Section: Microbiology

Original Research Article

DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

R. Synthia Selvakumari[1], Nirmaladevi Somasundaram[2], D. Sai keerthana[3], S.K.Vidhya[4], P.Sankar[5]

[1]*Assistant Professor, Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.*
[2]*Associate Professor, Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.*
[3]*Assistant Professor, Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.*
[4]*Assistant Professor, Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.*
[5] *Professor, Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.*

Received *: 19/12/2024*
Received in revised form *: 09/02/2025*
Accepted *: 25/02/2025*

Corresponding Author:
Dr.R. Synthia Selvakumari,
Assistant Professor,Department of Microbiology,Government Medical College and ESI Hospital, Coimbatore, Tamilnadu, India.
Email: smyrna2703@gmail.com

DOI: 10.70034/ijmedph.2025.1.216

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (1); 1150-1154

ABSTRACT

Background: Staphylococcus aureus is the leading cause of nosocomial and community acquired infections globally.The Macrolide - Lincosamide Streptogramin B (MLSB) antibiotics are commonly used to treat MRSA infections. Clindamycin is the most commonly prescribed (MLSB) antibiotics to for MRSA and resulted in Staphylococcal strains acquiring resistance against it. The aim of the study to detect Methicillin resistance and Inducible clindamycin resistance (iMLSB) among S. aureus isolates and to find the effectiveness of commonly used antibiotics and the correlation between Methicillin resistance and Inducible clindamycin resistance.

Materials and Methods: A cross sectional study was conducted from October 2023 to December 2023 in the Department of Microbiology, Tertiary care hospital among 200 samples. Staphylococcus aureus isolates were identified and antimicrobial susceptibility test was done by Kirby Bauer's disc diffusion method. Cefoxitin disc (30 µg) was used to detect Methicillin resistance. CLSI 2023 guidelines were followed for performing the tests and its interpretation. The data collected was entered in MS Excel windowsand the analysis was done through SPSS 16. p value <0.05 is considered as statistically significant.

Results: Total 35 staphylococcus isolates were obtained.Among them 14(40%) were MRSA and 21 (60%) were MSSA. The most common phenotype among Erythromycin resistant Staphylococcus aureus isolate were iMLSB 9 (25.7%) followed by MS 8(22.8%). Among Erythromycin sensitive isolates, S phenotype detected in 13(37.1%) of the samples.

Conclusion: Our study concluded by stating the prevalence of inducible clindamycin resistance (iMLSB) among S. aureus isolates was 25.7%.

Keywords: MRSA -methicilin resistant Staphylococcus aureus, D-test – Double Disc Diffusion test, iCR Inducible clindamycin resistance.

INTRODUCTION

Globally Staphylococcus aureusis a leading cause of nosocomial and community acquired infections.[1] The emergence of drug resistance among Staphylococci is of great concern. Methicillin resistant Staphylococcus aureus (MRSA) has emerged as a major problem of public health importance. β-lactam group of antibiotics are the most commonly used antibiotic for the treatment of Staphylococcus aureus infections.[2]However, Methicillin resistant S.aureus(MRSA) poses severe therapeutic challenges. The Macrolide – LincosamideStreptogramin B (MLSB) antibiotics are commonly used to treat MRSA infections and among them, Clindamycin is the most commonly used MLSB antibiotic in the treatment of Staphylococcal infections particularly in Methicillin resistant isolates due to its high bioavailability, the availability of both parenteral and oral formulations,

soft tissue permeability, inhibits toxin production, and it is relatively cheap.[4] However, the wide spread use of MLSB antibiotics has resulted in an increase in the number of Staphylococcal strains acquiring resistance against Clindamycin.[5]The resistance to Clindamycin depends upon the expression of rRNAmethylase (RM) by S.aureus, either produced constitutively or induced by an inducing agent. Erythromycin is known to be an effective inducer of the enzyme.

S. aureus isolates with constitutively expressing RM (strains termed cMLSB) resist Erythromycin and Clindamycin. Invitro Staphylococcal strains with inducible rRNAmethylase enzyme (iMLSB) shows resistance only to Erythromycin but are sensitive to Clindamycin.[6,7] In vitro routine tests for Clindamycin susceptibility may fail to detect inducible clindamycin resistance. The difference between MLSB and cMLSB can be detected by Double disc diffusion test called D test.

