Research Article

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DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* FROM NASAL CARRIERS AMONG DIABETIC FOOT PATIENTS

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ABSTRACT:

Staphylococcus aureus emerged globally as an important pathogen responsible for nosocomial and community acquired infections. The resistance to antimicrobial agents among staphylococci is an increasing problem worldwide. Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics. ***Aim:*** *This study was undertaken to detect the presence of inducible clindamycin resistance among isolates of Staphylococcus aureus from nasal carriers among diabetic foot patients.* ***Materials and Methods****: Among 95 S.aureus isolates were subjected to routine Antibiotic susceptibility testing by Kirby Bauer disk diffusion method. Inducible Clindamycin resistance was detected by Disc Approximation test (D-Test) as per CLSI guidelines.* ***Results:*** *Among 170 samples,95 Staphylococcus aureus was isolated ,32 [33.33%] showed D test positive indicating inducible MLSB[iMLSB] phenotype , 18[18.75%] showed Constitutive MLSB[cMLSB] phenotype and 45[47.9%] were D-test negative MS phenotype. Among the 95 Staphylococcus aureus isolates tested, 59 (61.45%) were found to be MRSA and 36(38.54%) were MSSA.* ***Conclusion:*** *This study showed that D-test should be used as a mandatory method in routine disc diffusion testing to avoid treatment failure.*

KEYWORDS:

Clindamycin resistance, MRSA, Inducible MLSB phenotype, Staphylococcus aureus.

INTRODUCTION

Methicillin- resistant *Staphylococcus aureus* (MRSA) first emerged as nosocomial pathogens in the early 1960s are of great concern to public health and highly reported in human clinical samples[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].

Erythromycin is an effective inducer of inducible MLSB resistance. It will induce production of the methylases, which allows Clindamycin resistance to be expressed[5]. Staphylococcal resistance to MLS antibiotics may be due to an active efflux mechanism encoded by msrA or may be due to ribosomal target modification mediated by erm genes affecting macrolides, lincosamides and type B streptogramins (MLSB resistance). 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[6,7,8].

The expression of the MLSB phenotype can be Constitutive (MLSBc) or Inducible MLSBi). Inducible resistance is observed when the inactive mRNA produced by the production of methylases becomes active in the presence of an inducer, while active methylase mRNA is produced in strains where constitutive expression is seen[9]. The strains carrying the inducible *erm* gene are resistant to the inducer and remain susceptible to non inducer macrolides and lincosamides. Low levels of erythromycin is an inducer of the MLSBi phenotype, which forms the basis of theD-test[10].

Inducible MLSB resistance can be detected by disc approximation test–(D test) by placing erythromycin and clindamycin discs in adjacent positions[11,12] . Hence this study was undertaken to detect the presence of Inducible clindamycin resistance among isolates of *Staphylococcus aureus* from nasal carriers among diabetic foot patients.

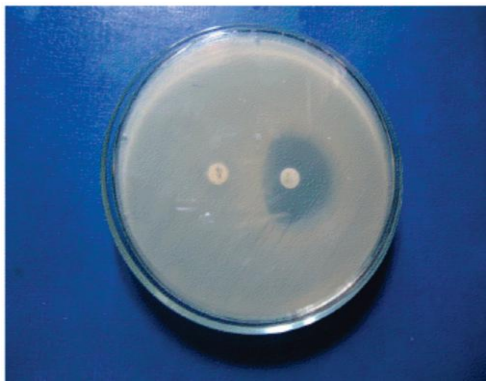
Materials and Method:

This study was undertaken from May 2014 to July 2014. A total of 170 nasal swabs were taken from diabetic foot patients admitted in our hospital during this period. Nasal samples were collected with sterile cotton wool swabs from patients both the left and right nares under aseptic conditions. Informed the patients before collecting the samples. **Inclusion criteria:** Staphylococcus aureus isolates were included. Coagulase negative staphylococcus and No growth samples were excluded from this study.

The isolates were first identified as *S.aureus* isolates by standard biochemical reactions. The confirmed isolates were routinely tested for antibiotic susceptibility by Kirby-Bauer's disc diffusion method. The drugs tested are as follows Oxacillin (1µg), Tetracycline (30µg), cotrimoxazole (25µg), Cephalexin (30µg), Cefoxitin (30µg), Clindamycin (2µg) and Erythromycin (15µg) as per CLSI guidelines. An inhibition zone of 10mm or less around oxacillin disc and 19mm or less around cefoxitin disc indicates MRSA.

Inducible resistance to clindamycin was tested by 'D Test' as per CLSI guidelines. Clindamycin and Erythromycin discs were placed adjacent to each other,the distance from egde to edge being 21±1mm on a Mueller-hinton agar. Following overnight incubation at 37^0c, Flattening of Zone (D-Shaped) around clindamycin in the area between two discs,indicated inducible clindamycin resistance.

