e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(10); 25-29

Original Research Article

Phenotypic Detection of Inducible Clindamycin Resistance in *Staphylococcus Aureus*: A Study in Central India

Anshul Gupta[1], Manoj Sahu[2], Amit Saxena[3], Mansi Gupta[4], Ronak Jain[5], Amardeep Singh Rai[6]

[1]Associate professor, Department of Orthopaedics, Govt. Bundelkhand Medical College, Sagar (M.P)
[2]Assistant Professor, Department of Anesthesia, Govt. Bundelkhand Medical College, Sagar (M.P)
[3]Assistant Professor, Department of Medicine, Govt. Bundelkhand Medical College, Sagar (M.P)
[4]Demonstrator, Department of Microbiology, Govt. Bundelkhand Medical College, Sagar (M.P)
[5]P.G Resident, Department of Orthopaedics, Govt. Bundelkhand Medical College, Sagar (M.P)
[6]Professor, Department of Microbiology, Govt. Bundelkhand Medical College, Sagar (M.P)

Received: 12-07-2023 / Revised 21-08-2023 / Accepted 20-09-2023
Corresponding author: Dr. Mansi Gupta
Conflict of interest: Nil

Abstract:

Introduction: Emergence of increasing resistance in *Staphylococcus aureus* has renewed the interest in Clindamycin (lincosamide) usage because of its good pharmacokinetic properties in the treatment of *Staphylococcal* infections especially in Methicillin Resistant *Staphylococcus aureus* (MRSA) infections. Erythromycin (macrolide) also used to treat *Staphylococcal* infections but it is a potent inducer of Clindmycin resistance. Combined use of these drugs pose a threat of treatment failure. Phenotypic detection of Erythromycin induced Clindamycin resistance by a simple D- test can be done in clinical laboratories to curb inappropriate use of these drugs in *Staphylococcal* infections including MRSA strains.

Objectives: To find out the burden of Inducible clindamycin resistance & its relation with MRSA.

Materials and Methods: A total of 132 clinical isolates of *S. aureus* between April 2014- June 2014 collected from various Clinical samples were processed to routine antimicrobial Suceptibility test using modified Kirby-Bauer disc diffusion method-using Cefoxitin (30µg) disc to detect MRSA. Erythromycin (15µg) & Clindamycin (2µg) disc placed at a distance of 15mm to detect Erythromycin induced Clindamycin resistance by D – test as per CLSI guidelines.

Results: Among 132 isolates, 24(18.18%) isolates showed inducible Clindamycin resistance, out of which 15 were MRSA strains. So percentage of inducible Clindamycin resistance was higher in MRSA (62.5%) as compared to MSSA (37.5%)

Conclusions: For optimum treatment of patients, D-test which is simple and feasible test should be used as routine lab method to detect inducible Clindamycin resistance in *Staphylococci.*

Keywords: Inducible Clindamycin Resistance, MRSA, D-test

Introduction

The rising prevalence of Methicillin resistance among *Staphylococci* is an increasing problem. [1] This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its good pharmacokinetic properties. [2,3] However, widespread use of MLSB antibiotics has led to rise in the number of *Staphylococcal* strains acquiring resistance to MLSB antibiotics. [3,4]

This resistance is brought about by two types of mechanisms: Target site modification by erm gene and efflux pump mechanism by "msr A" gene. Clindamycin resistance in *Staphylococcus* through target site modification by erm gene can be either constitutive (cMLSB) or inducible (iMLSB). [1]

In case of constitutive resistance, methylase is always produced, whereas in inducible resistance methylase is produced only in presence of an inducer like Erythromycin. [5,6] Isolates with constitutive resistance show invitro resistance to both Erythromycin and Clindamycin, while inducible resistance shows Erythromycin resistance and appears to be sensitive to Clindamycin in vitro, but in vivo therapy with Clindamycin may select erm mutants and leads to treatment failure. [1,5] In case of other mechanism of resistance mediated through msr A genes i,e active efflux of these drugs from bacterial cell. They are called as MS phenotypes

showing resistance to Erythromycin and sensitive to Clindamycin invitro, with successful treatment with Clindamycin invivo. [4] Therefore it is important to differentiate these phenotypes. The Clinical and Laboratory Standards Institute (CSLI) recommends D-test for detecting inducible resistance phenotypically.