D-test can help to determine whether Clindamycin could be used as a therapeutic option in S.aureus.[8]Sedighiet al have recommended to use D-test routinely in all microbiologic laboratories and not to apply Clindamycin in patients with infections caused by inducible resistant S. aureus to avoid treatment failure.They also suggested to avoid switch therapy from Erythromycin to Clindamycin.[9] The D-zone test has a high throughput reporting different types of phenotypic resistance in a single test. This method has a sensitivity of 100% when the Erythromycin disc (15ug) and the Clindamycin disc (2ug) are kept within in the distance of 15 mm.[10] The present study was aimed to find out the percentage of S. aureus having inducible clindamycin resistance (iMLSB)using D- test. The aim of the study to detect Methicillin resistance and Inducible clindamycin resistance (iMLSB) among S.aureus isolates and to findthe effectiveness of commonly used antibiotics and the correlation between Methicillin resistance and Inducible clindamycin resistance.

MATERIALS AND METHODS

Study Design: A cross sectional study.
Study site: Microbiology laboratory.

Study period	:2 months (October 2023 to December 2023).
Samples size	:200.
Study population	: Samples of patient attending tertiary care hospital.

Study Samples

Clinical samples including wound Swab, blood, pus, sputum, and body fluids received in the diagnostic Microbiology laboratory for Culture & Sensitivity were included in this study.

Procedure

All samples received were streaked on Mac Conkey agar and blood agar media and incubated at 37°C for 24-48 hrs.Colony morphology was observed, Grams staining was performed and Gram positive cocci in clusters were observed.The isolates were subjected to catalase test.The catalase positive isolates were further subjected to tube coagulase test as per standard laboratory protocols. The S. aureus isolates were collected and subjected for antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method.[6] The sensitivity pattern of the isolates to the drugs were observed.

An inhibition zone 22 mm or less around Cefoxitin disc indicates MRSA as per CLSI guidelines 2023. Inducible resistance to Clindamycin were tested by 'D test' as per CLSI guidelines.[7]

Quality control (QC) of the Erythromycin and Clindamycin discs were performed with S. aureus ATCC 25923, according to the standard disc diffusion QC procedure. Additional QC were performed with separate in-house selected S. aureus strains that demonstrated positive and negative D-test reactions. Different phenotypes were observed.

Sensitive (S) phenotype: Inhibition of growth around Erythromycin (zone size ≥23 mm) and Clindamycin (zone size ≥21 mm). Sensitive to both E and CD Constitutive MLSB phenotype (MLSBc): Presence of growth around Erythromycin (zone size ≤13 mm) and Clindamycin (zone size ≤14 mm). Resistant to both E and CD. Inducible MLSB phenotype (MLSBi): Presence of growth around Erythromycin (zone size ≤13 mm) and clearance around Clindamycin (zone size ≥21 mm), giving D shaped zone of inhibition around Clindamycin with flattening towards Erythromycin disc (D test positive). MS phenotype: Presence of growth around Erythromycin (zone size ≤13 mm) and inhibition of growth around Clindamycin (zone size ≥21 mm) and giving circular zone of clearance around Clindamycin (D test negative).

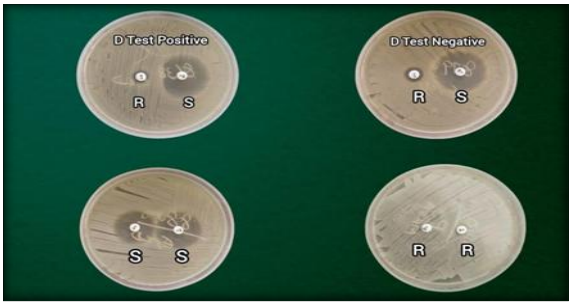

Figure 1: Different phenotypes of Staphylococcal isolates

Figure 1:a) MLSBiinducible clindamycin resistance (E=Resistant CD=Sensitive with positive D testb) MS- (E=Resistant and CD=Sensitive) ; c) S- (E=Sensitive and CD=Sensitive) ; d) MLSBc-constitutive clindamycin resistance (E=Resistant and CD=Resistant. E-Erythromycin&; CD-Clindamycin.

Statistical Analysis

The data were entered in spread sheet. Univariate analysis weresummarised using numbers and percentages. Bivariate analysis was done using Chi-

square statistics. p value <0.05 is considered as statistically significant.

