RESEARCH ARTICLE

Available Online at http://www.recentscientificcom

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 16, Issue, 04, pp.216-219, April2025

International Journal of Recent Scientific Research

ISSN: 0976-3031

Subject Area : Microbiology

PHENOTYPIC CHARACTERIZATION OF MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN B (MLSB) RESISTANCE AMONG METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES

Bagwan Asia[*1], Rajeshwari R.Surpur[2], Aaftab G P[3], Anand Kumar H[4] ,Preeti Sharma[5] and Maalappa[6]

Post Graduate Student., Prof &Head., Associate Professor., Professor, Assistant Professor and Tutor
Department of Microbiology, Navodaya Medical College, Hospital & RC, Raichur, Karnataka, India

DOI: http://dx.doi.org/10.24327/ijrsr.20251604.0038

ARTICLE INFO

Article History:

Received 12th March 2025
Received in revised form 25th March 2025
Accepted 13th April 2025
Published online 28th April 2025

Key words:

Staphylococcus aureus, MRSA, D-test, MLSB, Phenotypes, inducible clindamycin resistance.

ABSTRACT

Introduction: *Staphylococcus aureus* is the most common virulent pathogen and MRSA is a major cause of Hospital and Community acquired infections.Macrolide-Lincosamide-StreptograminB (MLS_B) group of antibiotics especially Clindamycin is the next preferred agent to treat MRSA infections due to emergence of vancomycin resistant strains, however resistance to these antibiotics is of major concern. The Objective of the study is to Detect and Characterize MLS_B resistance phenotypes among MRSA isolates. **Material &Methods:** A total of 1156 pus samples were processed by conventional methods for identification of *S.aureus*. Cefoxitin Disc (30mcg)(Himedia) used to detect MRSA by Disc Diffusion Method as per CLSI guidelines and further tested for Clindamycin Resistance by D test. **Results:** A total of 169 MRSA isolates tested for Clindamycin resistance by D- test shows 53 (31.3%) were of inducible clindamycin resistance (iMLSB phenotype), 42(24.8%) were of MS phenotype and 31 (18.3%) were of constitutive resistance (cMLSB phenotype). **Conclusion:** Clindamycin is a preferred drug due to its excellent pharmacokinetic properties. D test is simple, easy and reliable method to delineate inducible, constitutive, MS phenotypes of clindamycin resistance.

INTRODUCTION

Staphylococcus aureus, a Gram positive cocci is the most frequently encountered virulent pathogen from clinical specimens and causes minor skin infections like furuncles,cellulitis,abscess to life threatening infections like Toxic Shock Syndrome (TSS),Staphylococcal Scalded Skin Syndrome (SSSS),Endocarditis,Septicemia[1] and has developed multifaceted resistance mechanisms against various antibiotics by Active efflux systems that extrude antibiotics from the cell wall, Enzymatic inactivation of antibiotics, Alterations in target sites rendering antibiotics ineffective, Production of **β**-lactamases that hydrolyze **β**-lactam antibiotics [2] **.** Penicillin was the drug of choice to which resistance in Staphylococcus spp. was first detected in 1944, just a year after penicillin became a therapeutic agent. Later, methicillin-resistant Staphylococcus aureus (MRSA) emerged, with the first reported cases in the UK in 1961[3] .The emergence of Methicillin-Resistant *S. aureus* (MRSA) strains, often associated with nosocomial infections, has further complicated treatment options and is a major cause of Hospital and Community acquired infections with a high effect on patient morbidity and mortality [1].

Macrolide-Lincosamide-StreptograminB (MLS_B) group of antibiotics encompasses Macrolides (e.g-Erythromycin, Azithromycin, Spiramycin) Lincosamides (e.g-Clindamycin, Lincomycin) Streptogramin B (e.g-Quinupristin, Dalfopristin) and Clindamycin is the next preferred agent to treat MRSA infections due to emergence of vancomycin resistant strains [2] . Due to widespread use, staphylococcal strains have acquired resistance to MLS_B by mechanisms like Target site modification by "erm gene"that produces methylases, efflux pump mechanism by "msr A" gene, inactivation of lincosamide by chemical alteration by the "inuA gene" [2].

