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INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS

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ABSTRACT

Introduction: The resistance to antimicrobial agents among staphylococci is an increasing problem. This has led to renewed interest in the usage of macrolide- lincosamide- streptogramin B (MLSB) antibiotics to treat *Staphylococcus aureus* infections. Clinical failure has been reported due to multiple mechanisms that confer resistance to MLSB antibiotics.

Aims: The present study was aimed to detect inducible clindamycin resistance among *S. aureus* isolates and to study the relationship between clindamycin and methicillin resistance.

Materials and Methods: During a period of 6 months, a total 297 *S. aureus* isolates from various clinical specimens were included in the study. Antimicrobial susceptibility test was done by Kirby-Bauer's disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. For detection of inducible clindamycin resistance, D test using erythromycin and clindamycin as per CLSI guidelines was performed, and three different phenotypes were interpreted as MS phenotype (D test negative), inducible MLSB (iMLSB) phenotype (D test positive), and constitutive MLSB phenotype.

Results: Of the total 297 *S. aureus* isolates, majority were obtained from pus 35% (104), from swab 52% (153) followed by blood, tissue samples and body fluids 13% (40). Out of 297, 71% (211) were erythromycin resistant. Out of the total 297 isolates, 30.30% (90) were methicillin-resistant *S. aureus* (MRSA) and 69.69% (207) were methicillin-sensitive *S. aureus* (MSSA). MLSB phenotype in 13.46%, MS phenotype in 32.65%, and constitutive MLSB phenotype was observed in 24.91% of isolates. Inducible clindamycin resistance was more among MRSA than MSSA isolates.

Conclusion: D test should be included as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in staphylococci for the optimum treatment of patients.

Key words: Clindamycin, Erythromycin, methicillin-resistant *S. aureus*, methicillin-sensitive *S. aureus*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) are increasingly being reported as multidrug resistant with high resistance to macrolides (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin), leaving very few therapeutic options .[1] Newer antibiotics like vancomycin,linezolid, and quinupristin-dalfopristin have been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concerns over how long these uniform susceptibilities will hold good.[1-3] This has led to renewed interest in the usage of macrolide- lincosamide- streptogramin B (MLSB) antibiotics to treat *S. aureus* infections with, clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[4,5] MLSB antibiotics are structurally unrelated; however, they are related microbiologically because of their similar mode of action. They inhibit bacterial protein synthesis by binding to 23s rRNA, which is a part of large ribosomal subunit. They have a spectrum of activity directed against gram-positive cocci, gramnegative cocci and intracellular bacteria such as chlamydiae and rickettsiae.[6] For years, macrolides have been used as an alternative to penicillin and cephalosporins in the treatment of infections caused by gram positive bacteria, but the

worldwide development of macrolide resistance has now limited the use of these antibiotics. Macrolide resistance is by diverse mechanisms. The resistance to macrolide can be mediated by *msr*(*A*) gene coding for efflux mechanism or via *erm* gene encoding for enzymes that confer inducible or constitutive resistance to MLSB antibiotics. In constitutive resistance, r-RNA methylase is always produced (cMLSB); where as in inducible, methylase is produced only in the presence of an inducing agent (iMLSB).[7] Erythromycin is an effective inducer whereas clindamycin is a weak inducer. *In vitro*, *S. aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (iMLSB).[8] The treatment of patients harboring iMLSB staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure [9] The present study was aimed to detect inducible clindamycin resistance among *S. aureus* isolates and to study the relationship between clindamycin and methicillin resistance.

MATERIALS AND METHODS

The present study was a prospective study conducted during a period of 6 months from 1st January 2015 to 30th June 2015, on the patients admitted in Vadilal Sarabhai General Hospital, Ahmedabad. A total of 297 *S. aureus* isolates from various clinical specimens like pus, wound swab, aspirates, blood, body fluids, tissue, etc. were included in the study.General profile of patients is given in table-1.*S. aureus* isolates were identified by standard biochemical techniques.[10] Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method using various antimicrobial agents like penicillin G (10Units), cefoxitin (30 mcg), gentamycin (10 mcg), chloramphenicol(30 mcg), tetracycline (30 mcg), erythromycin (15 mcg),cotrimoxazole (25mcg), ciprofloxacin (5 mcg), vancomycin(30 mcg), linezolid (30 mcg) as per CLSI guidelines.[11] For quality control (QC), *S. aureus* ATCC 25923 was used. For detection of methcillin resistance, 30 mcg of cefoxitin disc was placed and plates were incubated at 35°C for 24 h. Isolates with zone diameters ≤22 mm were labeled as methicillin resistant.[11] For detection of inducible clindamycin resistance, a disk approximation test was performed by placing a 2 mcg clindamycin disc from 21 mm away from the edge of a 15 mcg erythromycin disc.[11] Following overnight incubation at 37°C, three different phenotypes were appreciated and interpreted as follows:

1. MS phenotype: S. aureus isolates exhibiting resistance to erythromycin (zone size ≤13 mm), while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin (D test negative).

