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Original Article

Inducible Clindamycin Resistance in Clinical Isolates of Staphylococcus Aureus

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ABSTRACT

Introduction: Increasing frequency of methicillin resistant infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of Macrolide-lincosamide-streptogramin (MLS) family of antibiotics like Erythromycin (a macrolide) and clindamycin (a lincosamide) to treat such infections with clindamycin being the preferred agent due to its excellent pharmacokinetics properties. The Clinical and Laboratory Standards Institute (CLSI) has recommended the erythromycin –clindamycin disc approximation test (D zone) to detect inducible clindamycin resistance. This study aimed to determine the levels of the macrolides-lincosamides-streptogramins B (MLSB) resistance phenotype in Staphylococcus aureus isolates from clinical samples.

Materials: A total of 156 strains of staphylococci, comprising 44 Methicillin resistant Staphylococcusaureus and 112 Methicillin sensitive Staphylococcusaureus isolates from various clinical samples, were identified by conventional methods. The double-disc test was applied by placing erythromycin and clindamycin discs on these isolates to investigate the inducible and constitutive MLSB resistance phenotypes and MS phenotype.

Results: Among them, 21.8% showed the constitutive and 15.4% the inducible phenotype, while 19.2 % were MS phenotype. Percentage of both inducible and constitutive Clindamycin resistance was higher amongst Methicillin resistant Staphylococcusaureus isolates as compared to Methicillin sensitive Staphylococcus aureus.

Discussion: By applying double-disc tests on a routine basis to detect inducible MLSB resistance, clindamycin can be effectively used on staphylococcal infections. Additionally, it can be used to survey the MLSB resistance of staphylococci strains from specific geographical regions or hospitals.

KEYWORDS: MRSA, D-test, Clindamycin, inducible MLSB resistance.

INTRODUCTION

The emergence of antibiotic resistance is a global public health problem. Increasing frequency of methicillin resistant infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of Macrolide-lincosamide-streptogramin (MLS) family of antibiotics like Erythromycin (a macrolide) and clindamycin (a lincosamide) to treat such infections with clindamycin being the preferred agent due to its excellent pharmacokinetics properties. However, this widespread use has led to an increase in the number of staphylococci strains resistant to MLS antibiotics.[1]

Macrolide antibiotic resistance in Staphylococcus aureus and coagulase-negative staphylococci (CNS) may be due to an active efflux mechanism encoded by msrA (conferring resistance to macrolides and type B streptogramins only) or may be due to ribosomal target modification affecting macrolides, lincosamides, and type B streptogramins (MLSB resistance). erm genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, thereby reducing binding by MLS agents tothe ribosome.[2,3] The msrA gene confers the so-called MS phenotype (resistance to erythromycin, inducible resistance to streptogramin B, and susceptibility to clindamycin) by efflux.[2,3] Strains with inducible MLSB resistance demonstrate in vitro resistance to 14- and 15-member macrolides(e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and

type B streptogramins; strains with constitutive MLSB resistance show in vitro resistance to all the agents.[2] Inducible clindamycin resistance is not recognized by using standard susceptibility test methods which may lead to therapeutic failure. The Clinical and Laboratory Standards Institute (CLSI) has recommended the erythromycin –clindamycin disc approximation test (D zone) to detect inducible clindamycin resistance.

The objective of our study was to determine the inducible clindamycin resistance among Staphylococcus aureus clinicalisolates using D-test.

MATERIALS AND METHODS

A total of 156 consecutive, non duplicate clinical isolates of Staphylococcus aureus were recovered from pus, swab, sputum, blood, CSF and other fluids except urine, which were received at the Microbiology Laboratory, Assam Medical College & Hospital over the period of six months.

All the isolates were identified by using standard microbiological methods. All the isolates were tested for susceptibility to erythromycin and other antibiotics of the panel by the modified Kirby-Bauer disc diffusion test. Methicillin resistance was detected by cefoxitin disc (30µg) on Mueller Hinton agar (MHA) supplemented with 2% NaCl.The isolates that were found to be erythromycin resistant by the modified Kirby-Bauer disc diffusion method were subjected to the D zone test for inducible clindamycin resistance as per the CLSI guidelines. The clindamycin (2µg) and erythromycin (15µg) discs were procured from HiMedia India, Pvt. Limited, Mumbai. The clindamycin (2µg) discs were placed at a distance of 15mm (edge to edge) from the erythromycin (15µg) discs on the same plate and were incubated at 37°C overnight. A flattening of the zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance.