*Corresponding author: **Bagwan Asia**

Department of Microbiology, Navodaya Medical College, Hospital & RC, Raichur, Karnataka, India

Aims and Objectives:

1. To Detect MRSA isolates from pus samples.
2. To Characterize MLS_B resistance phenotypes among MRSA isolates.

MATERIAL &METHODS

This is a Prospective Cross sectional study conducted over a period of 1 year from January 2024 to January 2025 in Department of Microbiology in tertiary care centre, Karnataka.

Inclusion Criteria:

1) Isolates of Staphylococcus from clinical Pus samples during routine diagnostic testing included in this study.
2) Both males and females and of all age groups were included in the study

Exclusion Criteria:

1) All Clinical samples other than Pus samples

2) Organisms other than Staphylococcus aureus were excluded in the study.

Sample size was calculated using the following formula

$n=Z^2 \times p \times q/e^2$

$=1.96^2 \times (0.197 \times 0.803) / 0.06^2$

$=169$

Where,

n =Sample Size

Z =1.96 for 95% Confidence Interval (CI)

P =Prevalence of Study was taken from Previous Study, 19.7% [1]

Q =1-p

e = margin of error, 6%

A total of 1156 Pus samples were included in study and further processed by Conventional methods like Culture, Colony morphology, Gram stain and then standard biochemical tests like Catalase test, Slide and Tube Coagulase test were done for identification .Antibiotic susceptibility testing were performed by Modified Kirby Bauer's Disc Diffusion method as per CLSI Guidelines [4].

Cefoxitin Disc Diffusion test for MRSA detection

Cefoxitin Disc (30mcg) (Himedia, Mumbai) was used to detect MRSA by Disc Diffusion Method as per CLSI Guidelines [4, 6]. Transmitted light was used to examine the light growth of methicillin resistant isolates.

D -Test[1,4,5,6]

A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate and then Clindamycin (2mcg) and Erythromycin (15mcg) discs placed at an edge to edge distance of 15mm on MHA plate followed by overnight incubation at 37 degree.

Flattening of zone around clindamycin in the area adjacent to the erythromycin producing D-shape indicates D-test positive, indicating inducible clindamycin resistance. All such isolates were reported as clindamycin resistant. The strains were interpreted as constitutive MLSB phenotype if resistant to erythromycin with zone size ≤13mm and clindamycin with zone size ≤14mm, and those strains that were resistant to Erythromycin with zone size ≤13mm and sensitive to clindamycin with zone size ≥21mm without D-zone was interpreted as MS phenotype (Table 1)

Table 1. Interpretation of D-Test [2]

Phenotype pattern	Erythromycin (E) Zone size	Clindamycin (CD) Zone size	D-Test Interpretation
Constitutive (cMLSB phenotype)	R(≤13mm)	R(≤14mm)	Growth up to E and CD discs
Inducible (iMLSB phenotype	R(≤13mm)	S(≥21mm)	D-Test +,Clear ,Flattened D shaped zone of inhibition around CD adjacent to E disc
MS phenotype	R(≤13mm)	S(≥21mm)	D-Test -,No D-zone, clear circular zone around Clindamycin disc

RESULTS

Out of 314 *S.aureus* isolates, a total of 169 which shows resistance to Cefoxitin with zone diameters of 21 mm or less considered as MRSA and majority of study group were male 98 (58%) and female of about 71 (42%). Table 2-3.

Table 2. Methicillin Resistance among *S.aureus* isolates

Total isolates	MRSA , n(%)	MSSA , n(%)
314	169(53.8%)	145(46.1%)

Table 3. MRSA prevalence in Males, Females

Prevalence of MRSA	Male ,n(%)	Female ,n(%)
169	98 (58%)	71 (42%)

Table 4. Clindamycin resistant phenotypes of MRSA by D-Test

Clindamycin resistance	Phenotype	No of isolates	percentage
E-R ,CD -S, D-test +	iMLSB phenotype	53	31.3%
E-R ,CD-S, D-Test -	MS phenotype	42	24.8%
E-R,CD-R	cMLSB phenotype	32	18.3%

Among MRSA isolates, 53 (31.3%) were of inducible clindamycin resistance (iMLSB phenotype) and is a predominant phenotype followed by 42(24.8%) of MS phenotype and 31(18.3%) were of constitutive resistance

