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PHENOTYPIC DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

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Abstract

Aim: Staphylococcus aureus is recognized as one of the leading pathogen causing skin and soft tissue infections. The resistance to antimicrobial agent among staphylococci is an increasing problem. Clindamycin is considered to be one of the alternative agents in these infections. To know the prevalence of inducible clindamycin resistance among Staphylococcus aureus isolates this study demonstrates a simple and reliable method- D test. **Materials and Methods**: A total of 300 staphylococcus aureus strains were been taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc. To detect inducible clindamycin resistance, 15 µg erythromycin and 2 µg clindamycin disc were placed on Muller Hinton agar plate at a distance of 15 mm apart (measuring edge to edge). If there is inducible clindamycin resistance, the erythromycin will diffuse through the agar and resistance to clindamycin will be induced, resulting flattening of clindamycin inhibition adjacent to erythromycin disc and giving D-shape to the zone. **Results**: Out of 300 Staphylococcus aureus isolates, 109 strains (36.33%) were found to be MRSA and 191 strains (63.67%) were Non MRSA. Of these, 90 strains (30%) were found to be resistance to erythromycin. These isolates were subjected to D tests shows 11 isolates resistance to both erythromycin and clindamycin indicating constitutive MLS_B phenotype and 79 isolates showed clindamycin susceptibility. Out of these 79 isolates, 47 isolates showed positive D-test indicating inducible MLS_B phenotype while 32 gave negative D test indicating MS phenotype.

INTRODUCTION

Gram positive organisms are one of the leading pathogens causing skin and soft tissue infections. Clindamycin is considered a useful alternate drug in penicillin-allergic patients in the treatment of skin and soft tissue infections caused by *Staphylococcus aureus.* Good oral absorption makes it convenient for outpatient prescription or as follow-up drug after intravenous therapy. Erythromycin (E) (a macrolide) and clindamycin (CD) (a lincosamide) represent two distinct classes of antimicrobial agents that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells.[1] Staphylococcal resistance to clindamycin may be inducible (iMLS$_B$ – inducible Macrolide-Lincosamide-Streptogramin B resistance) or constitutive. Staphylococcal resistance to macrolide-lincosamide-streptogramin B antimicrobials (MLS$_B$) is mostly mediated by erythromycin ribosome methylase (erm) genes. In particular erm (A) gene is the most predominant molecular mechanism among clinical isolated MLS$_B$-resistant *staphylococcus aureus* forms. The erm (A) gene encodes ribosomal methylase and the expression is regulated by translational attenuation. Ribosomal RNA methylation leads MLS$_B$ antibiotics not to binds to the ribosome, cross resistance and peculiar phenotypes, including inducible (i MLS$_B$) and constitutive (c MLS$_B$).[2,3] Inducible clindamycin resistance can be detected by a simple test known as Disk diffusion test (D test). Disk diffusion testing involved placing CD & E disk approximately 15 mm apart (measuring edge to edge) on a Muller – Hinton agar plate that has been inoculated with a *Staphylococcus aureus* isolates.[4].

MATERIAL AND METHODS

The present study has been carried out in Bacteriology laboratory of Microbiology Department of Sardar Patel Medical College, Bikaner (Rajasthan) from March 2009 to June 2010.

A total of 300 consecutive, non duplicate *staphylococcus aureus* strains were taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc.

Isolates were plated on nutrient agar plate and blood agar plate and were incubated at 37^0C for 24 hour. The plates were examined and colonies of *staphylococcus aureus* were

identified on the basis of colony morphology, gram staining, catalase test, coagulase test (tube & slide coagulase), mannitol fermentation. All the isolates were subjected to the antibiotic sensitivity by Kirby Bauer method. Mueller Hinton agar was used for sensitivity testing. To detect inducible clindamycin resistance, 15 µg erythromycin and 2 µg clindamycin disc were placed on Muller Hinton agar plate at a distance of 15 mm apart (measuring edge to edge). If there is inducible clindamycin resistance, the erythromycin will diffuse through the agar and resistance to clindamycin will be induced, resulting flattening of clindamycin inhibition adjacent to erythromycin disc and giving D-shape to the zone. Strains were resistance to both erythromycin and clindamycin were defined as showing constitutive MLS_B resistance, those showing flatting of the clindamycin zone adjacent to erythromycin disc were defined as having inducible MLS_B & those were resistant to erythromycin and sensitive to clindamycin were defined as showing the MS phenotype (Fiebelkorn *et al.*2003;2006).[5, 6]

