ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 11 | November - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATES RECOVERED FROM A TERTIARY CARE TEACHING HOSPITAL, CENTRAL GUJARAT

Microbiology

Dr Vidhi A Patel — Final year post-graduate, Department of Microbiology, Medical College Baroda & SSG Hospital, Vadodara, Gujarat.

Dr. Arpita T Parmar* — Final year post-graduate, Department of Microbiology , Medical College Baroda & SSG Hospital, Vadodara, Gujarat. *Corresponding Author

Dr. Tanuja B Javadekar — Professor & Dean, Department of Microbiology, Medical College Baroda & SSG Hospital, Vadodara, Gujarat.

Dr. Bithika Duttaroy — Professor & Head, Department of Microbiology, Medical College Baroda & SSG Hospital, Vadodara, Gujarat.

ABSTRACT

Introduction: Staphylococcus aureus is an important agent in hospital and community associated infections causing high morbidity and mortality. For resistance to Clindamycin can be constitutive or inducible. **Objective:** To determine the prevalence of Inducible Clindamycin resistance among Staphylococcus aureus isolates by D-test method. **Material and methods:** A total of 138 Staphylococcus aureus were obtained from various clinical samples . Antibiotic susceptibility test and D test were carried out as per Clinical Laboratory Standards Institutes (CLSI) guidelines M100. Flattening of inhibition zone (D-shaped) around Clindamycin was considered as Inducible Clindamycin resistance. **Result and Conclusion:** Out of 138 Staphylococcus aureus isolates,76 (55.1%) were Methicillin resistant and 62 (44.9%) were Methicillin sensitive. Inducible clindamycin resistance was detected in 58 (76.3%) MRSA isolates and 12 (19.3%) MSSA isolates. In conclusion, we found a high prevalence of inducible clindamycin resistance in our region.

KEYWORDS

Staphylococcus aureus, Clindamycin, Inducible resistance

INTRODUCTION:

Staphylococcus aureus (S.aureus) is recognized as one of the important organism causing hospital acquired and community-acquired skin and soft tissue infections in most parts of the world. It's increasing prevalence of methicillin resistance among Staphylococcus aureus is matter of concern and left with the very few therapeutic options1. The macrolide – lincosamide – streptogramin B (MLSB) family of antibiotic serves as one such alternative, with clindamycin being the preferred drug due to its excellent pharmacokinetic properties 2.

Clindamycin is a lincosamide antibiotic, developed in 1966 by chemically modifying the naturally occurring lincomycin3. Both MRSA and methicillin-sensitive S. aureus (MSSA) can be treated by Clindamycin, an MLSB antibiotic which is of low cost, and has fewer side effects and high bioavailability in both oral and parenteral forms4,5. Clindamycin is considered a useful alternative in Penicillin allergic patients for the treatment of skin and soft tissue infections caused by S.aureus. It has excellent tissue penetration except into the central nervous system, where it does not cross the blood-brain barrier, even in the presence of inflamed meninges 6. Good oral absorption makes it an attractive option for outpatient prescription or as a follow-up drug after intravenous therapy 7. This helps in the early shift to outpatient management of susceptible infection without the need for continued intravenous access 8.

Resistance to MLSB antibiotics occur by many different mechanisms. The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively (cMLSB phenotype) or inducibly (iMLSB phenotype). The erm genes codes for methylase enzyme which methylates and alters the target site of MLSB antibiotics i.e. the 23S ribosomal RNA 9,10.

It is very difficult to detect the inducible clindamycin resistance in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive in vitro when not placed adjacent to each other11. In such cases, in vivo therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure.

In case of another mechanism of resistance mediated through msrA genes i.e. efflux of antibiotic, staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro and the strain do not typically become clindamycin resistant during therapy 12.

This study helps to demonstrate a very simple and useful method of detecting inducible resistance to clindamycin in erythromycin resistant Staphylococcal isolates i.e." D test" as described by Fiebelkorn et al 13. Also, we tried to establish the relationship between methicillin-resistant Staphylococcus aureus (MRSA) and inducible clindamycin resistance.

MATERIAL & METHODS :

A total of 138 Staphylococcus aureus isolates were obtained from various clinical samples like pus, wound swab, aspirate , blood and sterile fluid received from various wards & ICUs of a tertiary care teaching hospital located in Central Gujarat during the period of January 2022 to May 2022. The isolates were subjected to antibiotic susceptibility testing on Mueller Hinton agar plate as per CLSI guidelines. The antibiotic susceptibility testing of Staphylococcus aureus isolates were carried out by Disc Diffusion method with using of Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Vancomycin (30µg), Linezolid (30µg) and D test carried out as per Clinical Laboratory Standards Institutes (CLSI) guidelines M100 14. A flattening of inhibition zone (D-shaped) around Clindamycin disc proximal to the Erythromycin disc was considered a positive result which indicates that the erythromycin has induced clindamycin resistance (D+ test). [Fig 1]

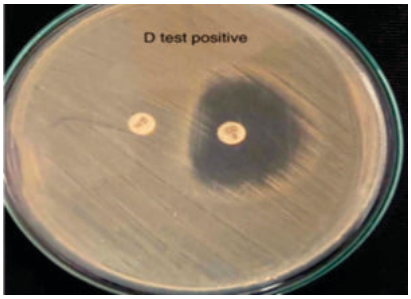

Fig 1. Showing D shaped zone around Clindamycin when Clindamycin and Erythromycin are placed at a distance of 15mm indicating D test positive.

