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Detection of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus*

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Clindamycin is commonly used in the treatment of Staphylococcus aureus. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to erm genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D test on routine basis. 140 Staphylococcus aureus isolates were subjected to routine antibiotic susceptibility testing including oxacillin (1μg) by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by D test, as per CLSI guidelines on Staphylococcus aureus isolates. 76 (54.28%) were Staphylococcus aureus and 64 (45.71%) were Methicillin resistant Staphylococcus aureus. In MRSA 07 (10.93%) isolates showed inducible clindamycin resistance, 08 (12.5%) showed constitutive resistance, 02 (3.1%)showed MS phenotype while in MSSA 02 (2.63%) isolates showed only inducible clindamycin resistance and not the constitutive resistance , 55 (72.36%) showed MS phenotype. Inducible resistance and Constitutive resistance were found to be higher in MRSA as compared to MSSA. The present study showed that, to avoid the therapeutic failure D test must be performed by all laboratories routinely.

Key words: Clindamycin resistance, Constitutive MLS_B phenotype, Inducible MLS_B phenotype, MRSA, MS phenotype.

Staphylococcus aureus infections are important causes of nosocomial and community acquired infections. Treatment of these infections is a growing problem due to increasing Methicillin resistance among Staphylococci.[1] The Macrolide-Lincosamide- Streptogramin B (MLS_B) family of antibiotics serve as an alternative treatment option, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[2] However, widespread use of MLS_B antibiotics has led to an increase in number of Staphylococcal strains acquiring resistance to MLS_B antibiotics.[3] Macrolide resistance may be due to enzymes encoded by a variety of erm genes- MLS_B phenotype or active efflux pump encoded by the mrsA gene- MS phenotype. MLS_B resistance can be either constitutive (c MLS_B) or inducible (i MLS_B). [4] In constitutive resistance, in vitro susceptibility tests show resistance to both erythromycin and clindamycin. In inducible resistance, in vitro susceptibility tests show resistance to erythromycin, but susceptibility to clindamycin, unless induced by erythromycin.

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$iMLS_B$ resistance can not be determined by using standard susceptibility test methods, but can be determined by erythromycin- clindamycin disc approximation test (D test).

The aim of the present study was to determine the prevalence of inducible clindamycin resistance in both Methicillin resistant and susceptible strains of Staphylococcus aureus in clinical isolates and also susceptibility pattern of the isolates in our hospital.

Subjects & Methods

The present study was conducted for a period of 2 years from January 2007 to December 2008 and included a total of 140 isolates of Staphylococcus aureus from specimens of Pus/ Wound swabs, Respiratory tract infections and Body fluids. The S. arueus were identified by using standard microbiological procedures.[5] Antibiotic susceptibility tests were performed by Kirby Bauer disc diffusion method on Mueller Hinton agar plates using Erythromycin (15µg),Clindamycin (2µg), Penicillin (10U), Cefazolin (30µg) Ciprofloxacin(5µg) Oxacillin (1µg), Trimethoprim-Sulfomethaxazole (1.25/23.75µg), Tetracycline (30µg) Vancomycin (30µg) and Teicoplanin (30µg) as per Clinical Laboratory Standards Institute (CLSI) guidelines Methicillin resistance was detected by Oxacillin disc diffusion method.[6]

To identify i MLS_B phenotype, the D test was performed. A lawn culture of the isolate which was adjusted to 0.5 McFarland's concentration was made on Mueller Hinton agar plate and discs of clindamycin (2µg) and erythromycin (15µg) were placed at a distance of 15mm (edge to edge) as per CLSI recommendations.

Four different phenotypes were interpreted as follows

1. D positive ($iMLS_B$ phenotype):-isolates showing resistance to erythromycin, while being sensitive to clindamycin with a D shaped zone of inhibition around clindamycin with flattening toward erythromycin disc. (Fig.1)
2. D negative (MSB phenotype):- No flattening of the clindamycin zone, resistant to erythromycin but susceptible to clindamycin. (Fig.3)
3. Constitutive resistance(c MLS_B phenotype):- Resistant to both erythromycin to clindamycin. (Fig. 2)
4. Sensitive phenotype: - Sensitive to both erythromycin to clindamycin.

Table 1. Distribution of MRSA and MSSA with different resistant phenotypes

Strains	No. of Isolates (%)	$iMLS_B$ phenotype (D +ve) (%)	$cMLS_B$ phenotype (%)	MS phenotype (D -ve) (%)	Sensitive phenotype (%)
MRSA	64 (45.71)	07 (10.93)	08 (12.5)	47(73.43)	02 (3.1)
MSSA	76 (54.28)	02 (2.63)	00	10 (13.15)	64 (84.21)
TOTAL	140	09 (6.42)	08 (5.71)	57 (40.71)	66 (47.14)

Table 2. Sensitivity of i MLS_B isolates to antimicrobial agents

Antimicrobial agents	No. of resistant strains	No.of sensitive strains
Oxacillin (1µg)	07	02
Penicillin(10U)	09	00
Cefazolin (30µg)	07	02
Vancomycin (30µg)	00	09
Teicoplanin(30µg)	00	09
Ciprofloxacin(5µg)	09	00
Trimethoprim- Sulfomethaxazole (1.25/23.75µg),	07	02
Tetracycline (30µg)	07	02

