

Original Research Article

Prevalence of constitutive and inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care institute in North India

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ABSTRACT

Background: Clindamycin is an important drug used in the treatment of Methicillin Sensitive *Staphylococcus aureus* (MSSA) as well as in Methicillin-resistant *Staphylococcus aureus* (MRSA) infections. This drug is widely used in the treatment of skin and soft tissue infections caused by them. Therapeutic failure caused by macrolide-lincosamine-streptogramin B constitutive and inducible clindamycin resistance (MLS_{BC} and MLS_{Bi}) is being more commonly reported.

Methods: The present study was conducted over a period of six months from October 2016 to March 2017 to know the incidence of MLS_{BC} and MLS_{Bi} in *Staphylococcus aureus* (*S. aureus*) isolates obtained in our hospital by D-test as per CLSI guidelines. A total of 130 isolates of *S. aureus* were obtained from different clinical specimens which included pus/ wound swab (n=266), urine (n=577), sputum (n=225), blood (n=221), throat swab (n=71), ear/eye discharge (n=21), high vaginal swab (n=20) and body fluids (n=50). All the isolates were subjected to antibiotic sensitivity testing by Kirby Bauer's disc diffusion method. Amoxycylav, Erythromycin, Clindamycin, Co-trimoxazole, Tetracycline, Ofloxacin, Gentamicin, Linezolid and Vancomycin were the antibiotics used.

Results: Out of 130 (8.9%) isolates of *S. aureus* obtained from 1451 clinical samples, 82 (63.1%) were found to be MSSA and 48 (36.9%) were MRSA. Among *S. aureus*, 43 (33.1%) isolates showed MLS_{BC} resistance, 22 (16.9%) isolates showed MLS_{Bi} resistance and 20 (15.4%) isolates showed MS phenotype. The remaining 45 (34.6%) isolates remained sensitive to Erythromycin. Among MSSA, MLS_{BC} were observed in 18 (22%) isolates and MLS_{Bi} in 9 (11%) while in MRSA, MLS_{BC} were observed in 25 (52.1%) isolates and MLS_{Bi} in 13 (27.1%) isolates. Almost all clinical isolates showed 100% sensitivity to Vancomycin and Linezolid in routine antibiotic susceptibility testing. Both MLS_{BC} and MLS_{Bi} resistance was significantly higher (p<0.05) in MRSA than in MSSA.

Conclusions: The study emphasizes the importance of conducting D test along with routine antibiotic susceptibility testing for better utilization of clindamycin in *S. aureus* infections.

Keywords: Clindamycin, Constitutive, Inducible, MRSA, MSSA, Phenotype

INTRODUCTION

Staphylococci is a member of the Micrococcaceae family commonly found on human skins and anterior nares and are capable of causing severe infections in humans. It is

known to cause skin, soft tissue, respiratory and urinary tract infections. *S. aureus* remains one of the versatile and dangerous pathogen in humans and both community and hospital acquired staphylococcal infections have increased steadily. Genes governing resistance to

antibiotics and producing virulence factors are present on both chromosome and extrachromosomal elements.¹ Resistant to MRSA is due to an additional penicillin-resistant peptidoglycan transpeptidase, PBP-2a encoded by *mec A* gene.² MRSA isolates possessing the gene encoding Panton-Valentine leucocidin are capable of causing severe infections and their numbers is increasing.³

Due to the increasing frequency of methicillin resistant infections and changing patterns in antimicrobial resistance there is renewed interest in use of macrolide - lincosamide -streptogramin (MLS_B) family of antibiotics such as erythromycin, clindamycin and dalbapristin/quinupristin.⁴ Clindamycin is the most preferred agent because of its good oral absorption, excellent tissue penetration and no need of renal dose adjustment. It suppresses production of Panton -Valentine leucocidin and other virulence factors in MRSA.³

Macrolide antibiotic resistance in *S. aureus* and CONS occur due to active efflux mechanism coded by *msr A* gene or due to *erm* genes. The gene *msrA* confers resistance to macrolide and streptogramins type B only while *erm* genes encode enzymes which are capable of conferring inducible (MLS_{Bi}) or constitutive (MLS_{Bc}) resistance to all the three group of drugs via methylation of the 23S rRNA.⁵ The enzyme encoded by *erm* gene called as 23S rRNA methylase renders affected ribosomes incapable of binding the MLS antibiotics and low levels of erythromycin act as the most effective inducer. Staphylococcal phenotypes observed in one study found an apparent inverse correlation between the resistance observed and the use of erythromycin in each hospital. Greatest erythromycin use yielded the lowest incidence of MLS_{Bc} and vice-versa.⁶