RESULTS

Out of 300 Staphylococcus aureus isolates, 109 strains (36.33%) were found to be MRSA and 191 strains (63.67%) were Non MRSA. Of these, 90 strains (30%) were found to be resistance to erythromycin. These isolates were subjected to D tests shows 11 isolates resistance to both erythromycin and clindamycin indicating constitutive MLS_B phenotype; 79 isolates showed clindamycin susceptibility. Out of these, 47 isolates showed positive D-test indicating inducible MLS_B phenotype while 32 gave negative D test indicating MS phenotype.

The overall percentage resistance for all three phenotypes was as follows:-

Inducible clindamycin resistance (47) – 15.67%

Constitutive clindamycin resistance (11) – 3.67%

MS phenotype (32) – 10.67%

Percentage of both inducible and constitutive resistance was higher among MRSA isolates as compare to MSSA (Table 1).

DISCUSSION

Resistance in Gram-positive bacteria not only increases morbidity and mortality, but also the cost of management of hospitalized patients. The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multidrug resistance organisms. There as many option available for treatment of MSSA and MRSA infections, with clindamycin being one of good alternatives.[F] Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses and no renal dosing adjustment are needed (Kasten, 1999).

Reporting *Staphylococcus aureus* strains as susceptible to clindamycin without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy.

Considering the high prevalence of clindamycin resistance among the clinical isolates, we feel that the laboratories should routinely test *Staphylococcus aureus* strains for inducible MLS_B. As the D-test is simple, inexpensive and easy to perform, it can be included as a part of routine antibiotic susceptibility testing. The benefit of routine D-testing is that we can clearly identify those strains that remain susceptible to clindamycin despite macrolide resistance.

In our study we found high prevalence of erythromycin resistance isolates [90(30%)]. Among these 47(15.67%) isolates tested positive for inducible clindamycin resistance by D test while rest of the isolates negative for D test, out of which 11 (3.67%) were shown to have constitutive clindamycin resistance and 32 (10.67%) showed true sensitive to clindamycin (MS phenotype). It was also observed that percentage of inducible clindamycin resistance and MS phenotype were higher among MRSA (39.45% and 23.85% respectively) as compare to MSSA (2.09% and 3.14% respectively). This was in concordance with a few of the studies reported before – Deotale *et al*[6] found inducible clindamycin resistance of 27.6% in MRSA and 1.6% in MSSA; Ajantha *et al*[7] found inducible clindamycin resistance of 21.1% in MRSA

and 4.19% in MSSA; Mohamed Rahabar *et al*[8] reported 22.6% in MRSA and 4% in MSSA.

Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, in vitro susceptibility testing for clindamycin may indicates false susceptibility by the broth microdilution methods and by disc diffusion testing with erythromycin and clindamycin disc on nonadjacent positions. However, if inducible clindamycin can we reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in those patients with true clindamycin-susceptible strains. In this study, we have described simple, reliable methods to detect inducible resistance to clindamycin in erythromycin-resistant isolates of *Staphylococcus aureus.*

Table 1: Distribution of isolates

Susceptibility pattern (Phenotype)	MRSA (%)	MSSA (%)	Total (%)
E-S, CD-S	30 (27.5%)	180 (94.24%)	210
E-R, CD-R (Constitutive MLS_B)	10 (9.25%)	1 (0.52%)	11
E-R, CD-S, D test positive (Inducible MLS_B)	43 (39.45%)	4 (2.09%)	47
E-R, CD-S, D test negative (MS phenotype)	26 (23.85%)	6 (3.14%)	32
Total	109 (36.33%)	191 (63.67%)	300

E-Erythromycin, CD-Clindamycin, S-Sensitive, R-Resistant

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