ORIGINAL ARTICLE

Inducible Clindamycin Resistance in Gram Positive Isolates Obtained from Clinical Samples in a Tertiary Care Hospital in Mumbai

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Abstract:

Background: Infections by Gram positive isolates are increasing due to which their antibiotic sensitivity pattern is changing. This has revived interest in Macrolide-Lincosamide Streptogramin Group B (MLSB) antibiotics. Misuse of MLSB antibiotics has increased resistance in Gram-positive organisms especially *Staphylococcus species* to these drugs. Clindamycin is an important drug for treatment of Gram-positive isolates. Hence detection of inducible clindamycin resistance in these clinical isolates is required to prevent therapeutic failure and avoid inadvertent use of this drug. *Aim and Objectives*: To detect inducible clindamycin resistance among Gram positive isolates obtained from clinical samples. *Material and Methods*: The study was carried out over a period of one year (Jan-Dec 2018). A total of 461 Gram positive isolates of *Staphylococcus species, Streptococcus pneumoniae* and *Beta-haemolytic Streptococcus* were identified from various clinical samples and antibiotic susceptibility done on Vitek2 Compact using GP ID, and 628 and ST01 cards respectively. According to CLSI 2017, D-zone test was performed for detection of inducible clindamycin resistance for strains resistant to erythromycin. *Results*: *Staphylococcus aureus* (SA) isolates were 59%, *Staphylococcus epidermidis* (SE) 21%, other Coagulase Negative *Staphylococcus* (CONS) 16%, *Streptococcus pyogenes* (Group A-beta haemolytic) 2%, *Streptococcus agalactiae* (Group B beta-haemolytic) 1% and *Streptococcus pneumoniae (*alpha *haemolytic)* 1%. Isolates of Methicillin Sensitive *Staphylococcus aureus* (MSSA) were 58% and Methicillin Resistant *Staphylococcus aureus* (MRSA) were 42%. Frequencies of MS (clindamycin sensitive) phenotypes, inducible clindamycin resistance (MLSBi) phenotypes and phenotypes showing constitutive resistance (MLSBc) were 44%, 12% and 3% respectively among MSSA and 34%, 39% and 8% respectively among MRSA. Among SE, MS, MLSBc and MLSBi phenotypes were 39%, 24% and 12% respectively and 8%, 44% and 30% respectively among other CONS. One isolate of *S. pyogenes* was of MLSBi phenotype and none among *S. agalactiae* and *S. pneumoniae*. *Conclusion*: The study emphasizes the significance of conducting D-zone test along with routine antimicrobial susceptibility testing to guide in therapy and avoid treatment failures.

Keywords: D-zone test, Gram Positive Isolates, Inducible Clindamycin Resistance

Introduction:

Gram positive organisms are known to cause infections particularly of skin and soft tissue. *Staphylococcus aureus* (SA) and Coagulase Negative *Staphylococcus* (CONS) are known to cause community-acquired and nosocomial infections world-wide [1]. Emergence of methicillin resistance among *Staphylococci* is intensifying [1]. An alternative to this issue is the Macrolide-Lincosamide Streptogramin Group B (MLSB) group of antibiotics which includes clindamycin. Clindamycin is preferred due to various reasons. It has low cost, lesser side effects, good availability, good tissue penetration, no need for renal adjustment, and usefulness in penicillin

allergic patients, ability to inhibit toxin production directly and it is not hampered by bacterial burden [2]. But widespread use of MLSB group has led to increase in resistance to these drugs among *Staphylococcal* isolates [3]. Resistance to MLSB class of antibiotics can occur due to three different mechanisms. These are target site modification mediated by erythromycin ribosomal methylases enzyme encoded by ermA/ermC genes, enzymatic inactivation of antibiotic and impermeability of macrolide efflux pumps which is encoded by Macrolide Streptogramin Resistance A (MSRA) gene [3]. When methylase is always produced, the resistance is MLSB constitutive (MLSBc) whereas it is MLSB inducible (MLSBi) when produced only in the presence of an inducer like macrolides [3, 4]. Once induced, cross resistance to other members like lincosamides and streptogramin B is conferred [4]. MLSBi strains are not easily detectable in vitro susceptibility tests and appear to be erythromycin resistant and clindamycin sensitive in routine laboratory tests. Hence the role of D-zone test is significant which helps to identify MLSBi strains. In D-zone test, erythromycin and clindamycin discs are placed in close proximity to each other. Erythromycin diffuses through the agar and resistance to clindamycin is induced resulting in flattening of lincosamide zone of inhibition adjacent to erythromycin disc forming a "D" shape to the zone [3]. This helps to avoid reporting of clindamycin as falsely sensitive and thereby helps to avoid its use in cases where resistance is mediated by *erm* gene. D test was found to be 100% sensitive when compared to *erm* and *msr* gene detection by Polymerase Chain Reaction (PCR) [5]. Also, wide variation exists in the rate of inducible clindamycin resistance in various places [5]. Hence it is important to find its frequency of MLSBi in our Institute.

