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PHENOTYPIC DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS* AUREUS: A STUDY FROM A TERTIARY CARE HOSPITAL IN WEST BENGAL

Microbiology

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ABSTRACT

Introduction: The increasing prevalence of methicillin resistance among *Staphylococci* is an alarming problem. MRSA isolates with inducible clindamycin resistance (iCR) are resistant to erythromycin and sensitive to clindamycin on routine testing. D-test can help to determine whether clindamycin could be used as a therapeutic option. Hence the present study was undertaken to detect incidence of inducible resistance to clindamycin in erythromycin resistant *S. aureus* isolates by D test and to study the association between clindamycin and methicillin resistance.
Materials and Methods: Two hundred and seven isolates of *Staphylococcus aureus* were tested for methicillin resistance using cefoxitin (30µg) disc by Kirby Bauer disc diffusion method. Inducible resistance to Staphylococci was tested by D test as per CLSI guidelines.
Results: Of the total 207 isolates tested, 126 were erythromycin resistant. Among the erythromycin resistant isolates, 37(29.3%) showed clindamycin resistance, 47(37.3%) showed constitutive resistance and the remaining 42(33.3%) were of MS phenotype. Inducible and constitutive resistance was higher in MRSA as compared to MSSA(32.8%,44.2% and 25%,28.5%) respectively.
Conclusion: The study emphasizes the fact that every microbiology laboratory should perform D test on routine basis. This will help in detecting inducible clindamycin resistance and thus prevent therapeutic failure.

KEYWORDS

Clindamycin, MRSA, D test, inducible resistance, Staphylococcus aureus

INTRODUCTION

Staphylococcus aureus is one of the most common pyogenic bacteria infecting man.[1] *S. aureus* is known for acquiring antimicrobial resistance promptly after the introductionof new antibiotics. Emerging resistance to methicillin in this organism has left us with very few therapeutic alternatives to treat the infections caused by them. Clindamycin in macrolide-lincosamide streptogramin B (MLS_B) family of antibiotics serves as one such alternative for treating both methicillin susceptible S. aureus (MSSA) and methicillin resistant S. aureus (MRSA) infections, due to its excellent pharmacokinetic properties. However, widespread use of MLS_B antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to MLS_B antibiotic.[2]

Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible.[3]The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_Bphenotype) or inducibly (inducible MLS_Bphenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other. In such cases, *in vivo* therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through msrA genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both *in vivo* and *in vitro* and the strain do not typically become clindamycin resistant during therapy.[4]Thus to avoid clinical therapeutic failure in the resistance case mediated by erm gene, it is very important to detect inducible clindamycin resistance phenotypes in vitro which can be made by erythromycin-clindamycin disc approximation test (D-test) as its sensitivity was found 100% in different studies when compared with erm and msr gene detection by polymerase chain reaction.[5-7]

The present study was aimed to detect incidence of inducible clindamycin resistance in erythromycin resistant *S. aureus* isolates by D test and to study the relationship between clindamycin and methicillin resistance.

MATERIALS AND METHODS

Bacterial isolates

The present cross-sectional study was conducted in the Department of Microbiology, North Bengal Medical College from November 2017 to April 2018. A total of 207 non-duplicate isolates of *Staphylococcus aureus* was isolated from different clinical samples (wound swab, aspirates, blood , urine and respiratory) of both genders and all age groups attending North Bengal Medical College.

Identification of the organism and testing for MRSA

The isolates were first identified as *S. aureus* by standard biochemical techniques[8] and then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates. Methicillin resitance was detected using cefoxitin disc diffusion test. Isolates with cefoxitin zone size ≥22 mm were considered methicillin susceptible (MSSA) and those with ≤21 mm were considered methicillin resistant (MRSA).[9]

Phenotypic detection of constitutive MLS_B,inducible MLS_B and MS phenotype

Inducible resistance to clindamycin was tested by 'D test' as per CLSI guidelines.[9] Briefly, erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) disc on a Mueller-Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. Following overnight incubation at 37°C, flattening of 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[10]:

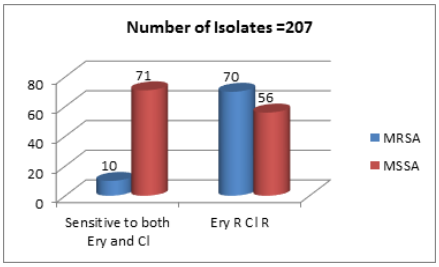
1. Constitutive MLS_B Phenotype - this phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with , circular shape of zone of inhibition if any around clindamycin.
2. Inducible MLS_B Phenotype - Staphylococcal isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.
3. MS Phenotype - Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype.

Quality control (QC) of the erythromycin and clindamycin discs was performed with *S. aureus* ATCC25923, according to the standard disc diffusion QC procedure. The data were analyzed using Statistical Package for Social Science, version 20. *p* value <0.05 was considered statistically significant.

RESULTS

Among 207 Staphylococcal isolates tested, 80 (38.6%) were MRSA, and 127 (61.3%) were MSSA. Out of these 207 isolates, 126(60.8%) were erythromycin resistant and mostly MRSA (MRSA 70 and MSSA 56). On the contrary, isolates sensitive to both Erythromycin and Clindamycin were mostly MSSA (55.11%). (Figure 1).

Figure 1: Distribution of MRSA among Erythromycin sensitive and resistant isolates.