Clindamycin was developed in 1966 by chemically modifying the naturally occurring lincomycin. It acts by inhibiting bacterial protein synthesis at the level of the 50S ribosome. It is capable of decreasing toxin production and increase microbial opsonization and phagocytosis at subinhibitory concentrations. It is well absorbed from gastrointestinal tract and achieves good concentration inside neutrophils, bones and joints. It is used in treatment of skin and soft tissue infections, abscesses, decubitus ulcers, osteomyelitis, head and neck, pleuropulmonary, abdominal and pelvic infections besides being an alternative in penicillin allergic patients.⁷

Strains with constitutive and inducible resistance to clindamycin have to be identified in the laboratory to avoid unnecessary use of clindamycin which may appear sensitive in vitro by the disk diffusion method. There are no studies about the prevalence of constitutive and inducible clindamycin resistance in this region. In this background, we conducted the study to estimate the prevalence of clindamycin resistance.

METHODS

The present observational study was conducted over a period of six months from October 2016 to March 2017. A total of 1451 clinical specimens such as pus/wound swab (n=266), urine (n=577), sputum (n=225), blood (n=221), throat swab (n=71), ear/eye discharge (n=21), high vaginal swab (n=20) and body fluids (n=50) were processed and *S. aureus* was isolated in 130 samples. Isolates were identified as *S. aureus* and methicillin resistant by standard conventional methods.⁸

Antibiotic sensitivity testing was done by Kirby Bauer's disc diffusion method on Mueller- Hinton agar plates using Amoxycylav (20/10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Co-trimoxazole (1.25/23.75 µg), Tetracycline (30µg), Ofloxacin (5 µg), Gentamicin (10 µg), Linezolid (30 µg) and Vancomycin (30 µg) (Himedia Lab, Mumbai). *S. aureus* ATCC 25923 was used for the purpose of quality control.

Phenotypic detection of inducible resistance to Clindamycin by D-test.

Clindamycin and Erythromycin disks were placed on Mueller Hinton agar plate separated by a distance of 15 mm between the edges. Plates were incubated at 37° C for 24 hours. Inducible resistance to Clindamycin was defined as blunting of the clear circular area of no growth around the Clindamycin disc on the side adjacent to the Erythromycin disk and was designated as D test positive. Absence of a blunted zone of inhibition was designated D-test negative.⁸

Three different phenotypes were interpreted as follows

- Constitutive MLS_{Bc} phenotype: Those 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.
- Inducible MLS_{Bi} phenotype: Those isolates showing resistance to Erythromycin (zone size <13 mm) and sensitive to Clindamycin (zone size >21 mm) giving D- shaped zone of inhibition around Clindamycin disc were labelled as MLS_{Bi} phenotype.
- MS phenotype: Those isolates showing circular zone of inhibition around clindamycin (zone size >21 mm) and resistance to Erythromycin (zone size <13 mm) was labelled as MS phenotype.

Statistical analysis

Data were entered and analyzed using SPSS (Statistical Package for Social Science) program version 24 and statistical significance was considered when p value was less than 0.05.

RESULTS

A total of 130 (8.9%) *S. aureus* were isolated from 1451 clinical specimens which included pus/wound swab (n=266), urine (n =577), sputum (n =225), blood (n

=221), throat swab (n =71), ear/eye discharge (n =21), high vaginal swab (n=20) and body fluids (n=50). Out of this 130 *S. aureus* strains isolated, 82 (63.1%) were MSSA and 48 (36.9%) were MRSA (Table 1).

Table 1: Phenotypic pattern of clindamycin resistance observed in MSSA and MRSA.

Isolates (n)	MLS _{BC} Phenotype n (%)	MLS _{Bi} Phenotype n (%)	MS Phenotype n (%)	Both Erythromycin and Clindamycin sensitive n (%)
<i>S. aureus</i> (n=130)	43 (33.1%)	22 (16.9%)	20 (15.4%)	45 (34.6%)
MSSA (n= 82)	18 (22%)	09 (11%)	12 (14.6%)	43 (52.4%)
MRSA (n= 48)	25 (52.1%)	13 (27.1%)	08 (16.7%)	02 (4.1%)

Table 2: Studies done in different places in India showing prevalence of constitutive and inducible clindamycin resistance.

