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"Prevalence of Inducible Resistance to Clindamycin in Staphylococcus Aureus Using D-Test at Rims Teaching Hospital, Raichur, India-A Retrospective Study"

Venkatesh Naik R*, Indushree M.C. and Basavaraj V. Peerapur
*Department of Microbiology, RIMS, Raichur, Karnataka, India *Corresponding author*

Abstract: *Staphylococcus aureus is recognized as causing nosocomial and community-acquired infections in every region of the world. Clindamycin is considered as useful alternate drug in penicillin-allergic patients in the treatment of skin & soft tissue infections caused by Staphylococcus aureus. Staphylococcus spp. can be resistant to erythromycin through either erm or msr A genes. Strains with erm-mediated erythromycin resistance may possess inducible Clindamycin resistance but may appear susceptible to Clindamycin by disc diffusion test. The objective of the present study was to know the prevalence of erythromycin-induced Clindamycin resistance among clinical isolates of S. aureus. A total of 192 S. aureus isolates from various clinical samples submitted in the Dept. of Microbiology, RIMS teaching hospital, Raichur were studied. Methicillin resistant S. aureus strains were identified by Cefoxitin disc diffusion method. Inducible Clindamycin resistance was detected by erythromycin and Clindamycin disc approximation test (D-zone test) as per CLSI guidelines. Among the 192 S. aureus isolates, 89 strains (46.35%) were detected as MRSA of which 41(51.89%) strains showed inducible Clindamycin resistance (D-test positive) and 21(20.79%) isolates out of 103 strains MSSA were D-test positive. 69(68.31%) isolates of S. aureus were sensitive to both erythromycin and Clindamycin. High prevalence of strains with inducible Clindamycin resistance particularly among MRSA indicates that inducible Clindamycin resistance testing (D-test) should be included as a part of routine antibiotic susceptibility. These isolates may be missed in routine antibiotic testing by disk diffusion method.*

Key words: *Clindamycin resistance, Erythromycin resistance, MRSA, MSSA, D test.*

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I. Introduction

Staphylococcus aureus is one of the most common bacteria infecting man [1]. It is also known to acquire antimicrobial resistance promptly after the introduction of new antibiotics [2]. Emergence of increasing resistance in Gram positive bacteria in the recent years has led to the use of the Macrolide, lincosamide and Streptogramin (MLS) antibiotics in the treatment of Gram positive infections [3]. Increasing frequency of Methicillin resistant staphylococcus aureus (MRSA) infections and changing patterns in antimicrobial resistance have led to renewed interests in the use of MLS antibiotics to treat such infections with Clindamycin being the preferred drug because of its excellent pharmacokinetic properties [4]. However, recently there has been increasing resistance pattern to MLS antibiotics because of their indiscriminate use. The determination of antimicrobial susceptibility pattern is very crucial for the optimal therapy of infected patients [5]. Erythromycin is an effective inducer of inducible MLSB resistance. It will induce production of the methylase, which allows CD resistance to be expressed. To detect inducible CD resistance strains, the disk approximation test (D-test) has been used by several authors [6-9].From past two to three decades; Clindamycin is being used to treat serious infections caused by Staphylococcus aureus. It is also found to be effective for many infections caused by community acquired Methicillin resistant Staphylococcus aureus [10].

Clindamycin belongs to Macrolide Lincosamide Streptogramin B (MLSB) family. It is the most commonly used antibiotic to treat infections with Methicillin Resistant Staphylococcus aureus [11]. It is also used as an alternate drug in patients allergic to penicillin to treat skin and soft tissue infections. Due to widespread use of MLSB antibiotics, Staphylococcal strains have acquired resistance to these antibiotics. This resistance is brought about by two types of mechanisms: Target site modification by erm gene and efflux pump mechanism by "msr A" gene. Target site modification by erm gene can be constitutive (cMLSB) or inducible (iMLSB) [12].

In case of constitutive resistance, methylase is always produced, whereas in inducible resistance methylase is produced only in presence of an inducer like Erythromycin [13, 14]. 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 [12, 15] .The msr A gene has specificity

for Macrolide and Streptogramin B and causes active efflux of these drugs from bacterial cell, but they have no action on lincosamide. They are called as MS phenotypes showing resistance to erythromycin and sensitive to Clindamycin in-vitro with successful treatment with Clindamycin in-vivo [14].Therefore it is important to differentiate these phenotypes. The Clinical and Laboratory Standards Institute (CSLI) recommends D-test for detecting inducible resistance phenotypically [16]. The aim of this study is to detect inducible Clindamycin resistance among the isolates of Staphylococcus aureus (S. aureus) by phenotypic method.