The present study was aimed to find out the burden of *S. aureus* having inducible clindamycin resistance (iMLSB) in our Health care settings (as no data available for this geographical area) using D-test and to ascertain its relationship with Methicillin-resistant *S. aureus* (MRSA).

Materials and Methods

During April 2014 to June 2014, 132 *S.aureus* isolates from various clinical samples like pus or wound swab, throat swab, catheter tip, HVS, aspirates, sputum, blood & body fluids from patients attending People`s College of Medical Science and Research Centre, Bhopal were evaluated and included in the study. The isolates were identified as *S. aureus* by conventional methodology. *Staphylococcus aureus* ATCC 25923 was used as quality control. MRSA detection was done with Cefoxitin (30 µg) disc using Kirby-Bauer disc diffusion method as per CLSI guidelines. [7]

The D-test was performed by placing the Erythromycin(15µg) and Clindamycin(2µg) discs adjacent to each other, the distance from edge to edge being 15mm on Muller Hinton agar plate & incubate at 37^0c for 18-24 hours. [5,8]

Following incubation a flattening of the zone (D shaped) around Clindamycin in the area between the discs where both drugs have diffused indicates that the organism has inducible Clindamycin resistance(iMLSB). The interpretation was done only for Erythromycin resistant *S. aureus* strains and all the sensitive strains were excluded.

Results

Out of total 132 *S.aureus* isolates, 88 isolates were Erythromycin resistant. Three different phenotypes of Erythromycin resistant strain were seen in the study in Fig 1,2,3. A total of 24(18.18%) isolates showed inducible Clindamycin resistance and 29(21.96%) isolates showing constitutive Clindamycin resistance, while MS phenotypes are 35(26.51%). These 24 inducible Clindamycin resistant strains isolated from different clinical samples(Table1), 15 were MRSA and 9 were MSSA. Percentage of inducible Clindamycin resistance was higher in MRSA 15(62.5%) as compared to MSSA 9 (37.5%). (3,8) (Fig 4)

Figure 1: D-test Positive (iMLSB Phenotype): Isolates showing resistance to Erythromycin(≤13mm) and sensitive to clindamycin(≥21mm) and showing D shaped zone of inhibition around Clindamycin with the flattening towards Erythromycin.

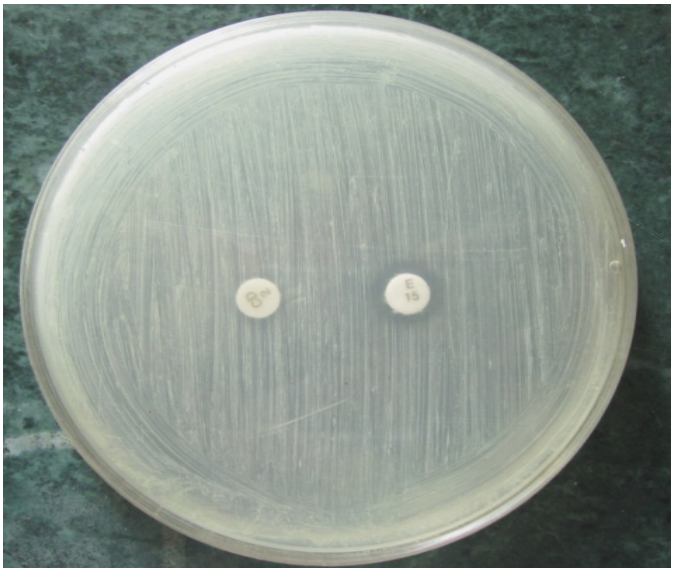

Figure 2: Constitutive Resistance (cMLSB Phenotype): Isolates showing resistance to both Erythromycin(≤13mm)and Clindamycin(≤14mm)with circular zone of inhibition around Clindamycin.