Material and Methods:

The present study is an observational study carried out in microbiology laboratory of our Institute during the period of January 2018 to December 2018. Various clinical samples like pus, wound swabs, urine, blood, body fluids, sputum, tissue, nasal swab, ear swab, throat swab and vaginal swab and catheter tip from both genders, all age groups and Out Patient Department (OPD), In Patient Department (IPD) and Intensive Care Unit (ICU) were processed for aerobic bacterial culture using standard operational procedures [6]. Isolates were identified and antibiotic susceptibility test done on Vitek2 Compact machine using GPID and 628 and ST01 cards respectively. *Staphylococcus aureus* ATCC 25923 was used for purpose of quality control. A total of 461 Gram positive isolates of *Staphylococcus species*, *S. pneumoniae* (alpha haemolytic *Streptococcus)*, *S. pyogenes* and *S. agalactiae* were isolated from these samples.

Phenotypic detection of inducible resistance to clindamycin by D-zone test:

Lawn culture of the isolate to be tested was made and clindamycin (2 mcg) and erythromycin (15 mcg) discs were placed on Mueller-Hinton Agar plate or Blood agar plate separated by a distance of 15 mm and incubated at 37^0C for 24 hours. Inducible clindamycin resistance was defined as blunting of zone around clindamycin disc on the side adjacent to erythromycin disc and was designated as D test positive. Absence of a blunted zone of inhibition was designated as D test negative [7].

Three different phenotypes based on D-zone test were interpreted as follows:

MLSBc phenotype:

Isolates showing resistance to both erythromycin (zone size<13 mm) and clindamycin (zone size <14 mm) discs were labelled as MLSBc phenotype.

MLSBi phenotype:

D test positive-Isolates showing resistance to erythromycin (zone size<13 mm) and sensitive to clindamycin (zone size>21mm) giving D shaped zone of inhibition around clindamycin disc on the side adjacent to erythromycin disc were labelled as MLSBi phenotype.

MS phenotype:

D test negative-Isolates showing resistance to erythromycin (zone size <13 mm) and circular zone of inhibition around clindamycin (zone size >21 mm) and was labelled as MS phenotype.

Only those isolates which were resistant to erythromycin (zone size <13 mm) were included in the study.

The study was approved by the Scientific and Ethical Committee of the Institute.

Data analysis:

Data were analysed using SPSS (Statistical Package for Social Science) program version 21 and statistical significance was considered when *p* value was less than 0.05.

Results:

A total of 461 Gram positive organisms were isolated of which SA were 272 (59%). One hundred and fifty eight (58%) were MSSA and 114 (42%) were MRSA. SE was 95 (21%).

Other CONS were 73 (16%) which included *Staphylococcus hominis* 24, *Staphylococcus haemolyticus* 14, *Staphylococcus warneri* 11, *Staphylococcus saprophyticus* 5, *Staphylococcus psedointermedius* 5, *Staphylococcus capitis* 5, *Staphylococcus lugdunensis* 4, *Staphylococcus lentus* 1, *Staphylococcus simulans* 1, *Staphylococcus caprae* 1, *Staphylococcus sieuri* 1 and *Staphylococcus cohnii* 1. *Streptococcus pyogenes* were 8 (2%), *Streptococcus agalactiae* 7 (1%) and *Streptococcus pneumoniae* were 6 (1%). Samples from which they were isolated were pus 135 (29%), wound swab 79 (17%), blood 75 (16%), nasal swab 48 (10%), urine 29 (6%), tissue 29 (6%), catheter tips 22 (5%), throat swab 19 (4%), sputum 7 (2%), body fluids 7 (2%), ear swab 6 (2%), and vaginal swab 5 (1%) (Fig.1). Isolates from male patients were 226 (49%) and female patients were 235 (51%). Sixty six (14%)