Author’s name (Place of study in India)	MSSA			MRSA		
	MLS _{BC} (%)	MLS _{Bi} (%)	MS Pheno type (%)	MLS _{BC} (%)	MLS _{Bi} (%)	MS Pheno type (%)
Mokta et al (Shimla)	13.4	9.3	6.7	29.2	28.1	13.4
Das et al (Dibrugarh)	16	8.9	21.4	36.3	31.8	13.6
Mittal et al (Lucknow)	4.5	8.4	16.1	8.6	44.8	13.3
Appalaraju et al (Coimbatore)	2.3	3.4	15.8	33.7	42.1	18.9
Nikam et al (Amaravati)	15	3	14	42.8	29.8	16.8
Supriyarajvi et al (Bikaner)	5	15.8	15.32	17.3	30.6	20
Present study (Pilkhuwa)	22	11	14.6	52.1	27.1	16.7

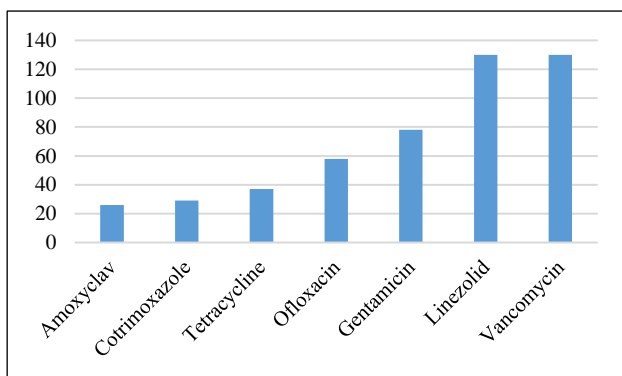


Figure 1: Antibiotic sensitivity pattern of *Staphylococcus aureus* (n=130) isolated from clinical specimens.

S. aureus was predominantly isolated from pus/wound sample (n =58) followed by sputum (n =19), urine (n =19), throat swab (n=11), high vaginal swab (n=9), eye/ear discharge (n=6), blood (n =5) and body fluids (n=3).

All *S. aureus* obtained from our study were found to be sensitive to vancomycin (100%) and linezolid (100%) followed by gentamicin (60%), ofloxacin (44.6%), tetracycline (28.5%), co-trimoxazole (22.3%) and amoxyclav (20%) (Figure 1).

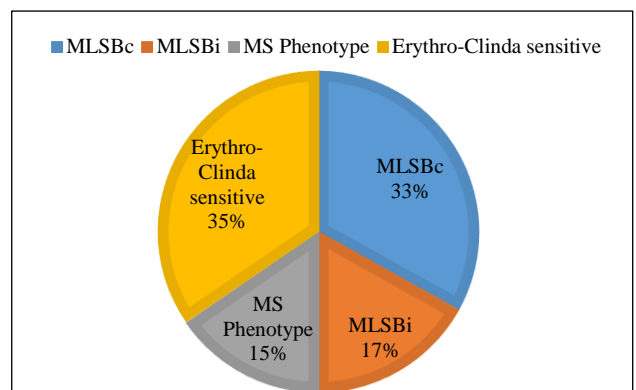


Figure 2: MLS_B resistance phenotype of *Staphylococcus aureus*.

In the present study, among *S. aureus* a total of 43 (33.1%) isolates were found to be positive for MLS_{BC} phenotype, 22 (16.9%) MLS_{Bi} and 20 (15.4%) were of MS phenotype (Figure 2). We found MLS_{BC} phenotype in 18 (22%) of MSSA and 25 (52.1%) of MRSA. MLS_{Bi} phenotype in 9 (11%) of MSSA and 13 (27.1%) of MRSA (Figure 3 and 4). The prevalence of MS phenotype was found in 12 (14.6%) of MSSA and 8 (16.7%) of MRSA (Table 1). Overall 45 (34.6%) isolates of *S. aureus* showed susceptibility to Erythromycin.

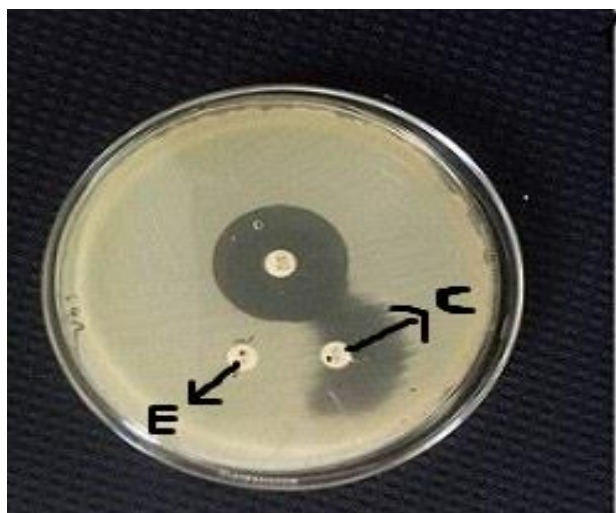


Figure 3: D test demonstrating a blunting of zone of inhibition around the clindamycin disc at 15mm distance from erythromycin disc that forms a D-shape (Inducible MLSB phenotype) with ceftiofur disc above (MSSA).



