Original Article

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Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* from Sub Himalayan Region of India

Microbiology Section

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ABSTRACT

Introduction: Clindamycin is an alternative antibiotic in the treatment of *Staphylococcus aureus* (*S.aureus*) infections, both in infections by methicillin susceptible and resistant (MSSA and MRSA) strains. The major problem of use of clindamycin for staphylococcal infections is the presence of inducible clindamycin resistance that can lead to treatment failure in such infections.

Aim: To determine inducible and constitutive clindamycin resistance among clinical isolates of *S. aureus* in a tertiary care centre of sub Himalayan region of India.

Materials and Methods: A total of 350 isolates of *S. aureus* from various clinical samples were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. Methicillin resistance was detected by cefoxitin (30µg) disc. All isolates were subjected to inducible clindamycin resistance was by Clinical Laboratory Standards Institute (CLSI) recommended D test.

Results: Among 350 *S.aureus* isolates, 82 (23.42%) were MRSA and 268 (76.57%) were MSSA. Erythromycin resistance was detected in 137 (39.14%) isolates. Erythromycin resistance in MRSA and MSSA was 71.6% and 29.36% respectively. Overall clindamycin resistance was seen in 108 (30.85%) isolates. Constitutive MSL_B phenotype predominated (29.62% MRSA; 13.38% MSSA) followed by $iMLS_B$ (28.39% MRSA; 9.29% MSSA) and MS phenotypes (13.58% MRSA; 6.69%MSSA). Both inducible and constitutive clindamycin resistance was significantly higher (p=0.00001, 0.0008 respectively) in methicillin resistant strains than in methicillin susceptible strains.

Conclusion: The present study gives a magnitude of clindamycin resistance among clinical isolates of *S. aureus* from this region of the country. Our study recommends routine testing of inducible clindamycin resistance at individual settings to guide optimum therapy and to avoid treatment failure.

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

INTRODUCTION

Staphylococcus aureus is one among the most common pyogenic bacteriae infecting man, causing both hospital and community acquired infections [1]. The increasing prevalence of resistance to most antimicrobial agents in *staphylococci* signifies the need for new effective agents to treat staphylococcal infections. Macrolides, lincosamides and type B streptogramin (MLS_B) are structurally unrelated but act through common mechanism of inhibition of protein synthesis, and are widely used to treat such infections [2].

Clindamycin (a lincosamide) in particular, is an attractive alternative for clinicians as it is available for parenteral and oral use, distributes well in tissues, and is bacteriostatic against *S.aureus* [3]. *Staphylococcal* strains resistant to MLS_B antibiotics have increased in number following the widespread use of these antibiotics for treating serious staphylococcal infections [4,5].

Resistance occurs by different mechanisms to these microbiologically related antibiotics. Resistance due to active efflux encoded by *msr* (A) gene confers resistance to macrolides and streptogramin B (MS phenotype) but not to clindamycin. Ribosomal target modification, another mechanism of resistance, confers resistance to macrolide, type B streptogramin and also to clindamycin (MLS_B phenotype). MLS_B resistance in *staphylococci* is either constitutive ($cMLS_B$), where rRNA methylase is always produced or inducible ($iMLS_B$), where methylase is only produced in the presence of an inducer, and is encoded by erm (A) or erm (C) gene [6,7]. Patients infected with $iMLS_B$ strains of *staphylococcus* if treated with clindamycin can develop constitutive resistance during therapy and subsequently result in treatment failure [8].

Detection of its three resistant phenotypes (MS, $iMLS_B$, $cMLS_B$) is crucial for guiding appropriate antimicrobial therapy. Constitutive resistance can be detected by routine disc diffusion method but it fails to detect inducible resistance ($iMLS_B$), which appears sensitive to clindamycin on routine testing, resulting in institution of inappropriate clindamycin therapy. Inducible resistance also cannot be detected by broth or agar dilution methods [2].

Erythromycin is an inducer of clindamycin resistance ($iMLS_B$), which induces production of erythromycin ribosomal methylase (erm) that allows expression of clindamycin resistance. Double disc diffusion (D test) is recommended by CLSI for detection of inducible clindamycin resistance [9]. A negative result for inducible clindamycin resistance (ICR) by D test confirms clindamycin susceptibility and provides a good therapeutic option, thus necessitates the detection of inducible clindamycin resistance [10].

