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Magnitude of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylocccus aureus* in a Tertiary Care Hospital

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Original Research Article

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Abstract: *Staphylococcus aureus* is associated with a variety of infections ranging from skin infections to life threatening illness. Therapeutic failure to Clindamycin has been reported due to mechanisms which confer resistance constitutively, or by the presence of low level inducers which can lead to therapeutic failure. Therefore, this study was undertaken to identify the strains that have the potential to become resistant during therapy. Inducible Clindamycin resistance was tested by the Clindamycin disc induction test (D test) as per the CLSI recommendations. The study showed 53.02% MRSA isolates and 22.73% inducible Clindamycin resistant isolates among them as compared to 10.25 % in MSSA isolates. We concluded that routine screening for inducible resistance to Clindamycin must be performed so that the drug is used effectively and for maximum clinical utility.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), *S.aureus*, inducible clindamycin resistance (iMLSB), D test, MS phenotype, (cMLSB).

INTRODUCTION

Most of the cluster-forming Gram-positive cocci of medical interest belong to genera Staphylococci, of which *Staphylococcus aureus* is the most important human pathogen. It is associated with a variety of infections ranging from skin infections to life threatening systemic illnesses. Community acquired bronchopneumonia is usually seen in elderly individuals and is associated with viral pneumonia as a predisposing factor [1]. Nosocomial pneumonia by *S aureus* occur in clinical setting of obstructive pulmonary disease, intubation and aspiration [2].

Underlying malignant diseases are recognized as important risk factors for the development of *S.aureus* bacteremia. Methicillin-resistant *Staphylococcus aureus* (MRSA) are increasingly being reported as multidrug resistant with high resistance to macrolide (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin), leaving very few therapeutic options [3]. This has led to renewed interest in the usage of macrolide lincosamide-streptogramin B (MLSB) antibiotics to treat Staphylococcal infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties [4, 5, 6]. Newer antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin have also been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concern[4]. The MS and iMLSB phenotypes are indistinguishable by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disk approximation test (D-test) and demonstration of resistance genes by molecular methods [7, 9, 10].

MATERIALS AND METHODS

This is a prospective study carried out for a period of one year (From 1st april 2016 to 31st march 2017). A total of 83 Staphylococcal isolates were recovered from various clinical samples at Department of Microbiology, SMS Medical College and attached group of hospitals, Jaipur (Rajasthan). Isolates were identified by using conventional methods (colony morphology, Gram stain, catalase test, slide and tube coagulase test, Hugh and Leifson's oxidation – fermentation test).

D-Test

Isolates obtained were tested for inducible resistance by the 'D test' as per CLSI guidelines using Erythromycin (15 µg) disc placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) 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).
- If the isolate was erythromycin resistant and clindamycin resistant, the isolate was considered to have the macrolide–lincosamide–Streptogramin B constitutive (cMLSB phenotype) [8]. The quality control of the erythromycin and clindamycin disc was performed with *S. aureus* ATCC 25923 [11, 12].

RESULTS

Out of the total 83 *Staphylococcus aureus* isolated in our study,44 (53.02%) were Methicillin resistant *Staphylococcus aureus* (MRSA) and 39 (46.98%) were Methicillin sensitive *Staphylococcus aureus* (MSSA). The difference in proportion was found to be statistically significant between the MRSA and MSSA (p value =0.003). The presence of iMLSB was confirmed by the D test. The overall prevalence of iMLSB among all *S.aureus* isolates was 14(16.87%).

Of 83 *Staphylococcal aureus isolates*, 59 (71.08%) were isolated from male patients and 24(28.92%) were females (Chi-square = 0.005 with 1 degree of freedom; P=0.944NS). Our study showed the highest percentage of *Staphylococcal aureus* in patients with the age group of 21-40 years (28 isolates) which was statistically significant (P=0.048) .Majority of *Staphylococcal aureus* were isolated from samples of IPD patients like pus and other body fluids 43(51.80%). Out of 44 MRSA isolated 10 (12.04%) exhibited the iMLSB (Chi-square = 1.490 with 1 degree of freedom; P = 0.222), 17(20.04%) and 2(2.41%) strains exhibited the constitutive phenotype and MS phenotype respectively (Chi-square=7.373 with 1 degree of freedom; P = 0.007) while 15(18.07%) exhibited sensitive phenotype. However in MSSA, 4(4.82%) showed iMLSB, 4(4.82%) showed the constitutive phenotype, 3(3.61%) strains showed the MS phenotype while sensitive phenotype was seen on 28(33.73%).

Table-1: Distribution of *Staphylococcus aureus* among different clinical specimens.

SPECIMENS	OPD	IPD	TOTAL
Blood	3	32	35(42.16)
Pus / other fluids	18	25	43(51.80)
CSF	0	5	5(6.02)
Total	21(25.30)	62(74.69)	

Table-2: Age-wise distribution of Staphylococcal isolates

Age Group (in years)	*S.aureus* isolates (%)
< 1year	17(20.48)
1-10 years	8(9.64)
11-20 years	4(4.82)
21-40 years	28(33.73)
41-60 years	22(14.45)
> 60 years	4(4.82)
Total	83(100)

Table-3: Sensitivity pattern of erythromycin and clindamycin among *Staphylococcus aureus* and distribution of D+ isolates

Organism	Total	E-S &CL-S	E-R&CL-S		E-R&CL-R(cMLSB)
			(iMLSB)D^+	(MS phenotype)D^-	
MRSA	44	15	10	2	17
MSSA	39	28	4	3	4

Table-4: Gender-wise distribution of Staphylococcal isolates

Gender	Number of isolates
Female	24(28.92)
Male	59(71.08)

Table-5: Occurrence of Multidrug resistant and extremely drug resistant case among Staphylococci

	MDR (%)	XDR (%)
Staphylococcus aureus	46 (17.69%)	06 (2.29%)

DISCUSSION

The increasing frequency of Staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin therapy to treat such infections [2]. Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost and excellent tissue penetration, and the fact that it accumulates in abscesses and no renal dosing adjustments are needed [9]. Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. Clindamycin is a good alternative for the treatment of both methicillin-resistant and susceptible Staphylococcal infections [10]. A study conducted in Turkey observed a prevalence of MLSBi as 21.9% [13]. Gadepalli *et al*., found 21 per cent inducible iMLSB phenotype [14]. Ö. K. Azap *et al*., observed 5.7% and 3.6% iMLSB phenotype in MRSA and MSSA isolates [15]. We found a high prevalence of 16.87% of MLSBi amongst all staphylococcal isolates.

CONCLUSION

Even though the overall prevalence of inducible clindamycin resistance among the isolates was found to be low in our set up, this study showed higher percentage of resistance to erythromycin and clindamycin. In the backdrop of this high resistance pattern and restricted range of antibiotics available for the treatment Staphylococcal infections and the known limitations of vancomycin, clindamycin should be considered for the management of staphylococcal infections. However, clindamycin resistance in the form of iMLSB and cMLSB limits the therapeutic options to the antibiotics like linezolid and vancomycin. The treatment of patients harboring iMLSB staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure. Clindamycin can be used to treat infections caused by MS phenotype without the risk of emergence of resistance during therapy. Therefore, to reduce the emergence of clindamycin resistance during therapy iMLSB resistant phenotype should be identified by D-test routinely in all the microbiology laboratories. To serve this purpose, D-test proves to be a simple, auxiliary, and reliable method to delineate inducible and constitutive clindamycin resistance

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