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Original Research Article

Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates

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ABSTRACT

Background: *Staphylococcus aureus* is a leading cause of nosocomial and community acquired infection in every region of world. Clindamycin is a frequent therapeutic option in the treatment of skin and soft tissue infection caused by *Staphylococcus aureus*. However resistance to this drug is again a problem which may be inducible or constitutive. The present study was carried out to determine the prevalence of inducible clindamycin resistance among Staphylococcus aureus and to find out the relationship between methicillin resistant *Staphylococcus aureus* and inducible clindamycin resistance.
Methods: A total of 177 *Staphylococcus aureus* isolated from different clinical samples were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. All were tested for Methicillin resistance by using cefoxitin 30 µg disc. Inducible clindamycin resistance was detected by 'D' test as per CLSI guidelines.
Results: Out of the 177 *Staphylococcus aureus* isolates, 77 (43.50%) were MRSA and 100 (56.50%) were MSSA. 101 (57.06%) isolates were erythromycin resistant. These erythromycin resistant isolates when subjected to 'D' test, 27 isolates showed MS phenotype, 26 showed inducible MLSB phenotype and 48 showed constitutive MLSB phenotype. Out of 77 MRSA isolates 23 (29.87%) showed Inducible MLSB phenotype and 33 (42.85%) showed Constitutive MLSB phenotype, while in 100 methicillin sensitive Staphylococcal isolates 03 (3%) showed Inducible MLSB phenotype and 15 (15%) showed Constitutive MLSB phenotype. The percentage of inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA isolates.
Conclusions: Study showed that 'D' test should be used routinely to detect inducible clindamycin resistance

Keywords: Constitutive clindamycin resistance, D test, Inducible clindamycin resistance, MRSA, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a leading cause of nosocomial and community acquired infection in every region of world. Increasing prevalence of methicillin resistance among *Staphylococci* is an increasing problem.[1] Clindamycin, a lincosamide antibiotic, a protein synthesis inhibitor, is a frequent therapeutic option for Staphylococcal infections, particularly for skin and soft tissue infections and as an alternative in penicillin allergic patients.[2] It has excellent tissue penetration except for central nervous system.[3] Clindamycin is also less costly than some of the newer agents that might be considered for these infections. Clindamycin may be able to inhibit production of certain toxins and virulence factors in *Staphylococci*.[4]

However, resistance to this drug is again a problem. Resistance to MLS_B can occur by two mechanisms: an active efflux mechanism encoded by the *msrA* gene and target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_B

phenotype) or inducibly (inducible MLS_B phenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive. 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* gene 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.[5] Inducible Macrolide – Lincosamide – Streptogramin B resistance can be detected by a simple test, known as Disk approximation test or D-zone test.[6]

The present study was carried out to determine the prevalence of inducible clindamycin resistance among Staphylococcus aureus in our geographic area isolated from various clinical samples, using 'D' test and to find out the relationship between methicillin resistant Staphylococcus aureus and inducible clindamycin resistance.

METHODS

This observational study was conducted in the Department of Microbiology at tertiary care hospital in Maharashtra state, from central India for a period of 6 month from January 2015 to June 2015, after obtaining the necessary permission from Institutional Ethical Committee (IEC).

A total of 177 isolates of Staphylococcus aureus were isolated from various clinical samples e.g. pus, blood, urine, sputum, body fluids, high vaginal swab, throat swabs, swabs from surgical and non-surgical wounds, referred for bacteriological cultures from patients of all age groups and both sexes from various departments. Isolates were identified on the basis of colony characteristics, Gram staining, catalase test, slide coagulase test, tube coagulase test, growth on mannitol salt agar and DNase test.[7]

In vitro antibiotic susceptibility pattern of *S. aureus* was carried out by Kirby Bauer disc diffusion method on Mueller Hinton agar using various drugs penicillin (10 U), Ampicillin (10 ug), gentamycin (10 ug), amikacin (30 ug), erythromycin (15 ug), ciprofloxacin (5 ug), vancomycin, linezolid (30 ug) and were screened for MRSA with 30 µg of cefoxitin disc as per Clinical and Laboratory Standards Institute (CLSI) guidelines, 2014.[6] The strains showing a zone diameter of less than or equal to 21 mm were considered as having mec-A mediated oxacillin resistance.[6] For quality control (QC) *S. aureus* ATCC 25923 was used.

D-zone test

All erythromycin resistant strains were tested for the presence of $iMLS_B$ resistance by D-zone test according to CLSI guidelines, 2014.[6] The test was done on Mueller Hinton agar with clindamycin (2 µg) and erythromycin (15 µg) placed 15 mm apart (edge to edge) on the same plate. Blunting of the circular zone of inhibition around the clindamycin disc on the side facing the erythromycin disc indicated the presence of $iMLS_B$ resistance. The results were interpreted into three phenotypes:

MS Phenotype

Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and giving a circular zone of inhibition around clindamycin were identified as having MS phenotype.