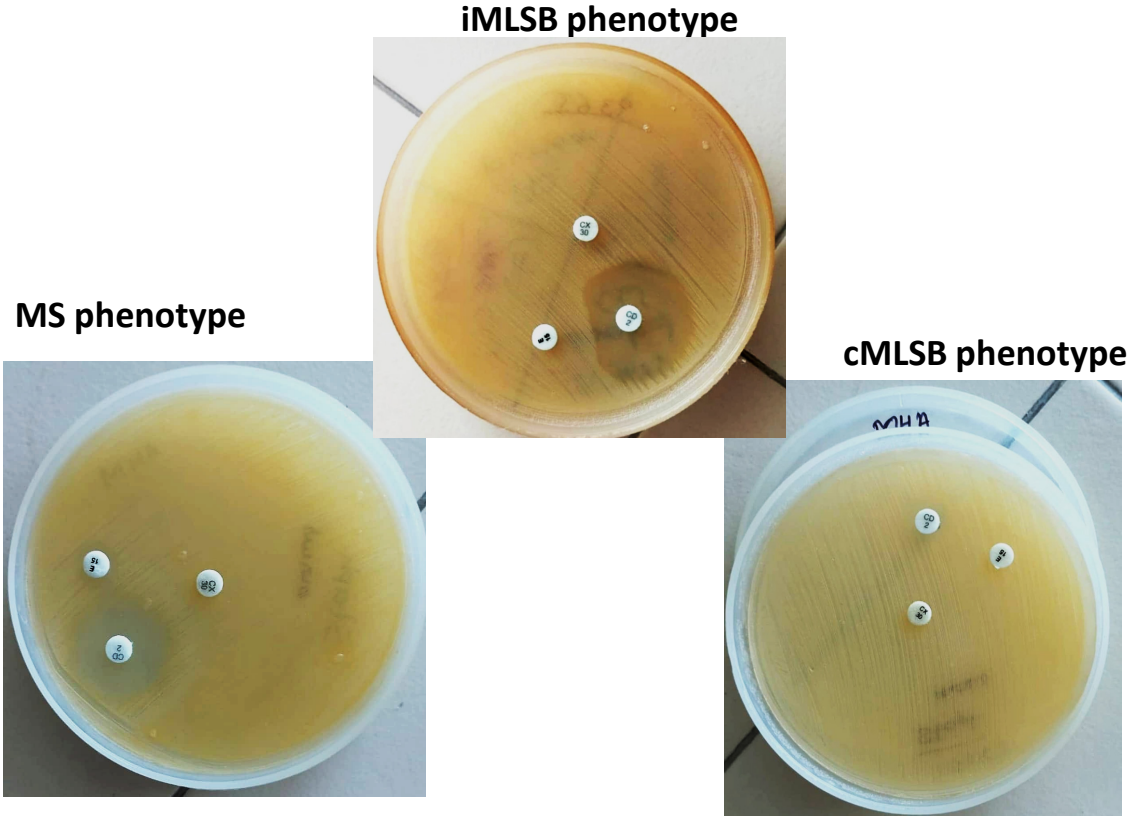
(cMLSB phenotype) by D-test.

DISCUSSION

Clindamycin remains a good alternative option for treating *S.aureus* infections by both MRSA and MSSA because of its good oral bio availability, however, recently there has been increasing resistance pattern to MLS antibiotics.

Isolates with constitutive resistance show in-vitro resistance to both Erythromycin and Clindamycin, while inducible resistance shows erythromycin resistance and appear to be sensitive to Clindamycin in vitro, but in vivo therapy with Clindamycin may select out erm mutants and leads to failure of treatment . MS phenotypes showing resistance to erythromycin and sensitive to Clindamycin in-vitro with successful treatment with Clindamycin in-vivo

- In the present study among 314 S.aureus isolates, 53.8% were MRSA detected which correlates with the result of Modukuru et.al[2]. Who has documented 56.32% MRSA.
- In the present study, Gender difference in the study group shows 58% males affected where as 42% female. Similar to Patel et al[8]. that shows 54% males and 46% females.
- In Venkatesh.et.al [10] 46.35% of MRSA isolated and were prevalent among Pus samples that nearly correlates to our study of 53.8% of MRSA from pus samples, highlighting its importance as a Pyogenic microorganism and it also correlates geographically and as iMLSB predominant phenotype in both studies.
- Present study showed 31.3% of inducible clindamycin resistance among MRSA. It shows concordance result with the study done by Sonagara P et al [1] *as* 19.71% whereas Venkatesh.et.al [10]shows 51.89% iMLSB phenotype and as predominant phenotype.
- Constitutive clindamycin resistance reported as 18.3% in present study which correlates with the study of Prabhu, *et al* [7] *as* 16.66% whereas Modukuru et al [2] reported 76.60% and as predominant phenotype.
- In present study MS phenotype found is 24.8%. Likewise, *Tiwari et al* [11] has reported 16% of MS Phenotype whereas Modukuru et al [2] reported 43.75% in study.



