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Original Research Paper

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PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG COMMUNITY AND HOSPITAL ACQUIRED STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL

Nivetha.R — MBBS student, Vinayaka Mission's Kirupananda Variyar Medical College, Salem

Venkata Raghavendra Rao.A* — MD Assistant Professor, Department of Microbiology Vinayaka Mission's Kirupananda Variyar Medical College, Salem * Corresponding Author

Reena Rajan — Senior Lecturer, Department of Microbiology, Penang Internationational Dental College, Salem

ABSTRACT

Introduction: Staphylococci are one among the most common pyogenic bacteria causing human infection. Clindamycin (a lincosamide) is a useful drug in the treatment of both methicillin sensitive and resistant strains of Staphylococcus. Isolates resistant to Macrolide, Lincosamide and type b Streptogramin (MLSB) antibiotics have increased in number following the widespread use of these antibiotics to treat serious staphylococcal infections.

Objectives: The present study is conducted to determine the prevalence of iMLSB resistance among clinical isolates of Staphylococcus aureus by D test.

Materials & Methods: 50 isolates of Staphylococcus aureus obtained from various clinical specimens were included in this study. Antibiotic susceptibility test was performed on these isolates by Kirby Bauer disc diffusion method according to CLSI guidelines. Isolates resistant to Erythromycin were subjected to D test to detect inducible resistance.

Results: Of the total isolates, 26(52%) were Erythromycin resistant. Among the Erythromycin resistant isolates, 7 isolates ,showed inducible clindamycin resistance (D test positive) and one isolate showed constitutive resistance and 5 isolates showed MS phenotype (D test negative).

Conclusion: Routine susceptibility testing for clindamycin may fail to detect inducible clindamycin resistance in Staphylococcus aureus, hence it is necessary to detect such resistance by D test.

KEYWORDS : Staphylococcus aureus, Clindamycin Resistance, D Test, Imlsb

INTRODUCTION:

Staphylococcus aureus is the most important human pathogen and has been recognised as an important cause of nosocomial and community acquired infections. Antibiotic resistance in *Staphylococcus aureus* signifies the need for new effective agents to treat infections. With the emergence of methicillin resistance among *Staphylococcus*, clindamycin is considered to be one of the alternative agents to treat these infections (1, 2). It has an excellent tissue penetration, accumulates in abscess and no dose adjustments are required in the presence of renal disease. The good oral absorption of clindamycin makes it an attractive option for use in outpatient or as follow up treatment after intravenous therapy (1, 3). Clindamycin acts by binding to the 50S ribosomal subunit of bacteria to inhibit its protein synthesis. Macrolide, Lincosamide and type b Streptogramin (MLSB) resistance in Staphylococci is either constitutive (rRNA methylase is always produced) or induced (methylase is only produced in the presence of an inducer) encoded by ermA or erm C gene (3).

Isolates of Staphylococci with constitutive resistance are resistant to both Erythromycin and Clindamycin and those with inducible resistance are resistant to Erythromycin and appear sensitive to Clindamycin. If Clindamycin is used for treatment of such isolates (iMLSB), selection for constitutive erm mutants occur which may lead to treatment failure. Thus the presence of inducible clindamycin resistance is one of the major concerns with respect to the use of clindamycin for the treatment of staphylococcus infection as these strains appear resistant to macrolide and susceptible to Clindamycin under standard testing condition (4, 5). Since Clindamycin has been increasingly prescribed by the physicians in clinical settings due to increasing incidence of community acquired MRSA, it is important to know the presence of inducible Clindamycin resistance in community settings as well as in hospitalized patients. (6) Erythromycin – Clindamycin disc approximation test is a simple reliable method to detect inducible Clindamycin resistance among Erythromycin resistant isolates of *Staphylococcus aureus*. For phenotypic detection of iMLSB strains, D test performed at 15 – 26 mm spacing is recommended by CLSI (2).

AIMS AND OBJECTIVES:

1. To study the antibiotic resistant pattern of *Staphylococcus aureus* isolates.
2. To determine the prevalence of iMLSB resistance among clinical isolates of *Staphylococcus aureus* by D test.