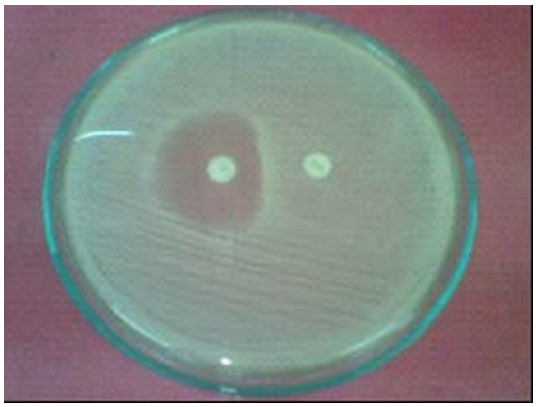

Fig. 1. D positive (i MLS_B phenotype)

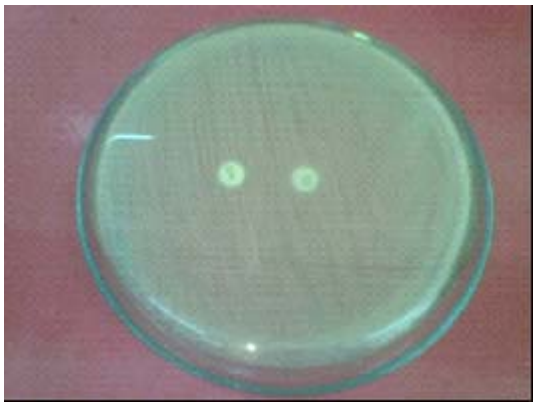

Fig. 2. Constitutive resistance (c MLS_B phenotype)

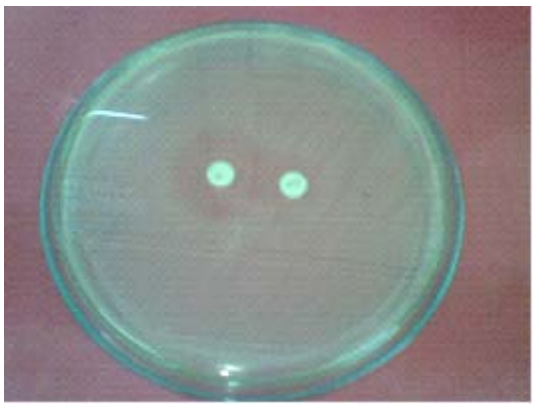

Fig. 3. D negative (MSB phenotype)

RESULTS

A total of 140 Staphylococci were isolated from various types of clinical specimens. Out of 140 isolates, 76 were Staphylococcus aureus and 64 were Methicillin resistant Staphylococcus aureus (MRSA).

The erythromycin and clindamycin resistance patterns of the isolates based on disc diffusion method are shown in the table- 1. $cMLS_B$ phenotype (12.5%) was predominant in MRSA. Sensitive phenotype was significantly higher in MSSA (84.21%). No $cMLS_B$ phenotype was detected in MSSA.

$iMLS_B$ phenotype (6.42%) was slightly higher than c MLS_B phenotype (5.71%) among the isolates.

The antibiotic susceptibility patterns of i MLS_B isolates are shown in the table.2. All isolates were susceptible to Vancomycin and Teicoplanin while all were resistant to penicillin and Ciprofloxacin. Sensitivity was least to Trimethoprim- Sulfomethaxazole and Tetracycline.

DISCUSSION

Due to the increasing Nosocomial MRSA infections which are multidrug resistant treatment option is very limited. Vancomycin due to its high cost and possibility of emergence of resistance; it is not widely used by clinicians.[7] Clindamycin due to its tolerability, cost, oral form and good tissue penetration remains alternative treatment option for skin and soft tissue infections. [8] But there are reports of $iMLS_B$ resistance in the clinical isolates[2-4,7-12]. So it is essential to detect the inducible resistance to avoid therapeutic failure in patients.

In our study $iMLS_B$ resistance was 6.42%. $cMLS_B$ resistance was 5.71%. Similar findings of higher inducible resistance were reported by Angel et al (64% vs. 00%) and Shantala G B et al (24.89% vs. 18.26%).[9,10] Deotale *et al.,* (14.5% vs. 3.6%). [11] However in MRSA $cMLS_B$ resistance (12.5%) was higher than that of $iMLS_B$ resistance (10.93%).Many studies have reported similar results- Gadepalli et al reported 38% cMLSB resistance and 30 % iMLSB resistance. [3] Gupta et al reported 46% cMLSB resistance and 20 % iMLSB resistance. [12] Debdas et al reported 23% cMLSB resistance and 18 % iMLSB resistance.[13]

Interestingly cMLSB resistance was not seen in MSSA. Similar result was reported by Angel et al only.

The antibiotic susceptibility patterns of i MLS_B isolates in this study showed that treatment options are very limited due to multidrug resistant strains. So clindamycin is the preferred drug for the treatment and for that D test must be performed to avoid therapeutic failure.

In conclusion, to avoid the therapeutic failure, the D test must be performed by all laboratories routinely while reporting the sensitivity against clindamycin in staphylococcal infections.

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