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Research Article

Phenotypic detection and incidence of inducible clindamycin resistance among *Staphylococcus aureus* from tertiary care hospital

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ABSTRACT

Background: Among few therapeutic alternatives available for treatment of erythromycin resistant *Staphylococcus aureus* infections, clindamycin has several advantages but major barrier in its usage is development of resistance especially inducible resistance due to *erm* genes resulting in treatment failure. *In vitro* routine tests for clindamycin susceptibility may fail to detect such resistance which can be detected by simple D test. 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 relationship between clindamycin and methicillin resistance.
Methods: 330 *S. aureus* isolates were subjected to routine antimicrobial susceptibility test by Kirby-Bauer's disc diffusion method using various antimicrobial agents. Erythromycin resistant isolates were studied for detection of inducible and constitutive clindamycin resistance by D test according to CLSI guidelines.
Results: Out of 152 erythromycin resistant *S. aureus*, 46 (30.2%) isolates showed inducible clindamycin resistance, 54 (35.5%) showed constitutive resistance while 52 (34.2%) showed MS phenotype. The percentage of inducible and constitutive resistance was more among MRSA (33.3% and 46.6%) than MSSA (25% and 19.3%) while the percentage of MS phenotype was more among MSSA (54.8%) than MRSA (20%).
Conclusions: D test should be used in routine disc diffusion testing for the detection of inducible clindamycin resistance for the proper treatment of the patient.

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

INTRODUCTION

The resistance to different antimicrobial agents among Staphylococci is an increasing problem. Among few therapeutic alternatives available for treatment of Staphylococcal infections, Clindamycin has several advantages but major barrier in its usage is development of resistance especially inducible resistance. Clindamycin belongs to the Macrolide, Lincosamide and Streptogramin (MLS) family. Structurally these antibiotics are unrelated. They act by inhibiting bacterial protein synthesis by binding to 23s rRNA, which is a part of the large ribosomal subunit. The mechanism of resistance to MLS antibiotics are target site modification, efflux of antibiotics, or drug modification.[1]

In target-site modification, methylation of the A2058 residue, located in the conserved domain V of 23s rRNA, takes place. This leads to cross resistance and formation of resistance pattern phenotype known as MLS_B encoded by *erm* (erythromycin ribosome methylases) genes (conferring resistance to macrolides, lincosamide and type B streptogramin).[2]

The expression of the MLS_B phenotype can be inducible ($iMLS_B$) or constitutive ($cMLS_B$). In inducible resistance

due to production of methylases, inactive mRNA produced which becomes active in the presence of an inducer, whereas in strains showing constitutive resistance active methylase mRNA is produced.[1] The strains carrying the inducible *erm* gene are resistant to the inducer but remain susceptible to non-inducer macrolides and lincosamides. For MLS_Bi phenotype, low levels of erythromycin are an inducer which forms the basis of the D test.[3] In the routine laboratory, strains with inducible clindamycin resistance are difficult to detect when erythromycin and clindamycin discs are not placed adjacent to each other. Such strains appear erythromycin resistant and clindamycin sensitive *in vitro* but *in vivo* therapy with clindamycin may select constitutive *erm* mutants which lead to clinical therapeutic failure. Clinically, bacterial strains exhibiting $iMLS_B$ have a high rate of spontaneous mutation to constitutive resistance and use of non-inducer antibiotics such as clindamycin can lead to selection of constitutive mutants at frequencies of 10^{-7} cfu.[4]

Other mechanism of resistance mediated through *msrA* genes (conferring resistance to macrolides and type B streptogramin only) i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vitro* and *in vivo* and the strain does not typically become clindamycin resistant during therapy.[4]

Hence the present study was carried out to detect incidence of inducible resistance to clindamycin in erythromycin resistant *Staphylococcus aureus* isolates by D test and to study the relationship between clindamycin and methicillin resistance in the tertiary care hospital of central India.

METHODS

This prospective study was conducted in department of microbiology of rural tertiary care hospital. The project was approved by institutional ethics committee. Total 330 *S. aureus* isolates from various clinical specimens like pus, wound swab, blood, body fluids, aspirates, urine, central line/neck line/umbilical catheter tips, etc. were included in the study. *S. aureus* isolates were identified by standard methods.[5] The isolates were screened for routine Antimicrobial susceptibility test by Kirby-Bauer's disc diffusion method using various antimicrobial agents like penicillin (5 µg), cefoxitin (30 µg), amikacin (30 µg), erythromycin (15 µg), cotrimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg)/norfloxacin (10 µg), Vancomycin (30 µg), linezolid (30 µg) as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[6] Staphylococcal isolates were screened for MRSA (Methicillin resistant Staphylococcus aureus) with 30 µg cefoxitin disc as per CLSI guidelines.[6] The plates were incubated at 33 to 35°C for 16 to 18 h; strains showing a zone diameter of less than or equal to 21 mm were considered as having mec-A mediated oxacillin resistance . For quality control, *S. aureus* ATCC 25923 was used.

