitutive Clindamycin

resistance were seen in significantly higher proportion among MRSA as compared to MSSA isolates ($p < 0.001$). Studies from different parts of India have reported 30% to 64% of the MRSA isolates to be of the iMLSB phenotype. In the present study, 32 (42.6%) of the 75 MRSA isolates were found to be of iMLSB phenotype which is higher than the findings of Deotale *et al.*, who reported 27.6 % iMLSB resistance in the MRSA isolates.^[13]

Table.1 Comparison of different types of MLSB resistance among *S. aureus* on D-test

PHENOTYPE (SUSCEPTIBILITY PATTERN)	MRSA (%) (n=75)	MSSA (%) (n=85)	TOTAL (%) (n=160)
Inducible Clindamycin resistance (ER-R, CL-S, D test + ve)	32(42.6%)	12(14.1%)	44(27.5%)
Constitutive Clindamycin resistance (ER-R, CL-R)	18(24.0%)	7(8.2%)	25(15.6%)
MS Phenotype (ER-R, CL-S, D test -ve)	10(13.3%)	8(9.4%)	18(11.2%)
Susceptible to Erythromycin & Clindamycin (ER-S, CL-S)	15(20.0%)	58(68.2%)	73(45.6%)

Fig.1



In this study, 13.3% MRSA belonged to MS phenotype as compared to 9.4% MSSA. Similar findings were made by Gadepalli *et al.*, reported 12.0% strains of the MS phenotype among the MRSA and MSSA each.

In present study, 46.8% of the total isolates of the *S. aureus* were MRSA. Other studies have also shown such a high prevalence of MRSA from various parts of the country ranging from 31% to 44%.^[15, 16] Lack of awareness, the indiscriminate and improper use of antibiotics before coming to the hospital might be the contributory factors for such a high prevalence of MRSA. Even though there are recent reports of the increase in emergence of Vancomycin resistance of *S.aureus* worldwide.^[17, 18]

As Clindamycin is one of the most commonly used antibiotics for MRSA isolates, the increasing Clindamycin resistance in the form of iMLSB and cMLSB limits the therapeutic options for MRSA to the antibiotics like linezolid and Vancomycin.

The inducible Clindamycin resistance can be easily missed by routine in vitro susceptibility tests, when the erythromycin and the Clindamycin discs are placed in non-adjacent positions. In view of the therapeutic implications, the D test is a simple, reliable and inexpensive test to perform along with routine susceptibility testing which delineates the inducible (iMLSB) and the constitutive (cMLSB) resistance.

The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible Clindamycin resistance is helpful in guiding anti-staphylococcal therapy. Use of D test in a routine laboratory will enable us in guiding the clinicians regarding the judicious use of Clindamycin.

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