Incidence of clindamycin resistance varies from place to place and therefore a local data is important to guide empirical treatment [11]. Data describing prevalence of clindamycin resistance among clinical isolates of *S. aureus* is lacking from our geographic area. We undertook this prospective cross-sectional study after approval from institutional ethics committee to estimate the percentage of constitutive and inducible clindamycin resistance among clinical isolates of *S.aureus* in a tertiary care centre of sub Himalayan region of India.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. The study included 350 non-duplicate isolates of *S. aureus* from

various clinical samples of indoor (IPD) and outdoor (OPD) patients received over a period of nine months (March 2014 to November 2014). Various clinical samples included pus, blood, urine, tracheal aspirates and cerebrospinal fluid (CSF), and distribution of *S. aureus* strains from origin of recovery is shown in [Table/Fig-1]. The organism was identified by conventional laboratory methods such as colony morphology, catalase test, slide and tube coagulase test and standard biochemical reactions [12].

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method and methicillin resistance was identified by using cefoxitin (30 µg) disc and interpreted as per CLSI guidelines [9]. Antibiotic discs used were penicillin G (10 units), cotrimoxazole (25 µg), tetracycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), linezolid (30 µg) and teicoplanin (30 µg). All isolates were subjected to inducible clindamycin resistance testing by CLSI recommended D test on Mueller Hinton agar by keeping erythromycin (15µg) disc and clindamycin (2 µg) disc at 15 mm apart (edge to edge) [9]. Blunting of the circular zone of inhibition around clindamycin disc towards erythromycin disc indicated the presence of $iMLS_B$ resistance and was reported as resistant to clindamycin. Quality control (QC) for erythromycin and clindamycin discs was done by using *S. aureus* ATCCC 25923 according to standard disc diffusion QC procedure. We interpreted the results as follows:

1. The isolate sensitive to erythromycin and clindamycin was considered susceptible phenotype,
2. Erythromycin resistant and clindamycin sensitive isolate (no D zone), MS phenotype,
3. Erythromycin resistant and clindamycin sensitive isolate (D zone present), $iMLS_B$ phenotype and
4. Erythromycin and clindamycin resistant isolate, cMLSB phenotype.

Statistical analysis was performed by using Epi info version 3.3.2, and P-values of < 0.05 were considered statistically significant.

RESULTS

Out of total 350 *S.aureus* isolates, 158 (45.14%) were from outpatients and 192 (54.85%) from inpatients. Eighty two isolates (23.42%) were MRSA. There was statistically significant difference in MRSA between inpatients (76.83%) and outpatients (23.17%) (p<0.0001). Overall erythromycin resistance was seen in 137 (39.14%) and clindamycin resistance in 108 (30.85%) isolates. Susceptibility to both drugs was found in 213 (60.85%) *S.aureus* examined. MS phenotype, $iMLS_B$ and $cMLS_B$ phenotypes were seen in 29 (8.28%), 48 (13.71%) and 60 (17.14%) isolates respectively. Clindamycin resistance, both inducible and constitutive, was significantly higher (p<0.05) in MRSA strains (28.39% and 29.62%) compared to MSSA strains (9.29% and 13.38%). Findings of D test are shown in [Table/Fig-2]. Detection of $iMLS_B$ and $cMLS_B$ phenotypes were greater in inpatients (19.79% and 23.43% respectively) than in outpatients (6.34% and 9.4% respectively), which was statistically significant (p<05). Penicillin, cotrimoxazole, tetracycline and gentamicin resistance was seen in 24 (70.57%) 187 (53.42%), 98 (28%) and 35 (10%) isolates respectively. No resistance was detected to teicoplanin and linezolid.

DISCUSSION

Resistance to most antibiotics used in the treatment of staphylococcal infections is an increasing problem and treatment options have become more limited. The changing pattern in antibiotic susceptibility has led to renewed interest in the use of clindamycin. Good oral bioavailability, low cost, excellent tissue penetration and the fact that it accumulates in abscesses makes it a good option to treat staphylococcal infections. It is a useful drug in the treatment of both methicillin sensitive and resistant staphylococcal strains [13]. It is indicated in skin and soft tissue infections, paediatric staphylococcal infections and patients allergic to beta lactam antibiotics [14]. Therapeutic failures caused by $iMLS_B$ resistant strains are now being reported commonly [15,16]. Routine antimicrobial sensitivity testing can detect $cMLS_B$ phenotypes but $iMLS_B$ resistance is missed if erythromycin and clindamycin discs are placed at non adjacent sites.