Inducible MLS_B Phenotype ($iMLS_B$)

Staphylococcal isolates showing resistance to erythromycin (zone size ≤13 mm) and sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were identified as having inducible MLS_B Phenotype.

Constitutive MLSB Phenotype (cMLSB)

Staphylococcal isolates which showing resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with a circular shape of zone of inhibition if any around clindamycin were identified as having Constitutive MLSB Phenotype.

RESULTS

Out of the 177 *Staphylococcus aureus* isolates, 77 (43.50%) were MRSA and 100 (56.50 %) were MSSA (Figure 1).

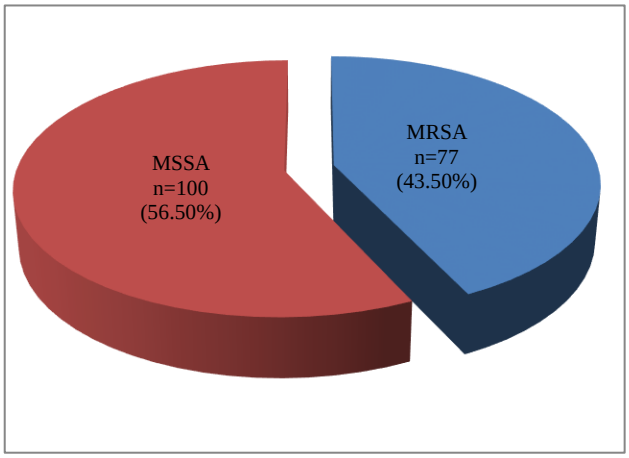

Figure 1: Prevalence of MRSA.

101 (57.06%) isolates were erythromycin resistant. These erythromycin resistant isolates when subjected to 'D' test, 27 isolates showed MS phenotype, 26 showed inducible

MLSB phenotype and 48 isolates showed constitutive MLSB phenotype (Table 1). Out of 77 MRSA isolates 23 (29.87%) showed Inducible MLSB phenotype and 33 (42.85%) showed Constitutive MLSB phenotype, while in 100 methicillin sensitive Staphylococcal isolates 03 (3%) showed Inducible MLSB phenotype and 15 (15%) showed Constitutive MLSB phenotype.

Table 1: Susceptibility to erythromycin (ERY) and clindamycin (CL) among erythromycin resistant *S. aureus* isolates.

Susceptibility pattern (Phenotype)	Number of isolates	Percentage
MS phenotype (E resistant and CL sensitive with D test negative)	27	26.73%
Inducible MLSB phenotype ((E resistant and CL sensitive with D test positive)	26	25.74%
Constitutive MLSB phenotype (E resistant and CL resistant)	48	47.52%
Total	101	100%

Table 2: Association of Clindamycin resistance with Methicillin resistance.

Susceptibility pattern	MRSA (n=77)	MSSA (n=100)
MS phenotype (E resistant and CL susceptible with D test negative)	13 (16.83%)	14 (14%)
Inducible MLSB phenotype (E resistant and CL susceptible with D test positive)	23 (29.87%)	03 (3%)
Constitutive MLSB phenotype (E resistant and CL resistant)	33 (42.85%)	15 (15%)
E sensitive and CL susceptible	08 (10.38%)	68 (68%)

The percentage of inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA isolates (Table 2, Figure 2).

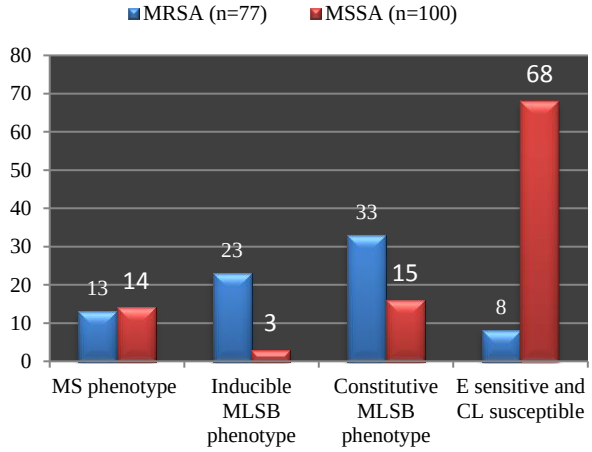

Figure 2: Association of Clindamycin resistance with Methicillin resistance.