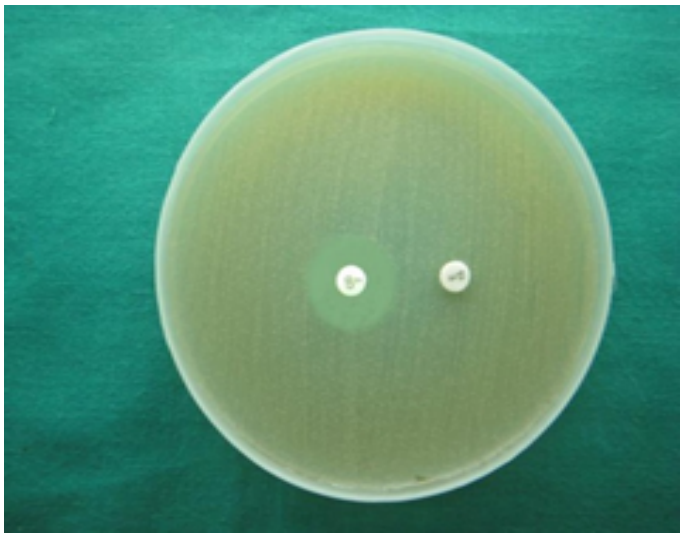

Figure 3: D-test Negative (MS Phenotype): Isolates showing resistance to Erythromycin (≤13mm) but susceptible to Clindamycin(≥21mm) and showing circular zone of inhibition around clindamycin.

Table 1: Showing distribution of inducible clindamycin resistant strains in clinical samples

Samples	Inducible Clindamycin resistant strains(n=24)
Pus	11(45.83%)
Urine	5(20.83%)
Blood	3(12.5%)
HVS	2(8.33%)
Catheter Tip	2(8.33%)
Sputum	1(4.16%)

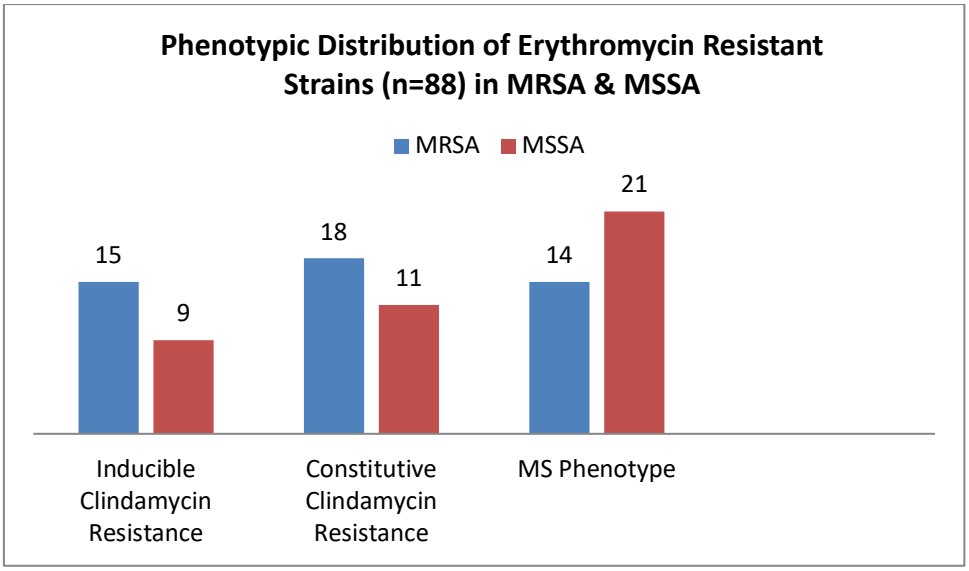

Figure 4 Showing distribution of Erythromycin Resistant Strains in MRSA &MSSA

Discussion

Clindamycin is one of the alternative drug for *Staphylococcal* infections [9] also used in patients allergic to penicillin to treat skin & soft tissue infections. [10] Due to widespread use of MLSB antibiotics, *Staphylococcal* strains have acquired resistance to these antibiotics. Clindamycin resistance can be constitutive($cMLS_B$ Phenotype) and inducible($iMLS_B$ Phenotype). erm genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation if 23S rRNA thereby reducing binding by MLS agents to the ribosome [5,11] Resistance to Clindamycin is due to erm genes or due to msr A gene. [8,12]