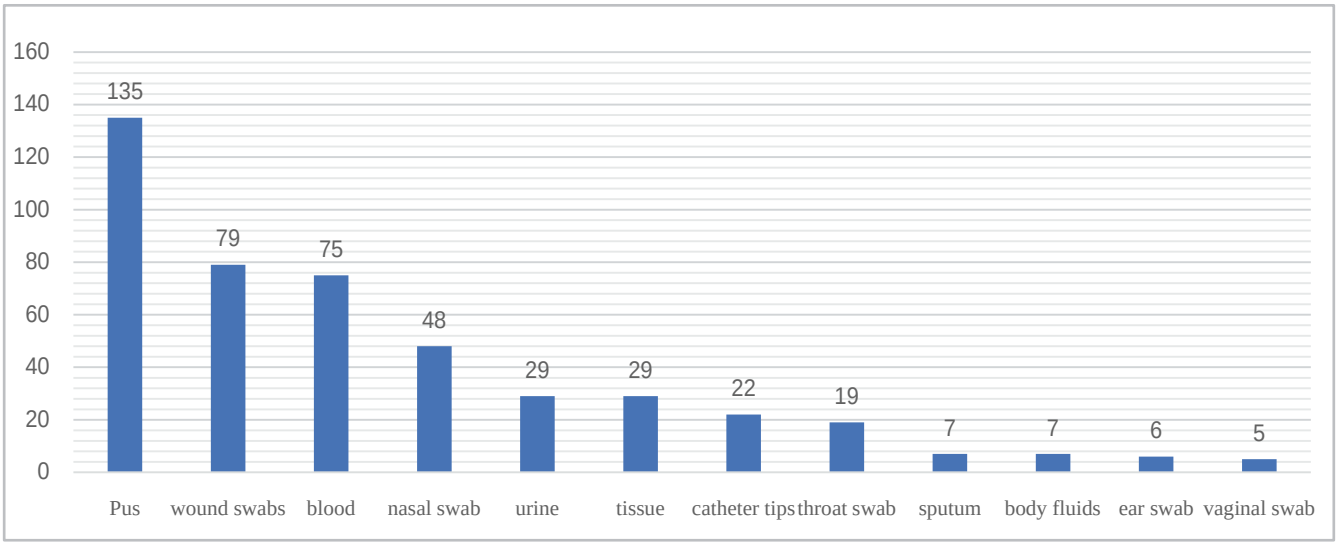

Fig. 1: Sample Distribution of Isolates

isolates were from age group of less than 18 years, 265 (58%) isolates from 19-65 years and 130 (28%) from age group above 65 years. Among MSSA, prevalence of MS, MLSBi and MLSBc phenotypes were 70 (44%), 19 (12%) and 5 (3%) respectively and 64 (41%) isolates were sensitive to erythromycin. Among MRSA, MS, MLSBi and MLSBc phenotypes were 38 (34%), 45 (39%) and 9 (8%) respectively and 22 (19%) isolates were sensitive to erythromycin. Among SE, 37 (39%), 11 (12%) and 23 (24%) isolates were MS, MLSBi, and MLSBc phenotypes respectively and 24 (25%) were sensitive to erythromycin. Among other CONS, MS, MLSBi and MLSBc were 6 (8%), 22 (30%) and 32 (44%) isolates respectively and 13 (18%) isolates were sensitive to erythromycin. Among *S. pyogenes*, 5 (63%) isolates were MS phenotypes, one (12%) MLSBi phenotype and none were MLSBc phenotype. Two isolates (25%) were sensitive to erythromycin. Among *S. agalactiae*, MS and MLSBc phenotypes were 2 (29%) and 1 (14%) respectively. None were MLSBi and 4 (57%) were sensitive to erythromycin. Among *S. pneumoniae*, 2 (33%) isolates were MS phenotype and 3 (50%) were MLSBc and none were MLSBi. One (17%) isolate was sensitive to erythromycin (Table 1). A comparison of various studies in India showing distribution of constitutive, MS and inducible phenotypes in SA isolates is shown in Table 2.

Table 1: Phenotypic Pattern of Clindamycin Resistance Based on D-Zone Test in Gram Positive Isolates

Isolate	Sensitive to erythromycin	MLSBc	MS	MLSBi	Total
Methicllin Sensitive Staphylococcus aureus **(MSSA)**	64 (41%)	5 (3%)	70 (44%)	19 (12%)	158
Methicillin Resistant Staphylococcus aureus **(MRSA)**	22 (19%)	9 (8%)	38 (34%)	45 (39%)	114
Staphylococcus epidermidis **(SE)**	24 (25%)	23 (24%)	37 (39%)	11 (12%)	95
CONS **(other than SE)**	13 (18%)	32 (44%)	06 (8%)	22 (30%)	73
Streptococcus pyogenes **(Group A Beta haemolytic)**	2 (25%)	0	05 (63%)	1 (12%)	08
Streptococcus agalactiae **(Group B Beta haemolytic)**	4 (57%)	1 (14%)	02 (29%)	0	07
Streptococcus pneumoniae **(Alpha haemolytic)**	1 (17%)	3 (50%)	02 (33%)	0	06

Table 2: Various Studies in India Showing Distribution of MLSBc, MS and MLSBi Phenotypes in SA Isolates