Erythromycin resistant Staphylococcus aureus were further studied for detection of inducible and constitutive clindamycin resistance by D test according to CLSI guidelines.[6] A 0.5 Macfarland suspension was prepared in normal saline for each isolate and inoculated on Muller Hinton agar plate. 2 µg clindamycin and 15 µg erythromycin disc were placed 15 mm apart edge to edge manually. Following overnight incubation at 37°C, three different phenotypes were appreciated and interpreted as follows:

1. MS phenotype: Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13 mm), while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin (D test negative).

2. Inducible MLS_B phenotype ($iMLS_B$): Staphylococcal isolates which showed resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

3. Constitutive MLS_B phenotype ($cMLS_B$): Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around clindamycin.

RESULTS

From 330 isolates of *S. aureus*, 164 isolates were MRSA and 166 isolates were MSSA. Out of total 330 isolates, 152 isolates were resistant to erythromycin (90 MRSA & 62 Methicillin sensitive Staphylococcus aureus - MSSA) (Figure 1). These 152 erythromycin resistant isolates were subjected to D test. D test showed two distinct phenotypes, D-zone phenotype (Figure 2a) in 42 (27.6%) isolates and D^+ phenotype (Figure 2b) in 4 (2.6%) isolates. For inducible clindamycin resistance, both these phenotypes were considered positive. Hence D test was positive in total 46 (30.2%) (30 MRSA & 16 MSSA) strains (Table 1). Constitutive clindamycin resistance (Figure 3) was shown by 54 (35.5%) isolates (42 MRSA, 12 MSSA). Out of 152 isolates, 52 (34.2%) isolates (18 MRSA, 34 MSSA) showed MS phenotype (Figure 4) (Table 1).

The percentage of inducible and constitutive resistance was more among MRSA (33.3% and 46.6%) than MSSA (25% and 19.3%) while the percentage of MS phenotype was more among MSSA (54.8%) than MRSA (20%) (Table 1). The susceptibility pattern of D test positive and D test negative strains showed that D test positive strains were more resistant to cotrimaxazole, ciprofloxacin and amikacin. All 152 strains were sensitive to vancomycin while 149 strains were sensitive to linzolid.

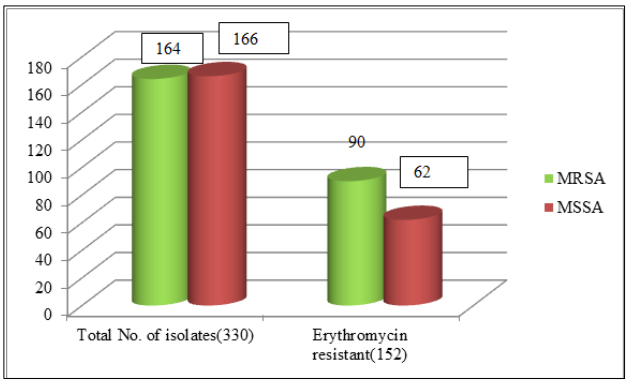

Figure 1: Distribution of *S. aureus* isolates.

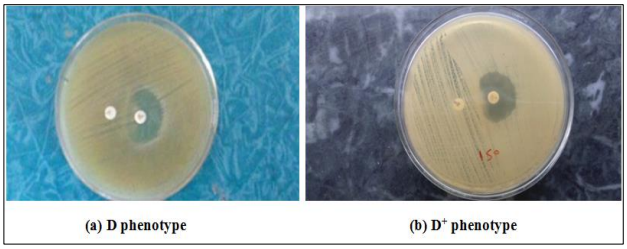

Figure 2: D test positive - showing iMLS$_B$ phenotype (Erythromycin resistant, clindamycin sensitive with flattening towards erythromycin disc).

Figure 3: Constitutive MLS$_B$ phenotype (Erythromycin resistant, clindamycin resistant).

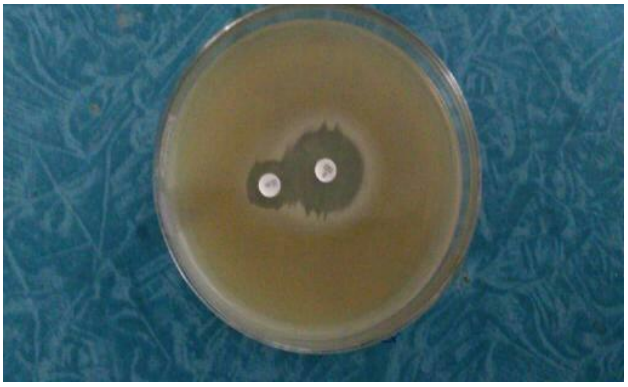

Figure 4: D test negative - (Erytromycin resistant, clindamycin sensitive) - MS phenotype.

Table 1: Distribution of MLS$_B$ phenotype among erythromycin resistant strains (n=152).

E-R strains	iMLS$_B$	cMLS$_B$	MS phenotype
MRSA (90)	30 (33.3%)	42 (46.6%)	18 (20%)
MSSA (62)	16 (25%)	12 (19.3%)	34 (54.8%)
Total (152)	46 (30.2%)	54 (35.5%)	52 (34.2%)

DISCUSSION

For the management of skin and soft tissue infections and serious infections caused by MRSA, MSSA and anaerobes, clindamycin is good option as it has several advantages.[7] However inducible or constitutive clindamycin resistance can develop in Staphylococcal isolates both *in vitro* testing and *in vivo* during clindamycin therapy leading to therapeutic failure.[8] Hence the prevalence of inducible resistance should be known, as it varies by geographical location, bacterial species, methicillin susceptibility and even from hospital to hospital.