DISCUSSION

In recent times, clindamycin has become an excellent drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients.[2] However, resistance to this drug is again a problem Since the iMLS $_B$ resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of *Staphylococcus aureus.* 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. Hence, Clinical and Laboratory Standards Institute (CLSI) recommends routine testing of all Staphylococcal isolates for iMLS $_B$.[6]

In present study we found high prevalence of erythromycin resistant *S. aureus* isolates, out of 177 isolates 101 (57.06%) were erythromycin resistant. Similar high prevalence of resistance to erythromycin has reported by Mittal et al. (44.2%) and Sasirekha et al. (41.17%).[8,9] Among 101 erythromycin resistant isolates 26 (25.74%) isolates tested positive for inducible clindamycin resistance by D-test. These findings are consistent with the study done by Deotale et al. and Mittal et al. who reported 27.6% and 23% of inducible clindamycin resistance.[5,8] These observations suggest that if D-test had not been performed, one- fourth of the erythromycin-resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

The incidence of MLS$_B$ resistance varies significantly by geographical region. Ajantha et al. reported a very high

frequency of inducible resistance (63%) in erythromycin resistant clindamycin sensitive isolates.[10] Constitutive resistance in our study was seen in 48 (47.52%) isolates, which was higher than inducible clindamycin resistance (25.74%). There are studies which reveal higher constitutive resistance in comparison to inducible resistance. Fiebelkorn et al. in their study found that out of 114 erythromycin-resistant *Staphylococcus aureus* isolates, 39 demonstrated constitutive resistances to clindamycin while 33 showed inducible resistance.[2] In the present study, 26.73% of erythromycin resistant Staphylococcal isolates showed true clindamycin susceptibility. Patients with infections caused by such isolates can be treated with clindamycin without emergence of resistance during therapy.

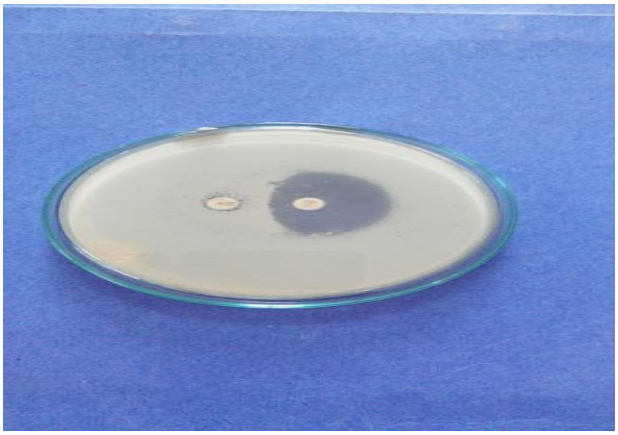

Figure 3: Positive 'D' test in iMLSB Phenotype.

Various authors have highlighted the relationship of MRSA and MSSA with different phenotypes of clindamycin and erythromycin resistant isolates. In our study percentages of inducible resistance and constitutive clindamycin resistance were higher amongst MRSA (30.26%, 43.42% respectively) as compared to MSSA (3%, 15.84%). This was in concordance with few of the studies reported before - Deotale et al. reported 27.6% in MRSA and 1.6% in MSSA, Gadepalli et al. found inducible resistance in 30% of MRSA and 10% in MSSA, Prabhu et al. showed it to be 20% in MRSA and 6.2% in MSSA.[5,11,12] On the contrary, Schreckenberger et al. and Levin et al. showed higher percentage of inducible resitance in MSSA (19–20 %) as compared to MRSA (7–12 %), 12.5 % MRSA and 68 % MSSA, respectively.[13,14] There are reports of decreased vancomycin susceptibility amongst MRSA i.e. VISA (vancomycin-intermediate *Staphylococcus aureus)* and VRSA (vancomycin-resistant *Staphylococcus aureus*). In our study we did not find any isolate showing resistance to vancomycin and linezolid.

Accurate susceptibility data are important for appropriate therapy decisions. The prevalence of inducible clindamycin resistance may vary from hospital to hospital. The true sensitivity to clindamycin can only be judged after performing D test on the erythromycin resistant isolates. From the current study, we can conclude that there is a fairly high percentage of inducible clindamycin resistance amongst the staphylococcal isolates. Use of D test in a routine laboratory will enable us in guiding the clinicians regarding judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not a suitable drug for D test positive isolates while it can definitely prove to be a drug of choice in case of D test negative isolates.

CONCLUSION

The pattern of macrolide resistance in *S. aureus* varies in different regions. Accurate susceptibility data are important for appropriate therapy decisions. The true sensitivity to clindamycin can only be judged after performing the D test therefore use of D test in a routine laboratory will help in guiding the clinicians regarding the judicious use of clindamycin.

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