CONCLUSION

Clindamycin, an access group of antibiotic is a preferred alternative drug in *S.aureus* infections due to its excellent pharmacokinetic properties and is treatment of choice in Children and penicillin-allergic individuals. However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug.

D -Test is simple, easy and reliable method to delineate inducible, constitutive, MS phenotypes of clindamycin resistance.False sensitive reports can lead to Clindamycin therapy failures and the selection of a constitutive resistant mutant in an iMLSB strain. So, it will be appropriate that all clinical laboratories test and report inducible clindamycin resistance.

To ensure effective treatment, it's crucial to detect inducible clindamycin resistance using the D-test. Using clindamycin on bacteria with inducible resistance can lead to the development of fully resistant strains, rendering treatment ineffective. This test prevents false sensitivity reports and potential treatment

failure.Accurate laboratory testing is vital to guide successful treatment strategies and avoid clinical failures.

References

1. Sonagara P, Shingala H. A Study of Inducible Clindamycin Resistance in Methicillin Resistant Staphylococcus Aureus in Tertiary Health Care Centre. *GAIMS J Med Sci* 2024;4(2):61-67.
2. Modukuru GK, Surya PMS, Kakumanu VR, Yarava S. Phenotypic Characterization of Macrolide-Lincosamide-Streptogramin B resistance in Staphylococcus aureus. *J Pure Appl Microbiol*. 2021;15(2):689-694. doi: 10.22207/JPAM.15.2.18
3. P C Appelbaum Microbiology of Antibiotic Resistance in Staphylococcus aureus *Clin Infect Dis*200745S16570
4. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing,32nd edition. CLSI Supplement M100. CLSI;2022.
5. Bailey & Scotts Diagnostic Microbiology .15th ed. *elsevier* publications; p.196,266.
6. Essentials of Antimicrobial Stewardship .1st ed. *Jaypee* publications;p.332-333.
7. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. *J Lab Physicians* 2011;3:25-7.
8. Patel M,Waites KB, Moser SA, Cloud GA, Hoesley CJ. Prevalence of Inducible Clindamycin Resistance among Community- and Hospital-Associated Staphylococcus aureus Isolates. *J Clin Microbiol* .2006;44(7):2481–4.
9. Leclercq R. Mechanism of resistance to macrolides and lincosamides. Nature of the resistance elements and their clinical implications. *Clin Infect Dis*.2002;34(4):482-492. doi: 10.1086/324626
10. Venkatesh Naik R, et. al. "Prevalence of Inducible Resistance to Clindamycin in Staphylococcus Aureus Using D-Test at Rims Teaching Hospital, Raichur, India-A Retrospective Study." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS),* 19(6), 2020, pp. 05-08.
11. Tiwari S, Rani E, Kumar A. Inducible and constitutive clindamycin resistance in *Staphylococcus aureus,*isolated from clinical samples. *IP Int J Med Microbiol Trop Dis* 2020;6(3):157-160.
12. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *Indian JMed Microbiol* 2010;28:124-6.
13. Ajantha GS, Kulkarni RD, Shetty J, Shubhada C, Jain P. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates by using the lower limit of recommended inter-disk distance. *Indian J Pathol Microbiol* 2008;51:376-8.
14. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in *Staphylococcus aureus*: A study from North India. *J Postgrad Med* 2009;55:176-9
15. Bingo S, Basak S, Das S, Kaushik P. Inducible clindamycin resistance in *Staphylococcus aureus* and its therapeutic implications. *Int J Curr Res.* 2017;9(10):59930-59933.

How to cite this article:

Bagwan Asia et al. (2025). Phenotypic characterization of macrolide-lincosamide-streptogramin b (mlsb) resistance among methicillin-resistant staphylococcus aureus (mrsa) isolates. *Int J Recent Sci Res*.16(04), pp.216-219.

****