II. Materials And Methods

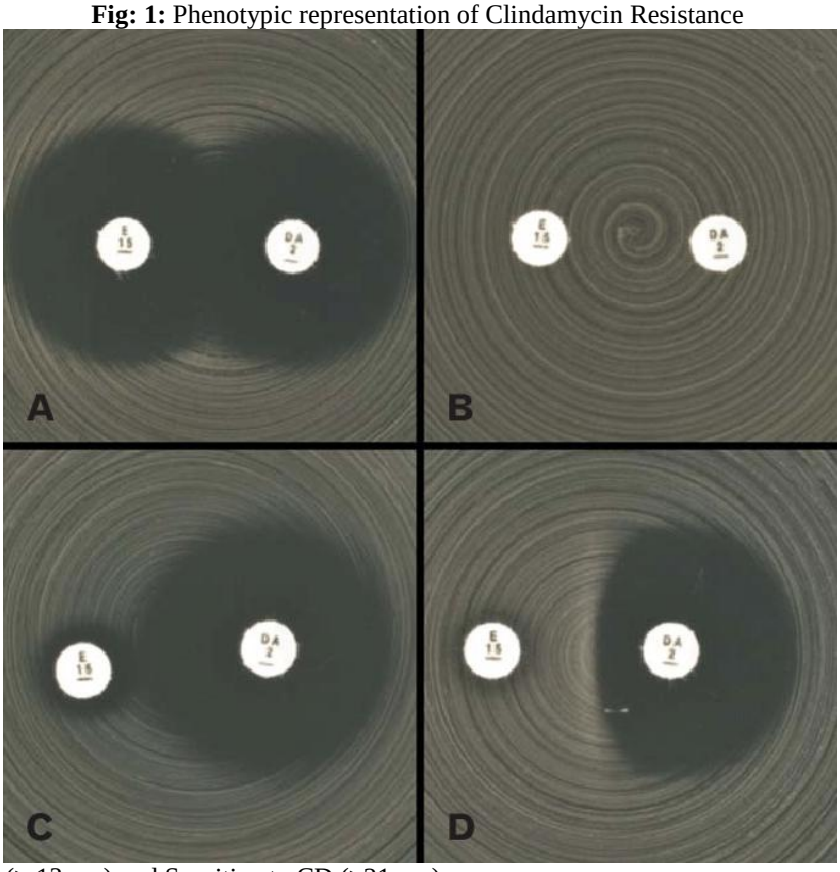
It is a retrospective study done during the period of January 2018 to July 2019, 192 S.aureus isolates from various clinical samples like pus or wound swab, aspirates, sputum, blood, body fluids, vaginal swab, cervical swab, throat swab and foreign body, collected from patients in the central laboratory attending RIMS from various parts of Raichur, were evaluated and included in the study. The isolates were identified as S.aureus by conventional methodology (Gram staining, colony morphology, Catalase test, Coagulase test, Mannitol fermentation test) [17]. Antibiotic susceptibility testing were performed by Kirby Bauer's disc diffusion method as per CLSI guidelines using antibiotics such as Penicillin(10units), Amoxyclav (30mcg), Gentamicin(10mcg), Tetracycline(30mcg), Doxycyclin (30mcg), Linezolid(30mcg), Cotrimoxazole(25mcg), Cefoxitin(30mcg), Erythromycin (15mcg), Clindamycin(2mcg), Ciprofloxacin(5mcg), Chloramphenicol (30mcg)[18] .

Methicillin resistance was determined using Cefoxitin (30mcg) disc and inducible resistance to Clindamycin was detected by D-test as per CLSI guidelines (2011) [18]. The D-test was performed by placing the Erythromycin (E-15mcg) and Clindamycin (CD-2mcg) discs side by side with edge to edge distance of 15mm on Muller Hinton agar plate [16]. Plates were analyzed after 18 hours of incubation at 37 ^{o} C Flattening of zone around Clindamycin in the area adjacent to the erythromycin producing D shape, indicates D-test positive, whereas complete zone indicates D-test negative (Fig :1)

Inclusion criteria: All Staphylococcus aureus isolated during study period.

Exclusion criteria: Organisms other than Staphylococcus aureus.

Fig: 1: Phenotypic representation of Clindamycin Resistance



A- Sensitive to E (> 13mm) and Sensitive to CD (>21mm)

B- Resistant to E (<13mm) and CD (<14mm) – Constitutive MLSB phenotype

C- Resistant to E (<13mm) and Sensitive to CD (>21mm) – MS phenotype (D test negative)

D- Resistant to E (<13mm) and Sensitive to CD (>21mm) with D shape (Flattening of zone towards E) – Inducible MLSB phenotype (D test positive).

III. Results

Among 192 S.aureus isolated from clinical specimens MRSA were found to be 89 (46.35%) and MSSA were 103 (53.64%). Our study showed four different phenotypic patterns in S.aureus isolate (Table 1).