Three different phenotypes were appreciated after testing and interpreted as follows-

The Inducible MLS_B phenotype:

Isolates which were resistant to erythromycin and with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc. (Fig1)

The Constitutive MLS_B phenotype:

Isolates which were resistant to both erythromycin and clindamycin with circular shape of zone of inhibition if any around clindamycin. (Fig 2)

The MS phenotype:

Isolates which were resistant to erythromycin and susceptible to clindamycin and giving circular zone of inhibition around clindamycin showing negative D test. (Fig 3)

RESULTS

One hundred and fifty six (156) isolates of Staphylococcus aureus were tested for susceptibility to Erythromycin, Clindamycin and other antibiotics by the modified Kirby-Bauer disc diffusion test as per CLSI guidelines. Out of them 28.2% isolates were found to be Methicillin resistant S. aureus (MRSA) whereas 71.7% isolates were Methicillin sensitive S. aureus (MSSA). Among them 56.4% isolates were resistant to Erythromycin. These isolates when subjected to D test showed 34 (21.8%) isolates resistant to both Erythromycin and Clindamycin indicating constitutive MLS_B phenotype; 24(15.4%) isolates showed positive D test indicating inducible MLS_B phenotype while 30(19.2%) gave negative D test indicating MS phenotype [Table 1]. Among the MRSA isolates 36.3 % had constitutive resistance, 31.8 % had the inducible MLS_B resistance and 13.6% had the MS phenotype. In MSSA, 16% and 8.9% isolates were found to have the constitutive and inducible MLS_B resistance phenotypes respectively, while 21.4 % exhibited the MS phenotype. Percentage of both inducible and constitutive Clindamycin resistance was higher amongst MRSA isolates as compared to MSSA.All the isolates of Staphylococcus aureus showed 100% sensitivity to Vancomycin and Linezolid.

DISCUSSION AND CONCLUSION

Clindamycin is a useful drug in the treatment of both methicillin susceptible and resistantstaphylococcal infections.[4]Accurate susceptibility data are important for proper treatment. However, false in vitro susceptibility results may be obtained by the disc diffusion testing with erythromycin and clindamycin discs in nonadjacent positions.[2,5]Hence, routine testing ofstaphylococcal isolates for inducible clindamycin resistance is recommended in the CLSI guidelines.

The overall inducible MLSB resistance phenotype in our study was 20.3 %. The constitutive phenotypepredominated over the inducible phenotype among both the MRSA (36.3 vs. 31.8%) and MSSA isolates (16.0 vs. 8.9%). The MS phenotype was found to be higher in MSSA as compared to MRSA (21.4% vs 13.6%).

This was in concordance with a few of the studies reported before - Yilmaz et al.found inducible resistance of 24.4% in MRSA and 14.8% in MSSA,[6]Gadepalli et al. howed it to be 30% in MRSA and 10% in MSSA.[7] On the contrary, Schreckenberger et al.[8] and Levin et al.[9] showed higher percentage of inducible resistance in MSSA as compared to MRSA, 7-12% in MRSA and 19-20% in MSSA; 12.5% MRSA and 68% MSSA respectively.Deotale et al.[10] in 2010 reported a distribution of 14.5% isolates inducible clindamycin resistance, 3.6% constitutive resistance while 14.1%

showed MS phenotype. Inducible resistance and MS phenotype were found to be higher in MRSA as compared to MSSA (27.6%, 24.3% and 1.6%, 4% respectively).

In the present study, a high percentage of erythromycin resistance was detected among Staphylococcus aureus isolates and several of them tested positive for inducible clindamycin resistance by D test . These observations suggest that had D test not been performed, nearly one fourth of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.The clinical microbiology laboratories should consider routine testing and reporting of inducible clindamycin resistance in S. aureus to prevent the possibility of clindamycin treatment failure.