Chi square: 38.82, p value:<0.00001(significant at p<.05)

Ery=Erythromycin,Cl=Clindamycin,R = resistant

126 erythromycin resistant isolates were subjected to D test.D test was positive in 37(29.26%) of the isolates (23 MRSA and 14 MSSA) indicating inducible CR. (Figure 2)

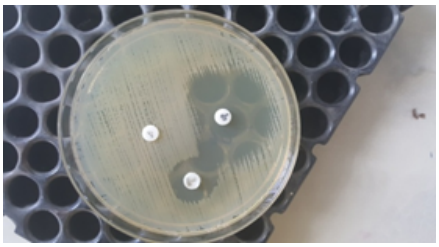


Fig 2: Inducible MLSB (D test +ve) in Methicillin Resistant Staphylococcus Aureus (MRSA)

Constitutive clindamycin resistance was shown by 47 (37.3%) isolates (31 MRSA and 16 MSSA) and MS phenotype was seen in 42 (33.33%) isolates (16 MRSA and 26 MSSA). The percentage of inducible and constitutive resistance was more among MRSA (32.8% and 44.2%) than MSSA (25% and 28.5%) while the MS phenotype was more among MSSA (46.4%) as compared to MRSA (22.8%) (Table 1)

Table 1: Distribution of MLSB phenotype among erythromycin resistant strains (n=126)

Phenotypes	MRSA(n=70)	MSSA(n=56)	Total
ERY- R, CL- R (cMLSB)	31 (44.2%)	16 (28.5%)	47 (37.3%)
ERY-R,CL-S (iMLSB ,D test +ve)	23 (32.8%)	14 (25%)	37(29.3%)
ERY-R,CL-S (MS,D test –ve)	16 (22.8%)	26 (46.4%)	42(33.3%)

Ery=Erythromycin,Cl=Clindamycin

Analysis shows that methicillin resistance is a significant factor in D test positive isolates as compared to D test negative isolates.(p <0.05)(Table 2)

Table 2: Comparison of D test +ve and D test –ve *Staphylococcus aureus* isolates by Chi square analysis

Phenotype	MRSA	MSSA
D +ve	23	14
D -ve	16	26

Chi square 4.55, p = 0.032 (Significant at p<0.05)

RESULTS

Clindamycin is a good alternative for the treatment of both methicillin-resistant and -susceptible staphylococcal infections. Also, clindamycin has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics.Clindamycin resistance can develop in staphylococcal isolates with the inducible phenotype, and spontaneous constitutively resistant mutants have been selected from such isolates both in vitro and in vivo during Clindamycin therapy.Failure to identify iMLS $_B$ resistance may lead to clinical failure of clindamycin therapy. Conversely, labeling all erythromycin-resistant *Staphylococci* as clindamycin-resistant prevents the use of clindamycin in infections caused by truly clindamycin-sensitive Staphylococcal isolates.[11] Hence, Clinical and Laboratory Standards Institute (CLSI) recommends routine testing of all Staphylococcal isolates for iMLS$_B$.[9]

In the present study, of the 207 isolates of *Staphylococcus aureus*, erythromycin resistance was seen in 126 (60.8%) isolates which is quite higher. This indicates higher use of macrolides like erythromycin as the first resort for empirical therapy in this region which has resulted in higher degree of resistant mutant. Study done by Pal *et al*(50.52%)[13] and Lyall *et al*(51.7%)[12] also reported higher percentage of erythromycin resistance. Low rate of resistance was reported by Prabhu *et al*. (28.4%)[10] and Ajantha *et al*. (15.7%)[14].

Inducible clindamycin resistance was observed in 29.3 %(37) of erythromycin resistant isolates. This emphasises the fact that if D test is not performed routinely, a significant number of cases will present with therapeutic failure. Different studies have found varying prevalence rates of inducible clindamycin resistance. Higher rate of inducible resistance was observed by Ajantha *et al*.[14](49%), Goyal *et al*. [15](50.6%), where as lower rate was observed by Prabhu *et al*. [10](10.5%).Our finding was similar to that reported by Sande *et al* [16](30.2%) and Lyall *et al* [12](33.3%). In this study, constitutive resistance was observed in 37.3 %(47) of the erythromycin resistant isolates which was almost similar to the finding by Sande *et al*.[16] (35.5%). Higher percentage was reported by Pal *et al*. [14](46.9%) whereas lower percentage was observed in studies by Patil *et al*. [17](3.55%) and Mittal *et al.* [18](6.15%).In the present study, 33.3% of erythromycin resistance *S. aureus* isolates showed true clindamycin susceptibility (MS phenotype). Lower incidence was observed by Patil *et al*. [17](15.33%) and Mittal *et al.* [18](15%).

In our study, inducible clindamycin resistance was seen in higher percentage of MRSA than MSSA(32.8% MRSA, 25% MSSA isolates) but the association between MRSA and inducible clindamycin resistance was not found to be statistically significant (Chi square 0.58, p value 0.44). Our finding was similar to that reported by Sande *et al* [16](33.3% in MRSA and 25% in MSSA) and Gadepalli *et al.*[19](30% in MRSA, 10 % in MSSA). Similarly constitutive resistance was also more in MRSA than MSSA (44.2% and 28.5% respectively)

Staphylococci exhibiting inducible resistance to MLS$_B$ antibiotics are now common in clinical practice. One should be cautious about using clindamycin in patients with major infections, especially where treatment is likely to be prolonged or infection difficult to eradicate as constitutive mutants can be selected during the course of clindamycin therapy in patients with MLSBi. Conversely labeling all erythromycin-resistant staphylococci as clindamycin resistant would prevent the use of clindamycin in infections caused by truly clindamycin susceptible isolates.[20]

CONCLUSION

The laboratories and also clinicians must be aware of the local prevalence of iMLSB isolates. As there is a high rate of clindamycin resistance in the staphylococcal isolates, the laboratories should routinely test *S. aureus* strains for iMLSB so that chances of therapeutic failure is decreased. As the D-test is simple, inexpensive and easy to perform, it can be included as a part of routine antibiotic susceptibility testing.

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