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Inducible Clindamycin Resistance Among Gram Positive Cocci in a Tertiary Care Centre

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Resistance in gram positive bacteria not only increases morbidity and mortality, but also the costs of management of hospitalized patients. The determination of antimicrobial susceptibility of a clinical isolates is often crucial for the optimal antimicrobial therapy of the infected patients. There have been reports indicating increase in the ratio of Staphylococci resistance to Macrolide-Lincosamide-Streptogramin B (MLS_B) group and failure in the treatment with clindamycin infection with microorganisms with inducible resistance to MLS_B group. The present study was undertaken considering the paucity data on inducible MLS_B from a tertiary care centre in Haldwani, (Nainital). A total of 182 gram positive cocci obtained from consecutive clinical specimens were included, consisting of 19.7% methicillin resistant *Staphylococcus aureus* (MRSA), 41.7% methicillin sensitive *S. aureus* (MSSA), 8.7% methicillin-resistant coagulase-negative staphylococci (MRCNS), 17.5% methicillin-sensitive coagulase-negative staphylococci (MSCNS) isolates and 12% *Streptococcus* spp. Of the 182 isolates, 61 (33.5%) had ER-R (erythromycin resistant) and CL-S (clindamycin sensitive) phenotype. Among 61 ER-R and CL-S isolates, 30 (49%) were recorded as inducible clindamycin resistance. Since the rate of inducible clindamycin is high (49% in our study), accurate reporting of inducible MLS_B would allow the clinician to retain confident in clindamycin.

Key words: MLS_Bi phenotype, Erythromycin, Clindamycin.

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[1]. Good oral absorption makes it an important option in outpatient therapy. It is a good alternative for the treatment of both methicillin-resistant and susceptible *Staphylococcal* infections[2]. However, important issue with clindamycin use is a risk of clinical failure during therapy due to inducible clindamycin resistance. The clindamycin resistance mechanism is primarily due to ribosomal modification by methylases encoded by *erm* genes. Methylation of 23S rRNA decreases the affinity for clindamycin, all macrolides and type B streptogramins[3]. Some of these enzymes are constitutively regulated, while others are inducibly regulated by translational attenuation of mRNA leader sequence in presence of erythromycin[4]. Constitutive resistance can be readily detected but inducible resistance is not detected by routine antimicrobial susceptibility test[1]. Inducible MLS_B can be detected by simple test known as D test[5]. The incidence of MLS_{Bi} is highly variable with

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regard to geographic locality[6]. Hence, local data regarding inducible clindamycin resistance is helpful in guiding clinician.

MATERIAL AND METHODS

A total of 182 consecutive gram positive isolates from clinical samples were identified by standard procedure[7] as 112 *S. aureus,* 48 coagulase-negative staphylococci (CNS) and 22 *Streptococcus* spp. Methicillin resistance was detected using 1µg oxacillin disc on Mueller Hinton agar supplemented with 2% NaCL followed by incubation at 35°C[5]. A total of 61 clinical isolates were selected based on erythromycin resistance (ER-R) and clindamycin sensitivity (CL-S). Using CLSI recommendation, these isolates were tested for inducible clindamycin resistance using D test keeping 15 mm inter-disc distance[5]. Briefly, erythromycin (15µg) disc was placed at distance of 15mm (edge to edge) from clindamycin dics (2 µg) on Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions. Following overnight incubation at 37°C, flattening of zone around clindamycin in area between the two discs indicated inducible clindamycin resistance. In our study four different phenotypes were appreciated after testing as follows:

MS phenotype

Isolates resistance to erythromycin but sensitive to clindamycin,

MLS_{Bi} phenotype

Isolates resistance to erythromycin while being sensitive to clindamycin and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.

MLSB constitutive phenotype

Isolates resistant to both clindamycin and erythromycin.

MLS_{Bi} and Constitutive phenotype

Isolates resistance to erythromycin and ingrowth within a larger clindamycin zone along with blunting of the outer zone towards erythromycin.