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Figure 1: Double-disc test (D test) demonstrating erythromycin discinduction of clindamycin resistance; a blunting of the zone of inhibition around the clindamycin disc is produced that forms a D-shape (Inducible MLS_B phenotype).

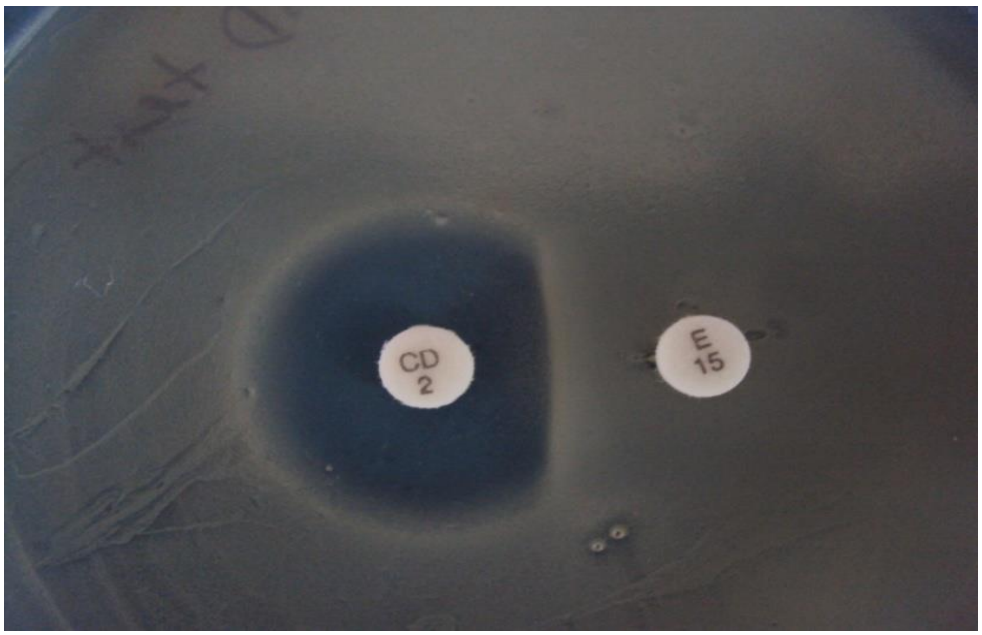

Figure 2: Double-disc test (D test) demonstrating resistance to both erythromycin and clindamycin with circular shape of zone of inhibition if any around clindamycin (Constitutive MLS_B phenotype).