RESULTS

Among 200 samples studied, pus was the most common specimen 128(64%) followed bySputum 40,Blood (23) and body fluid. Among the various samples the culture positivity of the samples were pus 74 (85.5%),OP 16 (21.6%) IP 58 (78.37%), sputum 10(11.49%) OP 2(,20%) IP 8(80%), blood 3(3.44%) OP (0,0%) IP (3,100%), body fluids 0(0%). [Table1, 2]

Table 1: Distribution of samples

	Pus	Sputum	Blood	Bodyfluids	Ear/eye swab	Total
OP	**23**	**13**	**1**	**1**	**0**	**38**
IP	**105**	**27**	**22**	**8**	**0**	**162**
Total	**128**	**40**	**23**	**9**	**0**	**200**

Table 2: Culture positivity percentage among various samples -200

	Pus	Sputum	Blood	Bodyfluids	Ear/eye swab
OP	16(21.6%)	2(20%)	0(0%)	0(0%)	0 (0%)
IP	58(78.4%)	8(80%)	3(100%)	0 (0%)	0 (0%)
Total	74(85.05%)	10(11.4%)	3(3.34%)	0 (0%)	0 (0%)

Among the 200 samples most of thesamples were from males 122. The most common age group was above 50 years were 77(63.2%) and below 50 years were 45(36.8%). Infemales 78 most common age group was above 50years were 44(56.4%) and below 50 years were34(43.6). [Table 3]

Table 3: Sample distribution among Males and females -200

	> 50years	< 50 years	Total
Males	77(63.2%)	45(36.8%)	122
Females	44(56.4%)	34(43.6%)	78

Among the 200 samples Staphylococcus aureus was isolated in 35(17.5%)samples. Pus was the most common sample from which 29(82.8%) followed by sputum 3(8.5%) blood 2 (5.7%) body fluid 1(2.8%).

Among the 35 isolates of S aureus 21(60%) isolates were Methicillin sensitive S aureus and 14 (40%)were Methicillin resistance S aureus. [Table 4, 5]

Table 4: Total number of Staphylococcus aureus isolates from specimens

	Pus	blood	sputum	Body fluids	Others	Total
Staphylococcus aureus isolates	29(82.8%)	2(5.7%)	3(8.5%)	1(2.8%)	0(0%)	35

Staphylococcus aureus isolate was iMLSB 9(25.7%), MS 8(22.8%), cMLSB 5(14.2%). Among Erythromycin sensitive isolates S phenotype detected in 13(37.1%). Gauravdalela et al., from Rajasthan also observed iMLSB phenotype as most common phenotype, their study showed a higher incidence of iMLSB (36.63%) as compared to cMLSB(32.67%) in the S.aureus isolates.[15]

Table 5: Staphylococcus aureus isolated from specimen and their Methicillin susceptibility

Sample	Total no of isolates	MRSA	MSSA
Pus	**29(82.8%)**	**12(86%)**	**17(81%)**
Sputum	**3(8.5%)**	**2(14%)**	**1(5%)**
Body fluid	**1(2.8%)**	**0(0%)**	**1(5%)**
Blood	**2(6%)**	**0(0%)**	**2(9%)**
Ear/eye swab	**0(0%)**	**0(0%)**	**0(0%)**
Total	**35(100%)**	**14(40%)**	**21(60%)**

The frequency of susceptibility pattern to Erythromycin as well as different patterns of susceptibility to Staphylococcus aureus was noted[Table 6].The inducible clindamycin resistance (D-test positive) was more commonly seen in MRSA as compared to Methicillin sensitive Staphylococcus aureus (MSSA). [Table 7]

Table 6: Phenotypes of Staphylococcus aureus isolates

	Phenotypes			
	cMLSB (E*-r,CD*-r)	**iMLSB (E-r, CD-s. D-test-pos-itive)**	**MS (E-r*, CD-s*. D-test-negative)**	**S (E-s,CD-s)**
Staphylococcus aureus	5(14.2%)	9(25.7%)	8(22.8%)	13(37.1%)

MRSA	3(8.5%)	7(20%)	2(5.7%)	2(5.7%)
MSSA	2(5.7%)	2(5.7%)	6(17.14%)	11(31.4%)

Table 7: Pattern of inducible clindamycin resistance(iMLSB) in Staphylococcus aureus with respect to methicillin susceptibility