Name of authors, place and year of study	MSSA			MRSA		
	MLSBc	MS	MLSBi	MLSBc	MS	MLSBi
Current study (Mumbai 2018)	3%	44%	12%	8%	34%	39%
Shetty (Uttar Pradesh 2017)	22%	14.6%	11%	52%	16.7%	27.1%
Rajani (Lucknow 2017)	43.7%	25%	31.2%	62.5%	0	37.5%
Singh (Jalna 2016)	4.7%	8.7%	8.7%	64.8%	8%	25%
Satish (2015)	11.8%	37.1%	8%	23.68%	21.93%	22.8%
Chudasama (Ahmedabad 2014)	9.61%	16.34%	15.38%	15.07%	25.39%	32.53%
Saxena (New Delhi 2014)	33.9%	53.5%	12.6%	47.4%	23.7%	28.9%
Jadhav (Pune 2011)	0	1.99%	1.66%	8.2%	19.31%	24.82%
Ciraj (Karnataka 2009)	0	-	12.9%	15%	-	38.4%

Discussion:

Infections by Gram positive cocci are increasing and so is the emergence of multidrug resistance among these organisms. This has led to scarcity of options to treat them [8]. Clindamycin is commonly used to treat skin and soft tissue infections caused by Gram positive organisms. Frequency of inducible resistance varies by geographic location, methicillin susceptibility, bacterial species and institute wise and hence every institute must be aware about its own rates [9, 10, 5]. Strains which are positive for inducible resistance appear sensitive to clindamycin *in vitro* but therapy with clindamycin may lead to treatment failure due to selection of constitutive *erm* mutants [11]. In our study, 58% of total *Staphylococcal* isolates were MSSA and 42% were MRSA. This was similar to study by Shetty *et al*. and Singh *et al*. where MSSA were 63% and 62% respectively and MRSA were 37% and 38% respectively [12,13]. Also, our results showed that erythromycin resistance was higher in MRSA (81%) than MSSA (59%) similar to those of Shetty *et al* (MRSA 95.9% and MSSA 47.6%) [12] and Singh *et al* (MRSA 97.7% and MSSA 22.1%) [13]. MS (44%) and MLSBi (12%) phenotypes predominated among MSSA isolates in our study as compared to MLSBc (3%). This was in accordance with studies of Jadhav *et al* (MS 1.99%, MLSBi 1.66%, MLSBc 0) [14] and Vyoma *et al* (MS16.34%, MLSBi 15.38%, MLSBc 9.61%) [15]. Ciraj *et al*, Jeevan *et al* and Satish *et al* showed MLSBi 12.9%, 11% and 8.1% among MSSA isolates respectively, which is comparable to our results [8,12,16]. We found that among MRSA isolates, MLSBi (39%) phenotypes predominated followed by MS 34% and MLSBc 8%.This was in accordance with studies by Jadhav

et al. (MLSBi 24.82%, MS 19.31%, MLSBc 8.2%) [14] and Vyoma *et al* (MLSBi 32.53%, MS 25.39%, MSLBc 15.07%)[15]. Studies by Ciraj *et al*. and Rajani *et al*. found MLSBi among MRSA to be 38.4% and 37.5% [8,17] comparable to our findings. Subramanium *et al*. found that MLSBi in MRSA isolates of their institute were 31.82% which was in accordance with our finding [18]. However, in a study by Sasirekha *et al*, percentage of MLSBi was more in MSSA (8.49%) as compared to MRSA (0.65%) [19]. Among all CONS (including SE) in our study, MLSBc, MS and MLSBi were 33%, 26% and 20% respectively. This was similar to results of Saxena *et al.* where these were 38.7%, 35.5% and 25.8% % respectively [20]. In our study, among SE isolates, 12% were MLSBi phenotypes. A study by Rajani *et al* found 4 out of 48 isolates (8%) of SE to be MLSBi phenotypes [17] and was similar to our results. This was noteworthy as inducible resistance among isolates of SE is significant in our study. In our study, among *S. pyogenes*, only one isolate was MLSBi and there were none among *S. agalactiae* and *S. pneumoniae*. These results of *Streptococci* were in compliance with those of Angel *et al* in which one isolate among *Strep-A*, none in *Strep-B* and *S. pneumoniae* showed MLSBi phenotypes [21]. As inducible resistance is not detected by routine antimicrobial sensitivity testing method, D-zone test forms a vital part of routine antimicrobial sensitivity test [22]. Without this, clindamycin resistant isolates can be misclassified as clindamycin sensitive, causing treatment failure [23].

Conclusion:

The D-zone test is easy to perform and it guides in deciding the use of clindamycin and consequently helps to avert therapeutic failure. By using clindamycin rightly, vancomycin use can be avoided and it can be reserved for other difficult cases. Keeping in mind the highly variable prevalence of inducible clindamycin resistance, local data regarding the same is useful in treatment.

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