Methicillin resistance was identified in 23.42% isolates of *S. aureus,* in concordance with other studies done in India [17,18]. High rate of methicillin resistance is noted among *S. aureus* isolates in developed nations [19]. There was statistically higher prevalence of MRSA in inpatients (76.83%) than in outpatients (23.17%) (p<0.0001). Significant difference of MRSA presence between inpatients and outpatients is reported in literature [20]. A high occurrence of MRSA in inpatient settings can be explained by the fact that organisms develop resistance in closed environment of hospitals and health care facilities due to selection pressure and their convenience in spreading between patients via the health care workers and instruments. Differences in the prevalence rate of MRSA among countries globally and different regions with in a country emphasize the importance of generating a local resistance data to guide empirical therapy.

Among 350 isolate studied, 39.14% were resistant to erythromycin which is comparable to an Indian study done by Deotale et al., [21]. In our study, $cMLS_B$ phenotype predominated (29.62%MRSA; 13.38% MSSA) followed by $iMLS_B$ (28.39% MRSA; 9.29% MSSA) and MS phenotypes (13.58% MRSA; 6.69%MSSA) which is in concordance with a study done in Greece [22].

Incidence of MLS_B phenotypes varies significantly by geographical regions. There are studies which reveal higher constitutive resistance in comparison to inducible resistance in *S. aureus* isolates. Fiebelkorn et al in their study found that out of 114 erythromycin resistant *S.aureus* isolates 39 (34.12%)were $cMLS_B$ while 33 (28.94%) were $iMLS_B$ [2]. In our study we found that 30.85% *S. aureus* isolates were clindamycin resistant; 13.71% were inducible and 17.14% constitutive resistant phenotypes. Angel et al., reported 23.24% inducible clindamycin resistance with no constitutive resistance, and Devdas et al., reported ICR in 6% and $cMLS_B$ resistance in 8% of *S. aureus* isolates, where as Deotale et al., found 3.6% constitutive and 14.5% inducible clindamycin resistance [21,23,24]. Therefore, studies depict a wide variation in incidence of clindamycin resistance among clinical isolates of *S.aureus* in different geographic areas.-

Relationship of MRSA and MSSA with different resistant phenotypes has been studied by different authors. In Europe, there is a high incidence (93%) of constitutive resistance in MRSA where as the ICR predominates in MSSA strains [25]. Azap et al., reported a high percentage of inducible resistance in MRSA (5-7%) than in MSSA (3.7%) [26]. From India, Debdas et al., reported that constitutive resistance was higher than inducible resistance in both MRSA and MSSA. In MRSA $cMLS_B$ was 23% and $iMLS_B$ was 18%; whereas in MSSA $cMLS_B$ was 3% and $iMLS_B$ two percent [24]. Likewise in our study, constitutive resistance was significantly higher than ICR both in MRSA and MSSA. A study from south India reported $cMLS_B$ in 32% and $iMLS_B$ in 14.2% *staphylococcus* isolates similar to the present study whereas contrary to our study all $iMLS_B$ phenotypes were MRSA [27]. Prevalence of clindamycin resistance among clinical isolates of *S.aureus* in various Indian studies is shown in [Table/Fig-3]. The difference in various resistant phenotypes in literature can be due to the difference in bacterial susceptibility in different geographical regions and also due to varying antimicrobial prescribing patterns of clinicians.