Methicillin Susceptibility	D-test		total	Chisquare value	p-value
	Positive	negative			
MRSA	**7(77.77%)**	**2(22.22%)**	**9**	**4.45679**	**0.03476**
MSSA	**2 (25%)**	**6 (75%)**	**8**		

DISCUSSIONS

Staphylococcus aureus was isolated most commonly from pus 29(82.8%) in the present study. Goudarziet al.,from Iran also isolated S.aureus from wound specimen (35%). Similarly Prasanthsingh et al., from Haryana, Mohanasoundaram et al., from Tamil nadu, Jangla et al., from Maharashtra reported pus as most common specimen reported S aureus 43%, 35% and 29% respectively.[1,13,17,18]

As per ICMR 2018-2022 antimicrobial resistance pattern report, the prevalence of MRSA from 2015 till 2022 in India were 37.5% in 2015, 32.9% in 2017, 38.6% in 2018, 42.6% in 2020 respectively. And in the present study prevalence was 40% and it was similar to the findings of Ajantha AS et al.,from Madhya Pradesh(64%), V Aruna et al.,form Tamil nadu (30.9%), Mohanasoundaram from Tamil nadu (39%) respectively.[5,11,12,17]

In the present study, Cefoxitin resistance was seen in (40%) samples. The sensitivity pattern of commonly used antibiotics were Doxcycline (100%),Cotrimoxazole (67%),Erythromycin (33%), Linezolid (100%), Tetracycline (100%), Vancomycin (100%), Clindamycin (67%) was observed.In the present study, the most common phenotype among Erythromycin resistant Staphylococcus aureus isolate was iMLSB9(25.7%), MS 8(22.8%), cMLSB 5(14.2%). Among Erythromycin sensitive isolates S phenotype detected in 13(37.1%). Gaurav dalela et al., from Rajasthan also observed iMLSB phenotype as most common phenotype, their study showed a higher incidence of iMLSB (36.63%) as compared to cMLSB(32.67%) in the S.aureus isolates.[15]

In present study inducible clindamycin resistance was observed in 25.7% isolates. This was similar to findings of V Aruna et al., from Tamil Nadu in Staphylococcus aureus (40%), Prasanthsingh et al., from Haryana (23%), Upadhya et al., from Karnataka in Staphylococcus aureus (33%).[12,13,16]This iMLSB phenotype widely varies on the basis of geographical location, patient age, type of clinical specimen, hospital environment, bacterial species and antibiotic susceptibility profile of bacteria. The emergence of MDR in Staphylococcus aureus has left limited options to the clinicians in selection of appropriate antibiotics. The inducible resistance may be missed by routine invitro susceptibility tests. D test is very simple, easy to perform and reliable method for detection of MLSBc and MLSBi resistance in resource limited setups. D test should be performed on all the Staphylococcus aureus isolates as a routine antimicrobial susceptibility testing. To avoid therapeutic failures of Clindamycin in Staphylococcal infections the D test to be performed as a routine test in all the laboratories.

CONCLUSION

According to our study findings, the prevalence of inducible Clindamycin Resistance (iMLSB) among S. aureus isolates was 25.7%.In future, Clindamycin can be kept as a reserve drug and advocated in severe MRSA infections. D test can be advised as a mandatory method in routine disc diffusion testing to detect inducible Clindamycin resistance in Staphylococcus for the optimum treatment of patients to avoid false susceptible results leading to treatment failure. Treatment with clindamycin to be avoided in patients with inducible clinamycin resistance.

Competing interest: There is no competing interest

Authors contribution: All authors in our study contributed to the data collection of the patients

Acknowledgement: The authors like to thank the Dean of the Medical College, Head of the Department of Microbiology, Government Medical College and ESI Hospital, Coimbatore, TamilNadu.