Figure 4: D test demonstrating resistance to both clindamycin and erythromycin (constitutive MLSB phenotype).

Both constitutive MLS_{BC} and inducible MLS_{Bi} Clindamycin resistance was significantly higher ($p < 0.05$) in MRSA than in MSSA in our study. MS phenotype in MRSA and MSSA was not statistically significant (Table 2).

DISCUSSION

Clindamycin is frequently used in treatment of skin and soft tissue infections because of its proven efficacy, safety and convenience of parenteral and oral administration in patients. But therapeutic failure caused by MLS_{Bi} strains is of great concern because these isolates appear as sensitive in routine antibiotic susceptibility testing. Hence there is a need to do D zone

testing which helps in identifying the cryptic resistant isolates.

Out of 130 isolates of *S. aureus*, we found that 17% were MLS_{Bi} strains and 33% were MLS_{BC} strains. Several studies have reported high levels of MLS_{BC} and MLS_{Bi} strains of *S. aureus*. Das et al found 21.8% isolates showed constitutive and 15.4% isolates showed inducible clindamycin resistance.⁹ Mokta et al found 13.7% MLS_{Bi} and 17.1% MLS_{BC} strains while in another study by Mittal et al reported 23.2% MLS_{Bi} strains and MLS_{BC} strains constituted only 6.1% among *S. aureus* isolates. In contrast to many other studies they found MLS_{BC} in MRSA very much lower.^{10,11} Study done in Tehran has found 7.5% MLS_{Bi} strains and 38.9% of MLS_{BC} strains of *S. aureus* but was lower than in coagulase negative staphylococci which showed 10.1% and 59.2% respectively.¹² Ghosh et al reported 23.9% of the tested isolates in their hospital were MRSA and 41.3% of *S. aureus* isolated belonged to MLS_{Bi} strains.¹³

In our study we found statistically significant MLS_{BC} and MLS_{Bi} strains in MRSA than in MSSA. Similar observations have been made by Appalaraju et al and Nikam et al. MLS_{BC} was detected in 33.7% and 42.8% while MLS_{Bi} in 42.1% and 29.8% isolates of MRSA respectively in their studies.^{14,15} Several studies done across the country have reported that constitutive and inducible MLS_B strains are seen more in MRSA than in MSSA strains.¹⁶⁻²¹ Since MLS_{Bi} strains cannot be detected by automated susceptibility testing or E-test, performing a simple, inexpensive, easy to perform and reproducible test such as D-test can be included as a part of routine antibiotic susceptibility testing.²²

Pus/wound sample accounted for the majority (n=58) from which *S. aureus* has been isolated and a high number of them showed MLS_{BC} (n=17) and MLS_{Bi} (n=15) clindamycin resistance. MS phenotype in our study was found to be 15.4% among *S. aureus* isolates. MS Phenotype was observed in 16.7% of MRSA and 14.6% in MSSA. All the isolates in our study showed susceptibility to vancomycin and linezolid which has also been reported in several other studies. In similarity to our study, resistance to other antibiotics has ranged from 18.8% to 80.1% and all isolates of *S. aureus* have been found sensitive to vancomycin and linezolid.^{4,13} Sensitivity of *S. aureus* including MRSA showed 90.2% sensitive to tetracycline and 48.4% to co-trimoxazole which in contrast our study showed only 28.5% and 22.3% respectively.²¹

Several reports of reduced susceptibility to glycopeptides have been reported. Emergence of vancomycin-intermediate *S. aureus* and more recently vancomycin-resistant *S. aureus* is an additional concern.²³⁻²⁵ Debnath et al has reported 7.22% MRSA strains resistance to linezolid.²⁶ Suggestions to use clindamycin, vancomycin and linezolid for MRSA as reserve drugs need to be emphasized in hospitals.²⁷

CONCLUSION

Though Clindamycin is an excellent drug in treatment of several infections, it still remains the priority drug in treatment of skin and soft tissue infections. The high prevalence of MLS_{BC} and MLS_{Bi} strains among clinical specimens in particularly pus/wound swab is a thing of concern. Studies done earlier have shown that for staphylococci, MLS_{Bi} phenotypes determined by disc diffusion methods correlated well with genotypes determined by hybridization techniques. Methicillin resistance in *S. aureus* is also in the rise in different regions across our country. In this background D-test done routinely with antibiotic susceptibility testing will help in guiding physicians properly and prevents therapeutic failure.

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