Three phenotypes were identified:

1. MS phenotype:

Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤ 13mm) while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of Inhibition around clindamycin was labelled as having MS phenotype.

2. Inducible MLSB (iMLSB) phenotype:

Staphylococcal isolates sharing resistance to Erythromycin (zone size ≤ 13mm) while being sensitive to clindamycin (zone size ≥ 21mm) and giving D shaped zone of Inhibition around clindamycin with flattening towards erythromycin disc was labelled as having iMLSB phenotype.

3. Constitutive MLSB (cMLSB) phenotype:

This phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤ 13mm) and clindamycin (zone size ≤14 mm) with a circular shape of the zone of inhibition around the discs.

RESULTS:

Out of 138 Staphylococcus aureus isolates, 76 (55.1%) were Methicillin resistant and 62 (44.9%) were Methicillin sensitive. Among MRSA strain 46 isolates (82.4%) were Erythromycin resistant and 30 isolates (52.6%) were Clindamycin resistant. A 38 isolates (22%) were Erythromycin resistant and 24 isolates (10.6%) were Clindamycin resistant among MSSA strain. Inducible clindamycin resistance was detected in 58 (76.3%) MRSA isolates and 12 (19.3%) MSSA isolates.

Table-1: Susceptibility to Erythromycin and Clindamycin among Staphylococcus aureus Isolates

Sr No.	Susceptibility pattern (phenotype)	Number of isolates	Percentage
1	ERY – S CL – S	47	34.1 %
2	ERY – R CL – R (cMLSB)	13	9.2 %
3	ERY – R CL – S (D test positive- iMLSB)	70	50.7%
4	ERY – R CL – S (D test negative MS)	8	6 %
Total		138	100 %

ERY – Erythromycin, CL – Clindamycin, S –Sensitive, R – Resistant, cMLSB – constitutive MLSB phenotype, iMLSB – inducible MLSB phenotype, MS– MS phenotype.

Table–2: Association of Clindamycin resistance with Methicillin resistance.

Clindamycin resistance	Methicillin resistance (Cefoxitin)		
	MRSA n=76	MSSA n=62	Total 138
ERY – S, CL – S	8	39	47
ERY – R CL – R cMLSB)	7	6	13
ERY – R CL – S (D test positive iMLSB)	58	12	70
ERY – R CL – S (D test negative MS)	3	5	8

ERY – Erythromycin, CL – Clindamycin, S –Sensitive, R – Resistant, cMLSB – constitutive MLSB phenotype, iMLSB – inducible MLSB phenotype, MS– MS phenotype, MRSA – Methicillin-resistant Staphylococcus aureus, MSSA – Methicillin sensitive Staphylococcus aureus.

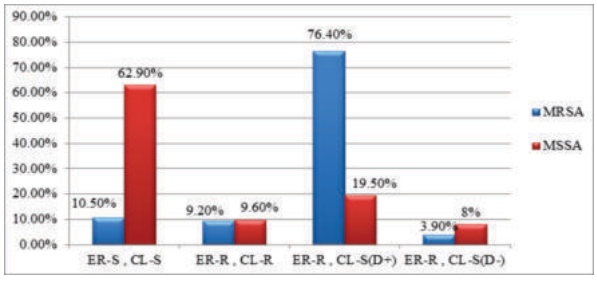

Fig 2 : usceptibility of S.aureus strain to Erythromycin (ER) & Clindamycin (CL)

DISCUSSION

Because of the increase in the resistance and emergence of multidrug-resistant organisms, Accurate antimicrobial susceptibility data of an isolate is crucial for appropriate therapy decisions. Empirical outpatient treatment options for Staphylococcal infections have become more limited as concern about the prevalence of MRSA has increased 15,16. Among the options available for MRSA and MSSA infections, Clindamycin has evolved much interest, as it is a very good alternative because of its excellent pharmacokinetic properties. 17

However, resistance to Clindamycin is highly variable, and the incidence of constitutive and inducible MLSB resistant phenotype varies by geographic regions and even between hospitals . So 'D test' becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of Staphylococcus aureus .18 It was also observed that percentages of inducible resistance and constitutive Clindamycin resistance were higher amongst MRSA as compared to MSSA (76.4%, 9.2% and 19.5%, 9.6%, respectively). This was not in concordance with few of the studies reported before. Some studies have showed higher percentage of inducible resistance in MSSA as compared to MRSA.12

Even though the overall prevalence of inducible Clindamycin resistance among the isolates was found to be low in our set up, this study showed higher percentage of resistance to Erythromycin and Clindamycin among MRSA as compared to other studies. 20, 21, 22 This indicates that there is wide use of erythromycin and Clindamycin for the treatment of staphylococcal infections in our set up, as wide consumption of macrolides results emergence of macrolide resistant Staphylococcus species due to selective pressure. 11,19 As this resistant patterns can be decreased by reduction in the use of macrolides.19 This study emphasizes the need to do likewise in our set up to reserve it as an alternative drug of choice for treating infection caused by MRSA. This study showed only 3.90% of MRSA among the erythromycin resistant isolates as MS phenotype (E-R, Clin-S) which means Clindamycin can be used as treatment option only for less number of MRSA which are erythromycin resistant. These findings further emphasize the need of D- test to be performed routinely in our set up to avoid clinical failure while using Clindamycin as an alternative to anti-MRSA antibiotics like Vancomycin and Linezolid.