MATERIAL AND METHODS:

A total of 50 isolates of *Staphylococcus aureus* from various clinical specimens such as pus, wound swab, blood, sputum, urine and body fluids were included in this study. The present study was conducted over a period of two months from May 2015 to June 2015 at the department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College, Salem. Urine samples were processed by semi quantitative method on Blood agar and MacConkey agar. Blood samples were inoculated onto Biphasic medium. All other samples were cultured on Blood agar and MacConkey agar and incubated at 37^{0}C for 24 -48 hours. After overnight incubation, staphylococcal colonies were identified by morphology, Gram staining, Catalase test, Coagulase test and Mannitol salt agar (7, 8).

Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) plates according to CLSI guidelines. Inoculum having bacterial count of 10^{5}CFU/ml was swabbed uniformly on MHA plates and incubated at 37^{0}C overnight. After incubation zone of inhibition was measured and results were interpreted as sensitive, intermediate and resistant. The following antibiotics were used : Ampicillin (10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Cotrimoxazole (1.25/ 23.75 µg), Vancomycin (30 µg), Linezolid (30 µg). Nitrofurantoin was used for isolates from urine sample. Methicillin resistance was detected by using Cefoxitin disc (30 µg). *Staphylococcus aureus* ATCC 25923 was used as control strain (9).

Detection of Inducible Clindamycin Resistance by D Test:

Isolates resistant to Erythromycin were subjected to D test to detect inducible resistance as per CLSI guidelines. A lawn culture of 0.5 Mc Farland equivalent suspension of organism was inoculated onto Muller Hinton agar plates. Erythromycin disc (15 µg) and Clindamycin disc (2 µg) were placed with 15 mm gap between the

edges on Muller Hinton Agar and incubated overnight at 37°C. Isolates showing blunted zone of inhibition around Clindamycin on the side adjacent to Erythromycin (D shaped inhibition zone) were considered as positive for inducible resistance (iMLSB) phenotype **(Fig.1)**. Absence of blunted zone (showing circular zone around clindamycin) was considered as D test negative (MS phenotype) **(Fig. 2)**. Those isolates with Erythromycin resistance (≤ 13 mm) and Clindamycin resistance (≤ 14mm) were considered as constitutive (cMLSB) phenotype (10) **(Fig.3)**.

Fig 1: Inducible Clindamycin Resistance (iMLSB) phenotype (D test +ve)

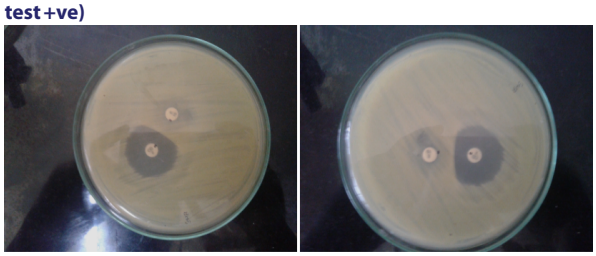


Fig 2: MS phenotype (D test -ve)

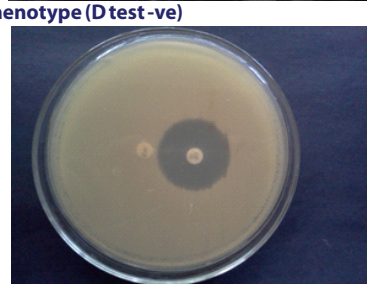
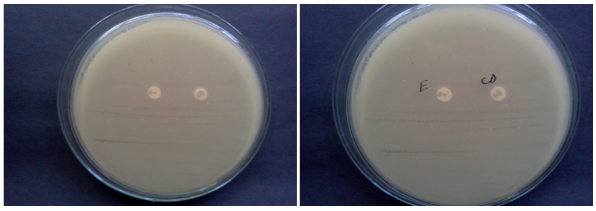


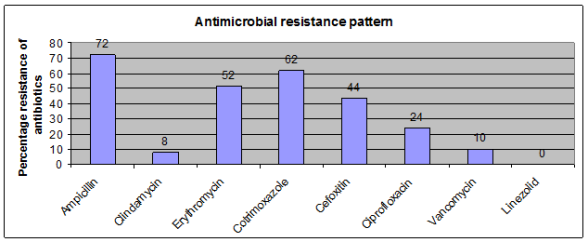
Fig 3: Constitutive Clindamycin Resistance (cMLSB) phenotype



