PHENOTYPIC DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE ISOLATES OF STAPHYLOCOCCUS AUREUS IN AROUND COIMBATORE

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Abstract: ***The goal of the present study was to phenotypic characterization of erythromycin-induced clindamycin resistance among clinical isolates of S. aureus. Among the, 39 procured isolates, 21 of were MRSA and 18 of were MSSA. These isolates were subjected to determine the Inducible clindamycin resistance by D-zone test. Among the 39 isolates, 23% of were MLSBc, 18% of were MLSBi, 26% of were MS phenotype and 33.3% of isolates sensitive to both erythromycin and clindamycin. The present study, MLSBi and MLS_B are greater in MRSA than MSSA. In this study, the prevalence of inducible clindamycin resistance was observed among S. aureus with an association between MRSA and inducible clindamycin resistance. The present study was indicated that D-test should be used as an important method in usual disc diffusion testing to detect inducible clindamycin resistance.***

Keywords: S.aureus, Clindamycin resistance, D test, MLSBc, MLSBi,

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most important and frequent bacteria that causing nosocomial and community-acquired infections in developing and developed countries. From the last 2 decades, the prevalence of both hospital and community acquired *S. aureus* infections have elevated and antibiotic treatment is increasingly being hampered by the spread of strains resistant to multiple drugs including methicillin (MRSA). Numerous studies on high level MRSA resistant to various antibiotics and antiseptics have been reported in out breaks with clones disseminating in the world (Gerald *et al*., 2009). The increasing incidence of MRSA was resistance to both betalactamase and aminoglycosides group of antibiotics (Anupurba *et al*., 2003).

This phenomenon was occurred by the usage of macrolide–lincosamide–streptogramin B antibiotics to cure *S. aureus* causing infections with clindamycin being the relevant agent due to its great pharmaceutical properties. However, the wide spread use of MLS_B antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLS_B antibiotics (Sasirekha *et al*., 2014).

Staphylococcus aureus with constitutive and inducible resistance to clindamycin have to be identified in the laboratory to avoid the irrelevant use of clindamycin which may appear sensitive in vitro by the disk diffusion method. There are no researches about the prevalence of constitutive and inducible clindamycin resistance in this region. (Jeevan and Zarrin, 2017). The current investigation was a goal to find out the percentage of *S. aureus* having inducible clindamycin resistance ($iMLS_B$) in our area using D-test method. Also, we proved to find the relationship between MRSA and inducible clindamycin resistance.

MATERIALS AND METHODS

TEST PATHOGENS

The clinical isolates of *S.aureus* were procured from Microtech, Microbiology Laboratory, and Coimbatore and used for the study. All isolates were inoculated onto Mannitol salt agar (MSA) and Chromogenic agar media. After 24 hrs, colony morphology was observed. For confirmation of the isolates, gram's stain, motility and biochemical characterization was carryout with according to previous studies (Koneman *et al.*, 1997).

PHENOTYPIC DETECTION OF INDUCIBLE RESISTANCE TO CLINDAMYCIN BY D-TEST

Isolates were tested for inducible resistance by the 'D test' as per the previous study of Sasirekha *et al*., 2014. Erythromycin (15µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2µg) on Mueller–Hinton agar plates previously inoculated with 0.5 McFarland bacterial suspensions. Plates were analyzed after 18 h of incubation at 37°C. Interpretation of the inhibition zone diameters was as follows: If an isolate was erythromycin resistant and clindamycin susceptible, with a D-shaped inhibition zone around the clindamycin disc, it was considered to be positive for inducible resistance (D test positive, iMLSB phenotype). If the isolate was erythromycin resistant and clindamycin susceptible, with both zones of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D test negative, MS phenotype), but to have an active efflux pump. If the isolate was erythromycin resistant and clindamycin resistant, the isolate was considered to have the macrolide–lincosamide–Streptogramin B constitutive (cMLSB phenotype).

RESULT AND DISCUSSION

The increasing frequency of Staphylococcal infections with MRSA was a crucial problem in every part of the world. The high percentage of methicillin resistance among *S. aureus* isolates have been cited in most of the countries. In the case of India, MRSA was noted in all over the country. The 27.4% of MRSA observed from Bangalore (Sasirekha *et al*., 2014) and recently 36.9% of MRSA from UP state (Jeevan and Zarrin, 2017). In the current study, among the 36 isolates, 21 (54%) of were MRSA and 18 (46.1%) were MSSA.

The Clindamycin is regularly used to treat MRSA causing skin and wound infections because of its reasonable cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses and no renal dosing adjustments are needed (Kasten 1999). However, this drug also resistance to MRSA isolates. This resistance of MLS isolates was not easily identified using standard disc diffusion methods and its prevalence differ according to a geographic area, D-test becomes an imperative part of regular antimicrobial susceptibility test for all clinical isolates of *S.aureus.* In India, huge occurrence of resistance to erythromycin has noted by Archana *et al*., (57%), Mittal *et al*. (44.2%) and Sasirekha *et al.* (41.17%).

In this study, all isolates were subjected to 'D' test. Of them, 39 isolates, 9 (23 %) of were showed resistant to erythromycin and clindamycin indicating constitutive $MLS_{Bc,}$ 7(18%) of were MLSBi and 10 (26%) were of MS phenotype. Out of 21 MRSA isolates, 28.5% of were MLSBc Phenotype, 23.8% of were inducible MLSB phenotype, 14.2% of were MS phenotype and 48% of were sensitive to both erythromycin and clindamycin (Table.1). In 2017, Jeevan and Zarrin were found to be 27.1% of MLSBi from clinical isolates. Same year Nikam *et al* found to be 30% of MLSBi and 43% of MLSBc.

While in 18 methicillin sensitive Staphylococcal isolates, 17% of were showed constitutive $MLS_{B,}$ 11.1% of were Inducible MLSB phenotype, 39% of isolates were MS Phenotype and 17% of were sensitive to both erythromycin and clindamycin. The percentage incidence of MLSBc and MLSBi was higher amongst MRSA isolates as compared to MSSA isolates. The similar study has also been found in India and other countries. In recently Jeevan *et al*., (2017) reported 17% were $MLSB_i$ strains and 33% were MLSBc strains. From a study in Nepal, the MLSBc phenotype predominated in *S aureus* strains (47% MRSA; 13% MSSA) and was followed by the $MLSB_i$ strains inducible (15% MRSA; 20% MSSA). This trend is in disagreement with other studies in India where the majority of MSSA had constitutive resistance (Ravisekhar *et al*., 2006; Patel *et al*., 2006).

The proper susceptibility information is important for appropriate therapeutic decisions. This inducible clindamycin resistance can be easily missed by regular susceptibility tests. Hence, D-zone test should be essentially carried out by the clinician so as to differentiate inducible MLSB resistance from that of constitutive MLSB resistance. In the present study, three different phenotyped were interpreted from erythromycin resistance isolates. These observations advocate that if D-test had not been performed, most of the erythromycin-resistant isolates would are misidentified as clindamycin sensitive leading to therapeutic failure.

Table 1. The distribution pattern for the three phenotypes among all isolates tested

Isolates (n)	MLSBc Phenotype (*n* &%)	MLSBi Phenotype (*n* &%)	MS Phenotype (*n* &%)	Erythromycin and Clindamycin sensitive (*n* &%)
S. aureus (n=39)	9 (23)	7 (18)	10 (26)	13 (33.3)
MRSA (n= 21)	6 (28.5)	5 (23.8)	3 (14.2)	10 (48)
MSSA (n= 18)	3 (17)	2 (11.1)	7 (39)	3 (17)

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