Figure:1-D-Test showing inducible clindamycin resistance



Three different phenotypic pattern were seen. This interpretation was done only for Erythromycin –resistant *S.aureus* strains and all the sensitive strains were excluded.

1. D-test Positive [iMLSB phenotype]: Isolates showing resistance to Erythromycin [Zone size≤13mm] and sensitive to clindamycin [≥21mm] and showing D shaped zone of inhibition around clindamycin with flattening towards Erythromycin.

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

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

4. Quality control of the erythromycin and clindamycin disks was performed with *Staphylococcus aureus* ATCC 25923. Results were tabulated and analysed statistically.

RESULTS:

Of the 170 samples processed ,95 were *Staphylococcus aureus ,69* were *CONS ,6* samples showed no growth.In 95 Staphylococcus aureus isolates ,32 [33.33%] showed D test positive indicating inducible MLSB[iMLSB] phenotype , 18[18.75%] showed Constitutive MLSB[cMLSB]phenotype and 45[47.9%] were D-test negative MS phenotype.

Out of 95, 59[61.45%] were oxacillin resistant and considered to be MRSA, 36[38.54%] were sensitive and considered to be MSSA.

Table-1 Depicting Different Phenotypes of *Staphlococcus* Isolates

PHENOTYPE	*Staphylococcus aureus Isolates(n=95)*
D-Test Positive(iMLSB)	32[33.33%]
D-Test Negative (MS)	45[45.7%]
Constitutive MLSB(cMLSB)	18[18.75%]

DISCUSSION

Macrolide inducible resistance to clindamycin was first recognized in the laboratory in early 1960's[13]. Clinical isolates resistant to clindamycin were first recognized in 1968[14].Clindamycin has been the drug of choice to treat serious infections caused by susceptible staphylococcus aureus and also for many infections caused by CA – MRSA[15,16]. Widespread use of MLSB antibiotics has led to an increase in number of Staphylococcal strains acquiring resistance to MLSB antibiotics.

Clindamycin is an efficient and economic lincosamide drug used for the treatment of staphylococci infection[17]. One macrolide resistance mechanism, modification of a drug binding site on the ribosome, results in resistance to macrolides, azalides, lincosamides, and group (B) streptogramins (MLSB). MLSB phenotypes can be either constitutive (MLSBc) or inducible (MLSBi). The inducible resistance to clindamycin (MLSBi) in MRSA can severely compromise therapy and can result in failure of clindamycin treatment of MRSA infections when nonsuitable therapy (e.g. erythromycin) is given [18].

S.aureus (and other Gram negative microbes) can be due to iMLSB genotypes or efflux pumps. The D-test, based on disc diffusion susceptibility testing, is recommended to determine if theiMLSB genotype is present. Isolates were also tested against erythromycin, clindamycin, and synercid for the characterization of MLSB phenotypic isolates. D-tests were performed on isolates exhibiting erythromycin resistance, to assay for the presence of inducible clindamycin resistance iMLSB phenotype[19].

In the present study, Out of 95 Staphylococcus aureus isolates, 32 [33.33%] showed D test positive indicating inducible MLSB [iMLSB] phenotype 18[18.75%] showed Constitutive MLSB [cMLSB] phenotype and 45[47.9%] were D-test negative MS phenotype.

Indian reports on inducible clindamycin resistance are scanty[20]. V Deotale et al found 45% of isolates to be D-test positive in their study[21]. Kavitha Prabhu et al,showed 37.52% of D-test positive isolates which is similar to our findings[22] . Another study done by Gadepalli Ravishekhar et al noticed 21% of iMLSB phenotypes in their study.

Various studies have shown the prevalence of cMLSB phenotype to be ranging from 11-27% and MS phenotype to be from 12-44%. The percentage of inducible clindamycin resistance was highest among MRSA [57.63%] as compared to MSSA[16.22%]. This correlates with studies done by various researchers elsewhere [23, 24.] In a European study, 93% of erythromycin-resistant MRSA and 44% of erythromycin-resistant MSSA exhibited constitutive resistance[25]. However the true incidence depends on the patient population studied the geographical region, the hospital characteristics and Methicillin susceptibility [26].

CONCLUSION

In conclusion, all clinical microbiology laboratories should report inducible clindamycin resistance in *S.aureus* and D-test can be used with routine antibiotic susceptibility testing. It is a reliable and cost effective method to detect inducible and constitutive clindamycin resistance routinely in clinical laboratories. Hence the early detection of clindamycin resistance helps the clinicians to use clindamycin judiciously for infections caused by truly susceptible strains of *S.aureus* thus avoiding treatment failure.

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