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Research Article

PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *Staphylococcus aureus* ISOLATED FROM VARIOUS CLINICAL SAMPLES AT TERTIARY CARE HOSPITAL, RAJKOT, WESTERN INDIA

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Abstract- Background: Multidrug resistant *Staphylococcus aureus* is a problem worldwide. This has led to renewed interest in usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat Staphylococcal infections. The resistance to macrolide can be mediated by msr A gene coding for efflux mechanism or via erm genes. *In vitro* tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance thus necessitating the need to detect such resistance by a simple D test on a routine basis. **Methodology:** 300 *S. aureus* isolates were subjected to routine antibiotic susceptibility testing including cefoxitin (30ug) by modified Kirby Bauer disc diffusion method. Erythromycin Inducible resistance to clindamycin in *S. aureus* was tested by "D test" as per CLSI guidelines. **Results:** Out of the 300 isolates; MS phenotype (MS Pheno) was seen in 10.3% (31) Erythromycin Inducible Clindamycin Resistance (iMLSb) is seen in 19% (58), constitutional (cMLSb) resistance was seen in 12% (36). Out of the total 58 Erythromycin Inducible Resistance Isolates, 63.79% (37) were associated with MRSA and 36.20% (21) were associated with MSSA. **Conclusion:** Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infections. This study showed that D test should be used mandatorily in routine disc diffusion test to detect inducible clindamycin resistance in *S. aureus* for optimum treatment of patients.

Keywords- Clindamycin resistance, Constitutive MLS_B, inducible MLS_B, MS MLS_B, MRSA

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Introduction

Staphylococcus aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections worldwide. The increasing prevalence of methicillin resistance among *S. aureus* is a major problem [1]. This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat *S.aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties [2,3]. However, widespread use of MLS_B antibiotics has led to an increase in the number of *S.aureus* strains acquiring resistance to MLS_B antibiotics [3,4].

Clindamycin resistance in *S.aureus* can be either constitutive or inducible [5]. The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively ($cMLS_B$) or inducibly ($iMLS_B$). Strains with inducible resistance to clindamycin are difficult to detect in the routine lab as they appear erythromycin resistant & clindamycin sensitive in vitro when not placed adjacent to each other. In case of another mechanism of resistance mediated through msr A genesi.e efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant & clindamycin sensitive both in vivo & in vitro & the strain don't become clindamycin resistant during therapy [3].

The present study was aimed to find out the percentage of *S.aureus* having inducible resistance in our geographic area using D-test. Also, we tried to ascertain the relationship between Methicillin Resistant *S.aureus* (MRSA) and inducible clindamycin resistance.

Materials & Methods

Total three hundred samples were taken from various clinical specimens like pus, aspirates, blood, urine etc. The isolated were first identified as *S.aureus* by standard biochemical techniques [6] and then subjected to antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using like penicillin G (10 units). Gentamicin (10ug), levofloxacin (5ug), ciprofloxacin (5ug), erythromycin (15ug), clindamycin (2ug), tetracycline (30ug), cotrimoxazole (1.25/23.75ug), linezolid (30ug), chloramphenicol (30ug) a per CLSI guidelines [7]. An inhibition zone of 19 mm or less around cefoxitin disc indicated MRSA.

Inducible resistance to clindamycin was tested by "D" test as per CLSI guidelines [7]. Briefly, erythromycin disc was placed at a distance of 15 mm (edge to edge) from clindamycin disc on a Mueller Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspension. Following an overnight incubation at 370C, Flattening of zone (D-shaped) around clindamycin in the area near the erythromycin disc, indicated clindamycin resistance.

Three different phenotypes were interpreted.

MS Phenotype: The isolate exhibiting resistance to erythromycin (zone size </=13mm) while sensitive to clindamycin (zone size >/=21mm) and giving circular zone of inhibition around clindamycin was having this phenotype.

Inducible MLS_B ($iMLS_B$) phenotype: The isolates showing resistance to erythromycin (zone size </=13mm) and giving a D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc was of this phenotype.

Constitutive MLS_B ($cMLS_B$) phenotype: This was named for those isolates, which showed resistance to both erythromycin (zone size </=13mm) and clindamycin (zone size </=14mm) with circular shape of the zone of inhibition if any, around clindamycin.

Quality control (QC) of the erythromycin & clindamycin discs was performed with *S. aureus* ATCC 25923, according to the standard disc diffusion QC procedure. Additional QC was performed with separate in-house selected *S. aureus* strains that demonstrated positive and negative D-test reactions.

Results

Three hundred *S. aureus* strains were tested for susceptibility to erythromycin & other antibiotics by routine disc diffusion testing; 125 (41.6%) of them were erythromycin resistant.

Out of the total Erythromycin 125 resistant isolates; MS phenotype (MS Pheno) was seen in 31 isolates (25%), Erythromycin Inducible Clindamycin Resistance (iMLSb) was seen in 58 isolates (46%), constitutional MacrolidLincosamide Streptogramin B (cMLSb) resistance was seen in 36 isolates (29%) [Table-1].

