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Original Article

Inducible clindamycin resistance in *Staphylococcus aureus* isolates from a rural tertiary care hospital, Kolar.

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Abstract

Background: Clindamycin is the commonly used drug for Methicillin Resistant *Staphylococcus aureus* (MRSA). Due to wide spread use of this antibiotic, it has developed resistance by different mechanisms and hence it is important to detect resistance to Clindamycin. The Clinical and Laboratory Standards Institute (CLSI) recommends D-Test for detecting inducible resistance phenotypically. D-Test is simple, reliable and easy to perform with high sensitivity and specificity. The present study was aimed to detect inducible clindamycin resistance among the isolates of *Staphylococcus aureus* (*S.aureus*) by phenotypic method. ***Methods:*** Two hundred and eighty five *S.aureus* isolates from various clinical samples were evaluated and methicillin resistance was determined using Cefoxitin (30 mcg) disc and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines (2011). ***Results:*** Among 285 *S.aureus* isolates inducible resistance was found in 38 (13.33%). Thirty three (23.07%) of 143 Methicillin Resistant *S.aureus* (MRSA) isolates and 5 (3.52%) of 142 Methicillin Sensitive *S.aureus* (MSSA) isolates showed inducible resistance. ***Conclusion:*** Our study showed that inducible clindamycin resistance is more in MRSA than MSSA, which can be detected by D-test and should be used as a routine test in all microbiology laboratories.

Key words: Clindamycin resistance, Constitutive resistance, Erm gene, MS phenotype, MRSA.

Introduction

Clindamycin belongs to Macrolides Lincosamide Streptogramin B (MLSB) family. It is the most commonly used antibiotic to treat infections with Methicillin Resistant *Staphylococcus aureus*[1] . 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)[2] .

In case of constitutive resistance, methylase is always produced, whereas in inducible resistance methylase is produced only in presence of an inducer like erythromycin[3,4]. Isolates with constitutive resistance show invitro 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[2,5] .The msr A gene has specificity for macrolides and streptogramin B and causes active efflux of these drugs from bacterial cell, but they have no action on lincosamides. They are called as MS phenotypes showing resistance to erythromycin and sensitive to clindamycin invitro with successful treatment with clindamycin invivo[4](Fig1).Therefore it is important to differentiate these phenotypes. The Clinical and Laboratory Standards Institute (CSLI) recommends D-test for detecting inducible resistance phenotypically[6] . The aim of this study is to detect inducible clindamycin resistance among the isolates of *Staphylococcus aureus (S. aurues)* by phenotypic method.

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Materials and methods

During the period of July 2011 to December 2011, 285 *S.aureus* isolates from various clinical samples like pus or wound swab, aspirates, sputum, blood and body fluids from patients attending R.L.Jalappa Hospital, Kolar 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)[7] . 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), Doxycycline(30mcg), Linezolid(30mcg), Cotrimoxazole(25mcg), Cefoxitin(30mcg), Erythromycin (15mcg), Clindamycin(2mcg), Ciprofloxacin(5mcg), Chloramphenicol(30mcg)[8] .

Methicillin resistance was determined using cefoxitin(30mcg) disc and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines(2011)[8]. 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[6]. Plates were analyzed after 18 hours of incubation at 35^{0}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 2) .

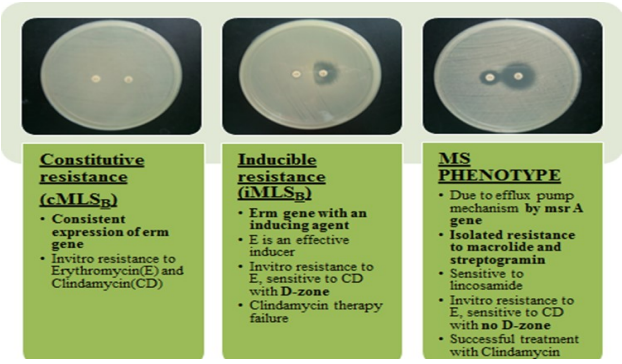


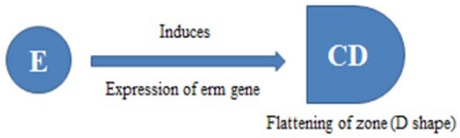
Fig:1 – Phenotypic representation of Clindamycin Resistance

Results:

Among 285 *S.aureus* isolated from clinical specimens MRSA were found to be 143 (50.18%) and MSSA were 142 (49.82%). Our study showed four different phenotypic patterns in *S.aureus* isolate (Fig 3): Thirty two (11.22%) showed sensitivity to both E and CD. One hundred and fourteen (40%) were resistant to both E and CD indicating constitutive resistance. Thirty eight (13.33%) showed resistance to E and sensitive to CD with D-test positive indicating inducible resistance to clindamycin. One hundred and one (35.43%) showed resistance to E and sensitive to CD with negative D-test showing MS phenotypic resistance.