RESULTS

A total of 182 gram positive isolates consisting of 19.7 % MRSA, 41.7 % MSSA, and 8.7% MRCNS, 17.5% MSCNS and 12% *Streptococcus* spp. were included. Susceptibility pattern of E and CL in gram positive cocci is shown in Table 1. Over all constitutive phenotype predominated over inducible phenotype (21.9% VS 16.5%). MSSA & MSCNS inducible phenotype were 18.4% and 18.7% respectively. Where as, MRSA & MRCNS inducible phenotypes were 16.4% and 12.5% respectively (Table 1). Among 61 ER-R and CL-S isolates, 30 (49%) were recorded as inducible clindamycin resistance. Out of 22 *Streptococcus* spp, 4 isolates of *Enterococcus* spp. showed ingrowth within a larger clindamycin zone along with blunting of the outer zone. Disk test was repeated for isolates showing ingrowth by selecting colonies in both confluent and light growth area to rule out mixed culture; repeat testing from single colonies revealed the same results.

Table 1. Susceptibility of E and CL in gram positive cocci

Organism	Total no. of isolates	MLS_Bi (D+ve) E-R, CL-S	MLS_Bc E-R, CL-R	E- R, CL- S (D-ve)	E-S, CL-R	E-S, CL-S
MRSA	36	06(16.6%)	08(22.2%)	06(16.6%)	02(5.5%)	14(38.8%)
MSSA	76	14(18.4%)	10(13.6%)	12(15.7%)	02(2.9%)	38(50%)
MRCoNS	16	02(12.5%)	08(50%)	0	02(12.5%)	04(2.5%)
MSCoNS	32	06(18.7%)	04(12.5%)	12(37.5%)	06(18.7%)	04(12.5%)
Enterococcus spp	10	0	06(60) +4 (40) *		0	0 0
Streptococcus spp	12	02(16.6)	0	01(8.33)	02(16.6)	07(58.33)
Total	182	30 (16.5)	40 (21.9%)	31(17.03%)	14(7.6%)	67(36.8%)

E- Erythromycin, CL – Clindamycin, R – Resistant, S – Sensitive, MLS_Bi- inducible macrolide, lincosamide and streptogramin type –B, MLS_Bc- constitutive macrolide, lincosamide and streptogramin type –B, *4 isolates with D zone effect

Fig. 1.

DISCUSSION

The prevalence of MLS_Bi varies from 7% to 94% [8]. Thus, the true incidence of clindamycin resistance depends on the patient population studied, as well as hospital characteristic and geographic factor. A study from CMC Vellore, India[2] has reported 64% MRSA positive for inducible clindamycin whereas only 5% MSSA showed the positive result. Another study from Delhi[1] has reported 30% MLS_Bi in MRSA strains and 10% in MSSA strains. In a study from Karnataka, India 38.4% MRSA and 12.9% of total MSSA were of the inducible MLS_B phenotype [6]

In the present study we observed 30 (16.5% of total isolates) isolates were inducible clindamycin resistant which is close with other studies[1,2] Among MSSA & MSCoNS inducible phenotype were 18.4% and 18.7% respectively. Where as, MRSA & MRCoNS inducible phenotypes were 16.4% and 12.5% respectively. Levin *et al* [9] also found more inducible MLS_Bi in MSSA as compare to MRSA. Study from Korea[4] has reported 32% of MRSA, 90% of MRCNS and 94% of MSCNS were inducible MLS_B. Interestingly, in present study 4 *enterococcus* spp isolates showed ingrowth within a larger clindamycin zone along with blunting of the outer zone. Enterococi are inheritantly resistance to clindamycin. The explanation for blunting of the clindamycin zone of inhibition by an erythromycin disk is (D zone effect) in 4 enterocci is unclear.

Failure to identify MLS_Bi may lead to clinical failure of clindamycin therapy. Conversely, labeling all erythromycin resistant *Staphylococcus* as clindamycin resistance preclude the use of clindamycin in infections caused by truly clindamycin-susceptible *Staphylococcal* isolates. Laboratories and also clinicians must be aware of the local prevalence of iMLSB isolates. In present study of the 61 clinical isolates, selected based on erythromycin resistance and clindamycin sensitivity, 30 (49%) were MLS_Bi. The high level of inducible clindmycin resistance in our institute raises concern that clindamycin treatment failure may occur. Since D- test is easy to interpret, reproducible and inexpensive it can be included as routine testing. The reporting of inducible MLS_B would allow the clinician to retain confident in clindamycin.

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