OBSERVATIONS AND RESULTS:

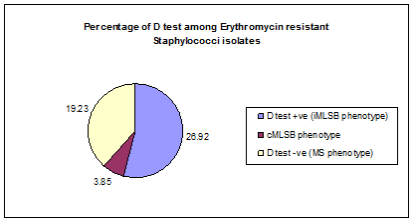
Out of 50 Staphylococcus aureus isolates , 26(52%) were Erythromycin resistant (≤ 13 mm) and 22(44%) isolates were resistant to Cefoxitin (≤ 19mm) by Kirby Bauer disc diffusion method. None of these isolates were found to be resistant to Linezolid. (Chart 1) Among the urinary isolates studied, 23.08% (3/13) were resistant to Nitrofurantoin.

Chart 1: Antimicrobial resistance pattern of Staphylococcus aureus from clinical samples.



Among the 26 Erythromycin resistant isolates, 26.92% (7/26) showed inducible clindamycin resistance (D test positive) and 3.85% (1/26) showed constitutive resistance. A total of 5(19.23%) isolates showed MS phenotype (D test negative). (Chart 2)

Chart 2: Percentage distribution of D - test among Erythromycin resistant staphylococcal isolates.



Out of 26 Erythromycin resistant isolates, 42.31% (11/26) were MSSA and 57.69% (15/26) were MRSA. Among the 15 MRSA isolates, inducible clindamycin resistance (D test positive) was 33.33% and constitutive clindamycin resistance was observed only in 1 isolate (6.67 %) .Among MSSA inducible clindamycin resistance (D test positive) was 18.18%.None of the MSSA isolates have shown constitutive resistance in our study **(Table 1).**

Table 1: D test among Erythromycin resistant Staphylococci isolates.

	D test -ve (MS phenotype)	D test +ve (iMLSB)	cMLSB
MSSA(11)	3/11 = 27.27%	2/11= 18.18%	0 (0%)
MRSA(15)	2/15=13.33%	5/15=33.33%	1/15=6.67
TOTAL(26)	5/26=19.23%	7/26=26.92%	1/26=3.85

DISCUSSION:

Macrolide - Lincosamide - Streptogramin group antibiotics have frequently been used for the treatment of Staphylococcal infection .Macrolide induced clindamycin resistance observed among clinical isolates of Staphylococci results in treatment failure with Clindamycin in vivo, as iMLSB resistance is not recognized by routine disc diffusion method. The prevalence of iMLSB resistance varies according to geographical location. The present study showed 26.92% isolates as having iMLSB. Among MRSA isolates 33.33% were observed as having iMLSB. A study from North India by Gupta et al (11) has showed 72% MRSA isolates as having iMLSB. Inducible Clindamycin resistance was found to be higher in MRSA isolates (33.33%) than MSSA isolates (18.18 %) in our study. This is comparable with another study from South India by Mallikarjuna Reddy et al (12) where inducible Clindamycin was observed in 46.34% of MRSA isolates. A similar study conducted by Levin et al (13) has showed high prevalence of inducible Clindamycin resistance among MSSA isolates. About 6.67 % MRSA isolates were found to be constitutive resistant strains in this study. Low incidence of constitutive Clindamycin resistance was reported by various Indian studies (Angel et al, Gadepalli et al) (14, 15). None of the MSSA isolates were found to be having constitutive resistance in our study which is in concordance with study conducted by Sreenivasalu Reddy et al (16). MS phenotype was observed in 19.23% of staphylococcal isolates in our study. About 7.97% isolates were found to be MS phenotype in North Indian study conducted by Amruth Krishnan et al (17).

Hence, Inclusion of routine double disk approximation test (D test) may be mandatory to prevent treatment failure.

CONCLUSION:

This study concludes that D-test should be used as a simple and reliable method to detect inducible and constitutive clinadamycin resistance among *Staphylococcus aureus* isolates. To take appropriate therapeutic decision, it is necessary to report Clindamycin susceptibility in clinical laboratory by D test which will prevent clinical failure of clindamycin therapy.

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