REFERENCES

1. Goudarzi M, Kobayashi N, Dadashi M, Pantůček R, Nasiri MJ, Fazeli M. Prevalence, genetic diversity, and temporary shifts of inducible clindamycinresistanceStaphylococcusaureusClones in Tehran, Iran: a molecular–epidemiological analysis from 2013 to 2018. Front Microbiol. 2020. https://doi.org/ 10. 3389/ fmicb. 2020. 00663.
2. Goudarzi M, Navidinia M, Beiranvand E, Goudarzi H. Phenotypic and Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Clones Carrying the PantonValentineLeukocid in Genes Disseminating in Iranian Hospitals.Microb Drug Resist.2018;24 (10) :1543-1551. doi: 10.1089/mdr.2018.0033.
3. Mama M, Aklilu A, Misgna K et al ., Methicillin- and Inducible Clindamycin - Resistant Staphylococcus aureus among Patients withWound Infection Attending Arba Minch Hospital,South Ethiopia. Int J Microbiol. 2019;2965490.doi:10.1155/2019/2965490.
4. Fishovitz J, et al ., Penicillin binding protein 2a of methicillin-resistant Staphylococcus aureus. IUBMB Life 2014; 66 : 572-7.
5. Ajantha GS, Kulkarni RD et al ., (2008) Phenotypic detection of inducible clindamycin resistance among Staphylococcus aureus isolates by using the lower limit of

recommended inter-disk distance. Indian J Pathol Microbiol51:376–378.
6. Gupta V et al ., Inducible clindamycin resistance in Staphylococcus aureus: a study from North India. J Postgrad Med.2009;55(3):176-179. doi:10.4103/0022-3859.57393.
7. Ahmed MO, Alghazali MH, Abuzweda AR, Amri SG. Detection of inducible clindamycin resistance (MLSBi) among methicillin-resistant Staphylococcus aureus (MRSA) from Libya. Libyan J Med. 2010; 5:1,4636. doi:10.3402/ljm.v5i0.463610.3402/ljm.v5i0.4636.
8. Lall M, Sahni AK. Prevalence of inducible clindamycinresistance in Staphylococcus aureusisolated from clinicalsamples. Med J Armed Forces India 2014; 70 : 43-7.
9. Sedighi I, Mashouf RY, Pak N, SeifRabiee MA. D-testmethod for detection of inducible clindamycin resistance in Staphylococcus aureus. Iran J Pediatr2009; 19: 293-7.
10. O'Sullivan MVN, Cai Y, Kong F, Zeng X, Gilbert GL. Influence of disk separation distance on accuracy of the disk approximation test for detection of inducible clindamycin resistance in Staphylococcus spp. J Clin Microbiol.2006;44(11):4072–6.
11. ICMR. Annual Report amtimicrobial Resistance Surveillaence Network 2022.
12. V Aruna, VM Theeba, S Arul Selvan, Rajesh Sengodan. Clindamycin Resistance Detection among MRSA at a Tertiary Care Hospital: A Cross-sectional Study.2024.
13. Prashant singh, Pratibha Mane, PoojaSingla, JyotiSangwan .Prevalence of Inducible Clindamycin Resistance in Staphylococcus: A Study from a Rural Teaching Tertiary Care Hospital in Southern Haryana. National Journal of Laboratory Medicine. 2021.
14. Juyal D, Shamanth AS, Pal S, Sharma MK, Prakash R, Sharma N. Theprevalence of inducible clindamycin resistance among staphylococci in a tertiarycare hospital - A study from the Garhwal hills of Uttarakhand, India. J ClinDiagn.
15. Phenotypic Expression of erm Gene Among Staphylococcus aureus.GauravDalela, Atul Vijay, Manoj Joshi.2016.
16. Upadhya A, Biradar S. The prevalence of inducible clindamycin resistance in Staphylococcus aureus in a tertiary care hospital in north-east Karnataka, India. Health Sciences: An International Journal. 2011;1(3):21–24. [GoogleScholar].
17. Mohanasoundaram KM. The prevalence of inducible clindamycin resistance among gram positive cocci which were isolated from various clinical specimens. Journal of Clinical and Diagnostic Research. 2011;5(1):38–40. [GoogleScholar].
18. Jangla SM, Naidu R, Machhi BS. Inducible clindamycin resistance in gram positive staphylococci obtained from clinical samples in a tertiary care hospital in Mumbai. Res. 2019.
19. Zorgani A., Shawerf O., Tawil K., El-Turki E., Ghenghesh K. Inducible clindamycin resistance among staphylococci isolated from burn patients. Libyan Journal of Medicine.2009;4 (3):104–106. doi: 10.4176/090128. [PMC free article] [PubMed] [CrossRef] [Google Scholar].
20. Abdelmawgoud Y. E., El-Latif A., Fawzy N. K., Elnagdy S. M. Prevalence of inducible clindamycin resistance and nanotechnological control of Staphylococcus aureus clinical isolates. Egyptian Journal of Botany. 2022;62(1):73–84. [Google Scholar].