Isolates with inducible Clindamycin resistance shows Erythromycin resistance and Clindamycin sensitivity in vitro, but in vivo, therapy with Clindamcin may select out erm mutants and lead to treatment failure which can be easily detected in lab phenotypically by simple D-test. [1,3,5,8]

The present study shows 88(66.66%) isolates are Erythromycin resistance and D- test was performed in all Erythromycin resistant strains. Inducible Clindamycin resistance found is 18.18% which is in concordance with Gupta V et al, Sireesha et al & Mohanasoundaram K M et al who reported inducible Clindamycin resistance was 18%,18% and 16% respectively in their studies. [13,14,15] Few other studies like Manjunath V et al (57%) shows high inducible Clindamycin resistance. [12]

In this study inducible and constitutive resistance was found to be higher in MRSA as compared to MSSA. [2,5] On the contrary, few studies show higher percentage of inducible clindamycin resistance in MSSA. [16,17] Inducible Clindamycin resistance was higher in pus samples followed by urine, blood samples etc.

Conclusions

For optimum patients care, D-test which is simple and feasible should be used as routine lab method to detect inducible clindamycin resistance in *staphylococci* and its implication as treatment outcome should be detailed out to physician.

References

1. Prabhu K, Rao S, Rao V. Inducible Clindamycin Resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians 2011;3:25-27.
2. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis. 2005;58:104–6. [PubMed]
3. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol. 2010;28:124–6. [PubMed]
4. 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. [PubMed]
5. Ciraj AM, Vinod P, Sreejith G, Rajani K. Inducible Clindamycin Resistance among clinical isolates of Staphylococci. Indian J Pathol Microbiol 2009;52:49-51.
6. Pal N, Sharma B, Sharma R, Vyas L. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. *J Postgrad Med.* 2010; 56(3):182-185.
7. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial sus-

ceptibility testing; Twenty-First Informational Supplement. CLSI Document M100-S21. Wayne PA: Clinical and Laboratory Standards Institute;2011.
8. Vidhya R, Parimala S, Beena P M. Inducible clindamycin resistance in Staphylococcus aureus isolates from a rural tertiary care hospital, Kolar. J Clin Biomed Sci 2013; 3:125-28.
9. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol. 2007;56:342–5. [PubMed]
10. Seifi N, Kahani N, Askari E, Mahdipour S, Naderi N M. Inducible clindamycin resistance in *Staphylococcus aureus* isolates recovered from Mashhad, Iran. Iran J Microbiol. Jun 2012; 4(2): 82–86.
11. Woods C R. Macrolide-Inducible Resistance to Clindamycin and the D-Test .Pediatr Infect Dis J 2009;28: 1115–1118.
12. Manjunath V, Singh E, Ramya T.G, Prakash M.R, Sharma A. D- Test- Its role in detection of inducible resistance to clindamycin in staphylococcus aureus with especial reference to MRSA. Int J Bio Med Res 2012;3:1430-1432.
13. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in *Staphylococcus aureus:* A study from North India. *J Postgrad Med.* July 2009;55(3):176-179.
14. Sireesha P, Setty C R Detection of various types of resistance patterns and their correlation with minimal inhibitory concentrations against clindamycin among methicillin-resistant Staphylococcus aureus isolates .IJMM 2012; 30(2): 165-169.
15. Mohanasoundaram KM. The prevalence of inducible clindamycin resistance among gram positive cocci from various clinical specimens. JCDR. 2011;5:38–40. 12.
16. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in Staphylococcus aureus and coagulase negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol. 2004;42:2777–9. [PMC free article] [PubMed]
17. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: Report of a clinical failure. Antimicrob Agents Chemother. 2005;49:1222–4. [PMC free article] [PubMed]