2. Inducible MLSB phenotype: iMLSB S. aureus isolates which showed resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

3. Constitutive MLSB phenotype: cMLSB S. aureus isolates which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape zone of inhibition around clindamycin.

RESULTS

Of the 297 *S. aureus* isolates, majority was obtained from swabs 52% (153), pus 35% (104) followed by tissue, blood and body fluids 13% (40). All the *S. aureus* isolates were sensitive to vancomycin, and linezolid.

Table 1: General profile of patients included in study (Total -297)

Details	Number (%)
Male	184(61.95)
Female	103(34.68)
Samples from various departments	
Surgery dept.	146(49.15)
Obs-gynec.dept.	42(14.14)
Orthopedic dept.	29(9.76)
Medicine dept.	17(5.72)
OPD	63(21.21)

Out of total 297 isolates, 71%(211) *S. aureus* isolates were resistant to erythromycin, 30.30% (90) were MRSA and 69.69% (207) were MSSA [Table 2]. Among the 297 isolates, D test was positive in 13.46% (40) (inducible MLSB Phenotype) and negative in 32.65% (97) isolates (MS phenotype). Constitutive MLSB phenotype was seen in 24.91% (74) isolates. Percentage of inducible phenotype resistance was more among the methicillin resistant than methicillin susceptible *S. aureus* isolates.

Table 2: Association of Clindamycin resistance with Methicillin resistance

Variable	MRSA (%)	MSSA (%)	Total (%)
ERY-S,CL-S	11(3.70)	75(25.25)	86(28.95)
ERY-R,CL-S D-test negative(MS phenotype	22(7.40)	75(25.25)	97(32.65)
ERY-R,CL-S D-test positive(Inducible MLSB phenotype)	24 (8.08)	16(5.38)	40(13.46)
ERY-R,CL-R (Constitutive MLSB phenotype)	33(11.11)	41(13.80)	74(24.91)

Table 3: Comparision with other studies

Variable	Present study	Mallikajurna et al[18]	Prabhu et al[16]	Kanwal et al[17]	Nilima et al[15]
Erythromycin resistance	71%	70.1%	28.4%	50.1%	30%
Inducible clindamycin resistance	13.46%	32.40%	10.5%	13.1%	42%
Inducible clindamycin resistance in MRSA	8.08%	17.59%	20%	33.2%	28.91%
Inducible clindamycin resistance in MSSA	5.38%	14.81%	6%	34.6%	3.16%
Constitutive MLSB resistance	24.91%	2.77%	9.47%	21.9%	11.85%
MS phenotype	32.65%	35.81%	8.1%	44.8%	45%

DISCUSSION

Clindamycin is used in the treatment of skin and soft-tissue infections, caused by staphylococcal species. Good oral absorption makes this drug an important option in outpatient therapy or as afollow-up after intravenous therapy. Clindamycin strain carrying inducible *erm* gene using clindamycin or any non-inducer macrolide can lead to clinical failure.[8,9,14] Constitutive mutants can be selected *in vitro* in the presence of clindamycin or any other non-inducer macrolide as they are widespread among methicillin-resistant strains.[7] *In vitro* routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D test on a routine basis**.**

Among the 297 *S. aureus* isolates studied, 71% isolates were erythromycin resistant, which is in concordance with study by Mallikajurna et al 70.1% [18] and Kanwal et al 50.1%[17]. inducible clindamycin resistance was observed in 13.46% isolates which was in concordance with study by prabhu k et al.10.5%[16] and Kanwal et al 13.1%[17].

The percentage of inducible resistance was higher among methicillin resistant (8.08%) than methicillin susceptible (5.38%) *S. aureus* isolates, which correlates with other studies [15,17,18,16] suggesting higher rate of inducible resistance in MRSA than MSSA. Constitutive (24.91%) and MS phenotype (32.65%) clindamycin resistance which correlates with study by Kanwal et al 21.9% and 44.8% respectively and study by Nilima et al. 11.81% and 45% respectively. This suggests variation in clindamycin resistance pattern and its relation with MRSA and MSSA in various geographical areas. [Table-3]

CONCLUSION

Reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand, negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.Use of D test in a routine laboratory enables us in guiding the clinicians in judicious use of clindamycin, as clindamycin is not a suitable drug for D test positive isolates; while it can definitely prove to be a drug of choice in case of D test negative isolates.

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