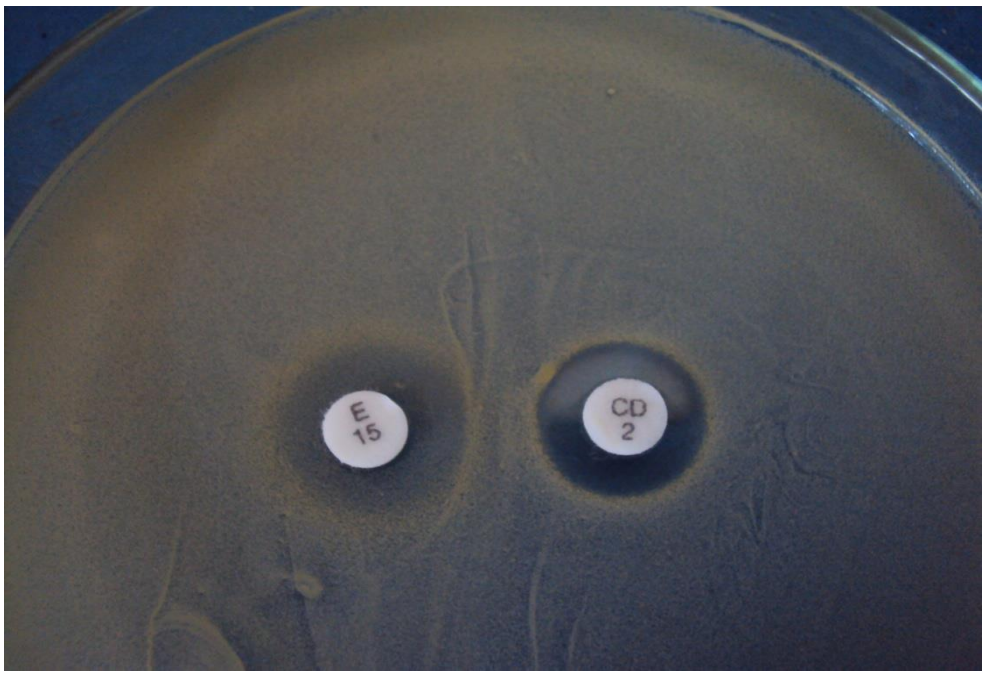

Figure 3:Double-disc test (D test) demonstrating MS phenotype(resistance to erythromycin and susceptible to clindamycin and giving circular zone of inhibition around clindamycin showing negative D test)

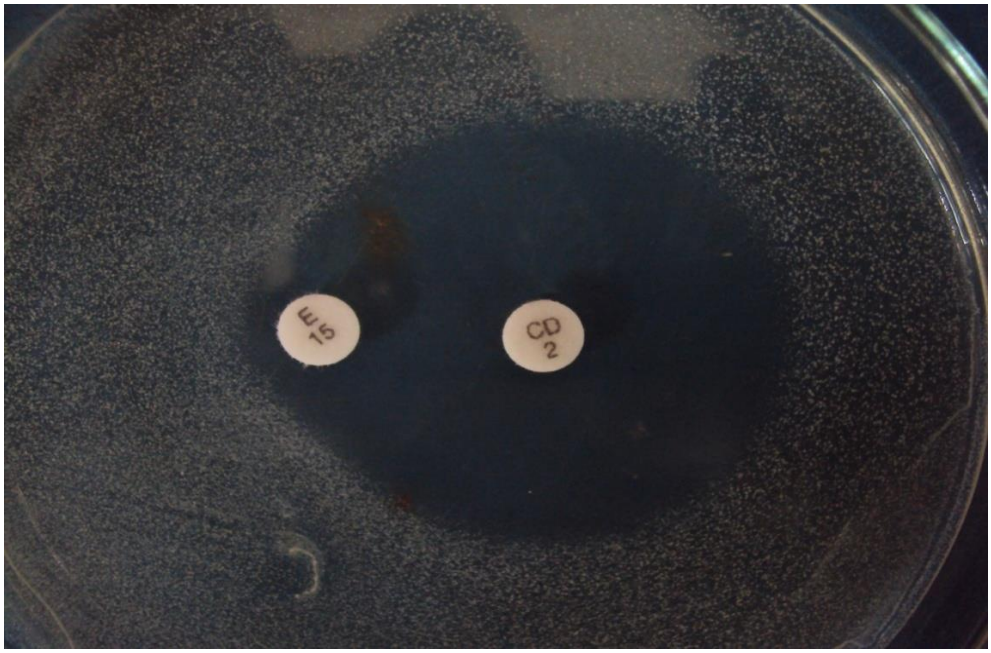

Table 1: Phenotypic characterization of isolates

Susceptibility pattern	MRSA(%)	MSSA(%)
Ery-S;Cl-S	8(18.1%)	60(53.5%)
cMLS$_B$ Phenotype Ery-R; Cl-R	16(36.3%)	18(16.0%)
iMLS$_B$ Phenotype Ery –R, Cl- S (D Test +ve)	14(31.8%)	10(8.9%)
MS Phenotype; Ery –R, Cl- S (D Test –ve)	6 (13.6%)	24(21.4%)
Total	44(28.2%)	112(71.7%)

S – susceptibility, R – resistance, Ery- Erythromycin, Cl- Clindamycin, cMLS$_B$- Constitutive resistance to Clindamycin, iMLS$_B$ – Inducible resistance to Clindamycin, MS phenotype- Resistant to Erythromycin, susceptible to Clindamycin. MRSA – Methicillin resistant S. aureus, MSSA – Methicillin sensitive S. aureus.

Table 2: Sources and categorization of Staphylococcus aureus isolates

Clinical sample types	MRSA	MSSA	Total
Pus	10	40	50
Swab	18	30	58
Sputum	6	14	20
Blood and other fluids	10	28	38
Total	44	112	156

MRSA – Methicillin resistant S. aureus, MSSA – Methicillin sensitive S. aureus

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