Out of the total 58 Erythromycin Inducible Resistance Isolates, 37 isolates (63.79%) were associated with MRSA and 21 isolates (36.20%) were associated with MSSA [Table-2].

Table-1 *Types of Erythromycin Resistant Staphylococcus aureus*

ERSA	No	%
iMLSb	58	46%
cMLSb	36	29%
MS Pheno	31	25%

Table-2 *Comparison of Erythromycin Inducible Clindamycin Resistance with Methicillin Resistant Staphylococcus aureus*

EICR	NUMBER	%
EICR+MRSA	37	63.80%
EICR+MSSA	21	36.20%

Discussion

In the present study, out of the total 300 isolates 58 samples (19.33%) showed erythromycin inducible clindamycin resistance. 31 isolates (10.33%) were MS phenotype wherein erythromycin was resistant but clindamycin was sensitive. 36 isolates (12%) were constitutional resistant types where both erythromycin and clindamycin were resistant. In the study carried out by Steward et al, $iMLS_B$ was maximum at 16.4% followed by $cMLS_B$ at 12.5% and the MS phenotype at 7.8% [8]. In the study of Swati, *et al*., also $iMLS_B$ was maximum at 34.6% followed by $cMLS_B$ at 27.8% and then MS phenotype at 13.4% [9]. Thus, these two studies are congruent with the present study. But in the study carried out by Dubey, *et al*., (2013) $iMLS_B$ ws maximum at 50.35% followed by MS phenotype at 34.55% and then $cMLS_B$ at 15.1% [10].

The incidence of constitutional and MS phenotype resistance may vary according to different geographical regions even from hospital to hospital or patient to patient. This variability is usually associated with the inconsistent use of erythromycin in different institutions; the origin of the isolate (nosocomial versus community acquired); patient age and clinical samples [Table-3].

Table-3 *Comparison of types of Erythromycin Resistant Staphylococcus aureus in Different Studies*

STUDY	YEAR	iMLSb	cMLSb	MS-Phenotype
Steward, *et al.*	2005	16.4%	12.5%	7.8%
Debasmita, *et al.*	2013	50.35%	15.1%	34.55%
Swati V Kant, *et al.*	2015	34.6%	27.8%	13.4%
Present Study	2016	19.33%	12%	10.33%

In the present study, out of the total 300 isolates studied, 41.1% showed Erythromycin resistance and 58.99% showed sensitivity to Erythromycin. Similar results were observed by Deotale, *et al*., (2010) with ERSA at 32.39% and ESSA at 67.61% [11], Kavita Prabhu, *et al*., (2011) with ERSA at 28.42% and ESSA at 72.57% [12], and Yoo Sang, *et al*., (2016) with ERSA at 45.6% and ESSA at 54.4% [13] [Table-4]. In the present study, out of the total 300 isolates of Staphylococcus aureus, 37 (12.33%) isolates of Erythromycin Inducible Clindamycin Resistant *Staphylococcus aureus*, were associated with MRSA. 21 isolates (7.0%) were associated with MSSA. Thus, Erythromycin inducible Clindamycin Resistance was seen more with Methicillin Resistant *Staphylococcus aureus* than with MSSA. Similar results were observed by Kavita Prabhu, *et al*., with EICR+MRSA at 20% and EICR + MSSA at 16% [12]; Mokta, *et al*., (2015) with EICR+MRSA at 28.39% and EICR + MSSA at 9.29% [14] and Suvarna, *et al*., (2015) with EICR+MRSA at 33% & EICR + MSSA at 25% [15] [Table-5].

Table-4 *Comparison of Erythromycin Resistant and Erythromycin Sensitive Staphylococcus aureus in Different Studies*

STUDY	YEAR	ERSA	ESSA
V Deotale, *et al.*	2010	32.39%	67.61%
Kavita Prabhu, *et al.*	2011	28.42%	71.57%
Yoo Sang, *et al.*	2016	45.6%	54.4%
Present Study	2016	41.1%	58.99%

Table-5 *Comparison of prevalence of Erythromycin Inducible Clindamycin Resistance with Methicillin Resistant and Methicillin Susceptible Staphylococcus aureus in different studies*

STUDY	YEAR	MRSA	MSSA
Kavita Prabhu, *et al.*	2011	20%	16%
Kiran, *et al.*	2015	28.39%	9.29%
Suvarna, *et al.*	2015	33%	25%
Present Study	2016	12.33%	7.0%

Conclusion

Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infections. This study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in *S. aureus* for optimum treatment of patients. Also, regular surveillance of hospital-acquired infections of multi-drug resistant *S. aureus* may be helpful in formulating & monitoring the antibiotic policy.

Application of research: In routine testing to detect erythromycin inducible clindamycin resistance.

Research Category: Clindamycin Resistance

Abbreviations:

$iMLS_B$: Inducible Macrolide-Lincosamide-StreptograminB
$cMLS_B$: Constitutive Macrolide-Lincosamide-StreptograminB
MRSA: Mehicillin Resistant *Staphylococcus aureus*
MSSA: Methicillin Susceptible *Staphylococcus aureus*
EICR: Erythromycin Inducible Clindamycin Resistance

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