There was a significant difference in detection of ICR in inpatient (19.79%) and outpatient isolates (6.34%) and in MRSA (28.39%) and MSSA (9.29%) isolates. This finding may have implications that outpatients with MRSA infections can reasonably be offered clindamycin treatment option due to the lower likely hood of these strains exhibiting $iMLS_B$ resistance. Two hundred seven (77.23%)

specimen	MRSA(n=82)		MSSA(n=268)		Total N (%)
	OPD	IPD	OPD	IPD	
Blood	3	24	22	51	100(28%)
Urine	5	6	27	4	42(12%)
Pus	11	27	89	70	197(56%)
Corneal scrapings	0	1	1	2	4(1%)
CSF	0	1	0	1	2(0.5%)
Synovial fluid	0	2	0	1	3(0.8%)
Tracheal aspirates	0	2	0	0	2(0.5%)
Total	19	63	139	129	350(100%)

[Table/Fig-1]: Distribution of MRSA and MSSA strains according to their origin. MRSA- methicillin resistant *Staphylococcus aureus*, MSSA-methicillin susceptible *Staphylococcus aureus*, OPD-outdoor department, IPD-indoor department, CSF-cerebrospinal fluid

Findings of the disc diffusion test	Erythromycin sensitive Clindamycin sensitive	Erythromycin resistant Clindamycin sensitive (D zone negative)	Erythromycin resistant Clindamycin sensitive (D zone positive)	Erythromycin resistant Clindamycin resistant
	No resistance n (%)	MS n (%)	$iMLS_B$ n (%)	$cMLS_B$ n (%)
Staphylococcus aureus (350)	213(60.85)	29(8.28)	48(13.71)	60(17.14)
MRSA (82)	24(29.26)	11(13.41)	23(28.04)	24(29.26)
MSSA (268)	189(70.52)	18(6.71)	25(9.32)	36(13.43)

[Table/Fig-2]: Findings of the disc diffusion test
MS_macrolide streptogramin B $iMLS_B$ inducible macrolide lincosamide streptogramin B phenotype, $cMLS_B$- constitutive macrolide lincosamide streptogramin B phenotype, MRSA- methicillin resistant *Staphylococcus aureus*, MSSA- methicillin susceptible *Staphylococcus aureus*

Author's name	MRSA			MSSA		
	$iMLS_B$ phenotype (%)	$cMLS_B$ phenotype (%)	MS Phenotype (%)	$iMLS_B$ phenotype (%)	$cMLS_B$ phenotype (%)	MS Phenotype (%)
Gadepalli et al., [4]	30	38	12	10	15	12
Gupta et al., [17]	20	46	16	17.3	10	37.3
Deotale et al., [21]	27.6	7.3	24.3	1.6	0	4
Pal et al., [28]	43.6	38.8	18.7	6.9	7.3	10.9
Debdas et al., [24]	18	23	48	2	3	6
Mittal et al., [29]	44.8	8.6	13.3	8.4	4.5	16.1
Lall et al., [30]	37.1	16.6	22.8	6	4.8	13.5

[Table/Fig-3]: Various studies from India showing prevalence of clindamycin resistance in *Staphylococcus aureus* isolates
MRSA-methicillin resistant *Staphylococcus* aureus, MSSA- methicillin sensitive *Staphylococcus* aureus

MSSA strains were found susceptible to clindamycin as opposed to 46.68% MRSA, suggesting a possible role of treatment with clindamycin in such strains. The occurrence of clindamycin resistance varies with geographic area, even with the same city, and methicillin susceptibility [11]. Hence, it should be determined at individual settings. We demonstrate ICR in a high percentage of MRSA and MSSA isolates, more so in inpatient setting, at our institute. This is alarming and raises concern that clindamycin treatment failure may occur without prior testing for inducible resistant phenotypes. Without the D test we would have wrongly reported 48 (13.71%) *S. aureus* isolates out of total 350 as clindamycin sensitive. Studies like ours should be necessary to generate local sensitivity data which help in guiding empiric therapy and formulate institutional antibiotic policy.

CONCLUSION

Treatment of staphylococcal infections has always been a challenge for the treating physician, particularly in the backdrop of changing resistance pattern. Keeping the mode of action, side effects and pharmacokinetics in mind of certain drugs like vancomycin and linezolid, clindamycin should be considered for the treatment of severe and resistant staphylococcal infections. Different studies done across the globe show that prevalence of inducible clindamycin resistance varies from place to place. Therefore, we recommend that whenever clindamycin is intended for treatment of staphylococcal infection the clinical microbiology laboratory should test the isolated organism for $iMLS_B$ by D test, before clindamycin susceptibility is reported. Present study giving a magnitude of clindamycin resistance among clinical isolates of *S. aureus* from this region of the country will help clinicians choose an appropriate therapy.

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