In the present study, out of 330 isolates of *S. aureus*, 46% were erythromycin resistance which is comparable to Mittal et al.[9] (44.2%). Higher percentage of erythromycin resistance was reported by Lyall et al.[10] (51.7%) and Pal et al.[11] (50.52%) whereas lower percentage was reported by Prabhu et al.[12] (28.4%) and Ajantha et al.[13] (15.7%). Inducible clindamycin resistance was observed in 30.2% isolates which is in accordance with Lyall et al.[10] (33.3%) whereas higher rate was observed by Goyal et al.[14] (50.6%), Ajantha et al.[13] (49%) and lower rate was observed by Prabhu et al.[12] (10.5%) and Ciraj et al.[15] (13.1%). In our study inducible clindamycin resistant strains showed two phenotypes, D (27.6%) and D$^+$ (2.6%) and both are considered to be positive D-zone test.[16,17] Pal et al.[11] reported D (18.13%) and D$^+$ (5.34%) phenotypes. In this study, constitutive clindamycin resistance was observed in 35.5% isolates. Higher percentage was reported by Pal et al.[11] (46.9%) whereas lower percentage was observed in studies by Patil et al.[18] (3.55%) and Mittal et al.[9] (6.15%). In the present study, 34.2% of erythromycin resistance *S. aureus* isolates showed true clindamycin susceptibility (MS phenotype). Lower incidence was observed by Patil et al.[18] (15.33%) and Mittal et al.[9] (15%). Patients with infections caused by such isolates can be treated with clindamycin without emergence of resistance during therapy.

Regarding relationship between clindamycin and methicillin resistance, the percentage of inducible clindamycin resistance in MRSA and MSSA in our study was found to be 33.3% and 25% respectively which is comparable to Gadepalli et al.[19] (30% in MRSA, 10 % in MSSA) and Ciraj et al.[15] (38% in MRSA & 12.9% in MSSA). Very high incidences of inducible clindamycin resistance among MRSA were noted by Angel et al.[20] (64% in MRSA and 5% in MSSA) and Mittal et al.[9] (44.8% in MRSA and 8.4% in MSSA). However Schreckenberger et al.[17] and Levin et al.[21] reported higher

percentage of inducible resistance in MSSA as compared to MRSA (7-12% in MRSA and 19.20% in MSSA, 12.5% in MRSA and 68% in MSSA respectively). In the present study, constitutive resistance in MRSA and MSSA was found to be 46.6% &19.3% respectively. Shrestha et al.[22] found constitutive resistance of 44.4% in MRSA &2.7% in MSSA while study by Gadepalli et al.[19] reported 38% in MRSA & 15% in MSSA. Lower percentage of constitutive resistance was found by Prabhu et al.[12] (16.7% in MRSA &6.2% in MSSA) and Patil et al.[18] (9.6% in MRSA & 0% in MSSA) (Table 2).

So in this study, the percentage of inducible and constitutive resistance was found to be more among MRSA (33.3% and 46.6%) than MSSA (25% and 19.3%) while the percentage of MS phenotype was more among MSSA (54.8%) than MRSA (20%).

Table 2: Showing comparison with various studies regarding rate of inducible and constitutive resistance to clindamycin in MRSA and MSSA.

Author's name	MRSA			MSSA		
	iMLS$_B$ (%)	cMLS$_B$ (%)	MS phenotype (%)	iMLS$_B$ (%)	cMLS$_B$ (%)	MS phenotype (%)
Gadepali et al. (2006)	30	38	12	10	15	12
Angel et al. (2008)	64	0	12	5	0	25
Gupta et al. (2009)	20	46	16	17.3	10	37.3
Shrestha et al. (2009)	39.7	44.4	11.1	0	2.7	13.7
Pal et al. (2010)	43.6	38.8	18.7	6.9	7.3	10.9
Prabhu et al. (2011)	20	16.7	13.3	6.2	6.2	6.2
Lyall et al. (2013)	33.2	22.1	44.6	34.6	7.5	46
Mittal et al. (2013)	44.8	8.6	13.3	8.4	4.5	16.1
Patil et al. (2014)	24.6	9.6	16.2	3.16	0	14.7
Present study	33.3	46.6	20	25	19.3	54.8

CONCLUSION

D-test is a simple, cost effective, reliable and easy to interpret with high sensitivity and specificity.[4,7] When correlated with detection of *erm* and *msr* genes by Polymerase Chain Reaction (PCR), sensitivity of D-test performed at 15-20 mm disk spacing was 100%.[23] Hence every laboratory should perform D test on a routine basis for detection of inducible clindamycin resistance as for D test positive isolates, clindamycin is not a suitable drug however in patients with true clindamycin-susceptible strains, it can be used safely and effectively.

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