Among the MRSA isolates 7 (4.90%) were sensitive to both E and CD, while 58 (40.55%) were constitutively resistant. Thirty three (23.07%) showed inducible resistance and 45 (31.46%) showed MS phenotype. Among MSSA, 25 (17.60%) were sensitive to both E and CD, while 56 (39.43%) were constitutively resistant. Five (3.52%) showed inducible resistance and 56 (39.43%) showed MS phenotypic resistance.

Principle of D-test:



Interpretation of D-test:

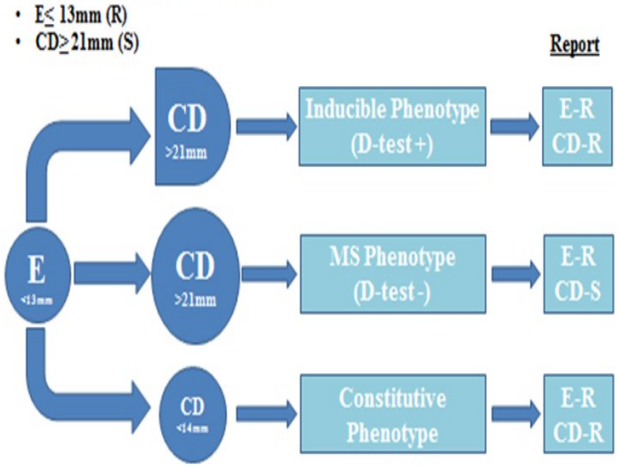


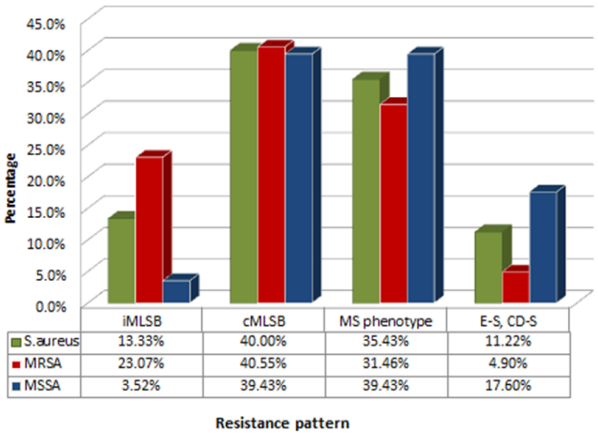
Fig:2 – Principle and interpretation of D-Test

Three different phenotypes were seen and interpreted as follows

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

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

C) Resistant to E(<13mm) and CD(<14mm) – Constitutive MLSB phenotype.



	iMLSB	cMLSB	MS phenotype	E-S, CD-S
S.aureus	13.33%	40.00%	35.43%	11.22%
MRSA	23.07%	40.55%	31.46%	4.90%
MSSA	3.52%	39.43%	39.43%	17.60%

Fig:3 – Represents the percentage of resistance

Discussion

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[9]. Clindamycin remains the good alternative option for treating *S.aureus* infections by both MRSA and MSSA because of its good oral bio availability[5,9]. However due to widespread use of clindamycin, resistance has been reported in the recent years with different mechanisms[2,5,10]. 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[1,4]. Reporting *S.aureus* as susceptible to CD without checking for inducible resistance may results in inappropriate treatment and can lead to treatment failure[5].

In the present study, resistance of *S.aureus* to erythromycin was 88.77% (253/285). Among them inducible clindamycin resistance (D-test positive) was 13.33% and MS phenotype (D-test negative) was 35.43% and constitutive resistance was 40%. It was found that inducible clindamycin resistance is more in MRSA(23.07%) compared to MSSA(3.52%). This is in concordance with few studies reported in India. Deotale et al[2] found 27.6% iMLSB in MRSA and 1.6% in MSSA. Gupta et al[3] 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.

We found MS phenotype is more in MSSA(39.43%) than MRSA(31.46%). This is similar to the study done by Gupta et al[3] and Shantale et al[10] 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 40.55% in MRSA and 39.43% in MSSA. The other studies done in India showed 16.66% in MRSA and 6.15% in MSSA by Prabhu et al[5] and Shantale et al[10] 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 laboratory, which helps the clinicians in avoiding treatment failure with Clindamycin.

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