CONCLUSION:

Accurate susceptibility data are important for appropriate therapy decisions. The pattern of macrolide resistance in S. aureus varies in different regions. Depending upon this the prescription rate will not be uniform in different regions. It is kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending upon the antimicrobial susceptibility results. However, expression of inducible resistance to Clindamycin could limit the effectiveness of this drug. So, clinical microbiology laboratories should report inducible clindamycin resistance in S. aureus, and D-test can be used as a simple, auxiliary and reliable method to describe inducible and constitutive Clindamycin resistance in routine clinical laboratories.

REFERENCES:

1. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol. 2007; 56;104106.
2. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible Clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol. 2010;28;124-126.
3. Mallamgunta S , Naidu KH, Ramakrishna N . Detection of inducible clindamycin Resistance among staphylococcus isolated from patients attending a tertiary care hospital in South India 2020;9(2): 105-109.
4. Timsina R, Shrestha U, Singh A & Timalsina B, Inducible clindamycin resistance And erm genes in Staphylococcus aureus in school children in Kathmandu, Nepal Future Science OA; January 2021;7(1).
5. Baral R, Khanal B. Inducible clindamycin resistance in Staphylococcus aureus strains isolated from clinical samples. Int. J. Biomed Res. 8(2), 81–84 (2017).
6. Upadhya A, Biradar S. Prevalence of inducible clindamycin resistance in Staphylococcus Aureus in a tertiary care hospital in north-east Karnataka, India. Int Health Sci Int J. 2011;1;21-24.
7. Banik A, Khyriem A B, Gurung J, Lyngdoh V W. Inducible and constitutive clindamycin Resistance in Staphylococcus aureus in a northeastern Indian tertiary care hospital. J Infect Dev Ctries. 2015;9(7)725-731.
8. Juyal D, Shamanth A, Pal S, Sharma M, Prakash R, Sharma N. The prevalence of inducible clindamycin resistance among Staphylococci in a tertiary care hospital – A Study from the Garhwal Hills of Uttarakhand, India. J Clin Diagn Res. 2013;7;61-65.
9. Jessica M, et al. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13:42–51.

10. Kishk RM, Anani MM, Nemr NA , Soliman NM , Fouad MM. Inducible clindamycin Resistance in clinical isolates of staphylococcus aureus in Suez Canal University Hospital, Ismailia, Egypt. J Infect Dev Ctries 2020; 14(11):1281-1287.
11. Adhikari, R.P., Shrestha S., Barakoti A. et al. Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infect Dis 2017;483 (2017). 10.1186/s12879-017-2584-5.
12. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J lab physicians. 2011;3(1):25–7.
13. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disc diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative Staphylococci. J Clin Microbiol. 2003;41;4740-4744.
14. Clinical and Laboratory Standards Institute (CLSI) M100. Performance standards for antimicrobial susceptibility testing 31st edition. CLSI 2021.
15. Ajantha G, Kulkarni R, Shetty J, Shubhada C,Jain P. Phenotypic detection of inducible Clindamycin resistance among Staphylococcus aureus isolates by using the lower limit of recommended inter-disc distance. Indian J Pathol Microbiol. 2008;51;376-378.
16. 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;2481-2484.
17. Lavallee C, Rouleau D, Gaudreau C, Roger M, Tsimiklis C, Locas MC, Gagnon S, Delorme J, Labbe AC. Performance of an Agar Dilution Method and a Vitek 2 card For detection of Inducible clindamycin resistance in Staphylococcus spp. J Clin Microbiol. 2010;48;1354-1357.
18. Singh T, Deshmukh AB, Chitnis V, Bajpal T. Inducible clindamycin resistance among Staphylococcus aureus isolates in a tertiary care hospital. Int J Health Allied Sci. 2016;5;111-114.
19. Appelbaum PC. Microbiology of antibiotic resistance in Staphylococcus aureus. Clin Infect Dis. 2007;45:S165–70.
20. Baiu SH, Al-Abdli NE. Inducible clindamycin resistance in methicillin resistant Staphylococcus aureus. Am J Infect Dis Microbiol. 2016;4(1):25–7.
21. Koppada R, Meeniga S, Anke G. Inducible clindamycin resistance among Staphylococcus aureus isolated from various clinical samples with special reference to MRSA. Sch J App Med Sci. 2015;3(6D):2374–80.
22. Sah, et al. Inducible and constitutive clindamycin resistance in Staphylococcus aureus: an experience from western Nepal. Int J Biomed Res. 2015;6(05):316–9.