Table 1: Phenotypic pattern of inducible Clindamycin resistance

Phenotype pattern	MRSA %(n= 89)46.35%	MSSA%(n= 103)53.64%	Total %(192)
E–R, CD-R (Constitutive MSLB)	10(12.65%)	2(1.98%)	12(6.25%)
E-R, CD-S, (D-Test positive-Inducible MLSB)	41(51.89%)	21(20.79%)	62(32.29%)
E-R, CD-S, (D-Test negative- MS)	9(11.39%)	11(10.89%)	20(10.41%)
E-S, CD-S	29(36.70%)	69(68.31%)	98(51.04%)

Sensitivity to both E and CD was seen in 98 (51.04%), 12 (6.25%) were resistant to both E and CD indicating constitutive resistance. 62 (32.29%) showed resistance to E and sensitive to CD with D-test positive indicating inducible resistance to Clindamycin. 20(10.41%) showed resistance to E and sensitive to CD with negative D-test showing MS phenotypic resistance

Among the MRSA isolates 29 (36.70%) were sensitive to both E and CD, while 10(12.65%) were constitutively resistant. Forty one (51.89%) showed inducible resistance and 9(11.39%) showed MS phenotype. Among MSSA, 69 (68.31%) were sensitive to both E and CD, while 2(1.98%) were constitutively resistant, 21 (20.79%) showed inducible resistance and 11 (10.89%) showed MS phenotypic resistance.

Specimen wise distribution among clinical samples was around 91 (88.34%) 0f MSSA and 57 (64.04%) of MRSA was prevalent among Pus samples. Erythromycin resistance was 94 (48.95%) prevalent in all clinical samples. (Table 2)

Table 2: Specimen wise distribution of MSSA, MRSA and Erythromycin resistance

Clinical specimen	MSSA (n=103)	MRSA (n= 89)	E- Resistance (n= 94)
Pus and wound swab	91	57	55
Urine	0	2	1
Blood	7	20	11
Body fluids	0	1	0
Vaginal swab	2	2	21
Cervical swab	0	1	1
Foreign body	0	1	1
Sputum	2	4	3
Throat swab	1	1	1

IV. Discussion And Conclusion

S.aureus is one of the most common bacteria causing various suppurative infections and encountered frequently in the laboratory. Increasing prevalence of MRSA among S.aureus is a major problem, which shows resistance to most of the cell wall acting antibiotics. This has led to renewed interest in the MLSB antibiotics [19]. Clindamycin remains the good alternative option for treating S.aureus infections by both MRSA and MSSA because of its good oral bio availability [15, 19]. However due to widespread use of Clindamycin, resistance has been reported in the recent years with different mechanisms [12, 15, 20]. So it is important to detect the type of resistance. Erm gene encodes for methylase enzymes causing methylation of 23s r RNA, which reduces binding of the drug to rRNA target. If erm gene is consistently expressed it results in constitutive resistance and if it is induced by an inducing agent it produces inducible resistance [11, 14]. Reporting S.aureus as susceptible to CD without checking for inducible resistance may results in inappropriate treatment and can lead to treatment failure [15].

In the present study, Resistance of S.aureus to erythromycin was (94/192) 48.95% where as Vidhya R et al, ref [21] 88.77% (243/285). Among them inducible Clindamycin resistance (D-test positive) was 62(32.29%) where as 13.33% ref [21] and MS phenotype (D-test negative) was 20(10.43%) where as 35.43% ref [21] and constitutive resistance was 12(6.25) where as 40% ref [21]. In this study it was found that inducible Clindamycin resistance is more in MRSA 41(51.89%) compared to MSSA 21(20.79%). This is in concordance with few studies reported in India. Deotale et al [12] found 27.6% iMLSB in MRSA and 1.6% in MSSA. Gupta et al [13] showed it to be 20% in MRSA and 17.33% in MSSA. Prabhu et al [5] showed 20% in MRSA and 6.15% in MSSA.

In our study MS phenotype is little more in MRSA (11.39%) than MSSA (10.89%). Where as in Gupta et al [3] and Shantale et al [20] who showed 37.3% MS phenotype in MSSA and 16% in MRSA and 16.34% in MSSA and 15.07% in MRSA respectively. Constitutive resistance in our study was found to be 12.65% in MRSA and 1.98% in MSSA. The other studies done in India showed 16.66% in MRSA and 6.15% in MSSA by

Prabhu et al [15] and Shantale et al [20] showed 25.39% in MRSA and 9.61% in MSSA which is similar to our study showing cMLSB is more in MRSA than MSSA.

In our study, we found that inducible and constitutive Clindamycin resistance is more in MRSA than in MSSA and the MS phenotype varies with the local strains. Inducible resistance due to erm gene can be detected by D-test in Staphylococcus aureus isolates and can be used as a routine test in all microbiology laboratories, which helps the clinicians in avoiding treatment failure with Clindamycin.

Statistical analysis:

The data were entered and analyzed using Statistical Package for Social Sciences SPSS software version21.0. P - Value < 0.05 was considered statistically significant

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