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Original Research Article

Inducible Clindamycin Resistance among Staphylococcal Isolates from Different Clinical Samples

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ABSTRACT

Introduction: Clindamycin is a preferred therapeutic option in the treatment of both methicillin susceptible and resistant staphylococcal skin and soft tissue infections. However, a major concern regarding its use for staphylococcal infections is the possible presence of inducible resistance to clindamycin. The present study was aimed to determine the incidence of constitutive and inducible clindamycin resistance in *Staphylococcal* isolates in our hospital.
Material & methods: A total of 233 consecutive, non-duplicate staphylococcal strains were isolated from various clinical specimens, both from inpatients and outpatients. Antibiotic susceptibility tests were performed using Kirby-Bauer disc diffusion method. Methicillin resistance was detected by oxacillin disc on Mueller-Hinton Agar (MHA) plate supplemented with 2% NaCl. D-test was performed on all erythromycin-resistant and clindamycin-sensitive isolates to detect inducible clindamycin resistance.
Results: Among 233 *Staphylococcal* strains, 86 (36.9%) were found to be Methicillin-resistant *Staphylococcus aureus* (MRSA), 97(41.63%) were found to be Methicillin-sensitive *Staphylococcus aureus* (MSSA), and 50 (21.45%) were coagulase negative Staphylococci (CoNS). Inducible CL resistance (MLSBi), was detected in 55 of 233 isolates (23.6%). MRSA isolates showed higher inducible resistance ($p < 0.0001$) to clindamycin as compared to methicillin-sensitive *S.aureus* (MSSA) and coagulase negative Staphylococci (CoNS).
Conclusions: The study strongly recommends the routine testing of *in vitro* inducible clindamycin resistance in *Staphylococcal* isolates as it will help in the optimal treatment of patients.
Key words: Methicillin-resistant Staphylococcus aureus, Methicillin-sensitive Staphylococcus aureus, Coagulase negative Staphylococci (CoNS), Clindamycin, D-test

INTRODUCTION

Staphylococcus aureus and coagulase negative staphylococci are recognized to be the very common organisms causing nosocomial and community acquired infections. Treatment of these infections is a growing problem because of changing patterns in antimicrobial resistance, particularly increasing methicillin resistance among Staphylococci. To treat such infections macrolide–lincosamide-streptogramin (MLS) antibiotics are used widely. However their widespread use has led to an increase in the number of staphylococci strains resistant to macrolide–lincosamide-streptogramin (MLS) antibiotics. [1- 3]

Macrolide resistance may be due to an active efflux mechanism encoded by msr (A) conferring resistance to macrolides and type B streptogramins but not to clindamycin (MS-phenotype). Another mechanism is a ribosomal target modification that affects activities of macrolides and type B streptogramins and also to clindamycin (MLS_B-resistance). [4]

MLS_B-resistance in staphylococci is encoded by erm(A) or erm (C) and can either constitutive ($cMLS_B$) or inducible ($iMLS_B$). [5] Constitutive resistance can be readily detected , but inducible MLS_B resistance is not recognized by routine susceptibility test methods including standard broth based or agar dilution susceptibility tests. [6]

Erythromycin is an effective inducer of $iMLS_B$ resistance. It will induce production of methylase, which allows clindamycin resistance to be expressed, which forms the basis of the D-test. To detect inducible CL resistance strains, the disk diffusion induction test (D-test) has been used by several authors. [7-9] Accurate antibiotic susceptibility data of the infecting organism is an essential factor in making appropriate decisions. If failed to identify inducible CL resistance, it leads to incorrect laboratory reports and treatment problems. [10, 11] Thus the aim of the present study was to detect the inducible clindamycin resistance in Staphylococci in our geographical area using D-test.

MATERIALS AND METHODS

The study was conducted from August 2010 and July 2011 at Dr. Vasantrao Pawar Medical College, Hospital and Research Center, Nasik. A total of 233 consecutive, non duplicate clinical isolates of staphylococci recovered from different clinical samples like pus, blood, urine, sputum. Throat swabs, CSF and others were tested.

All the Staphylococcal species were first indentified by standard biochemical techniques. [12] All identified species then subjected to Antibiotic susceptibility testing by the Kirby-Bauer disc diffusion method. Antibiotic discs used were ampicillin (10 µg), amoxiclavulanic acid (20/10 µg), cefepime (30 µg), ceftriaxone (30 µg), cephotaxime (30 µg), cefoperazone-sulbactam (75/30 µg), cephalexin (30 µg), ciprofloxacin (5 µg),) doxycycline (30 µg), erythromycin (15 µg), linezolid (30 µg), netilmicin (30 µg), piperacillin-tazobactum (100/10µg) and vancomycin (30µg). Staphylococcus ATCC 25923 was used as the control strain for the disc diffusion method.

Methicillin resistance was detected by using oxacillin (1 µg) on Mueller-Hinton agar plate supplemented with 2% NaCl followed by incubation at 35 ^{0} C. [13]

The isolates that were found to be erythromycin resistant by the Kirby-Bauer disc diffusion method were subjected to D zone test for inducible clindamycin

resistance as per the CLSI guidelines. [13] The clindamycin (2g), erythromycin (15g) and all other antibiotic discs were procured from Himedia India, Private Ltd.

D-zone test: - A lawn culture of the isolate which was adjusted to 0.5 Mcfarland's concentration was made on a Mueller Hinton agar plate and discs of CL (2µg) and ER (15µg) were placed at a distance of 15mm (edge to edge) as per the CLSI recommendations, along with routine antibiotic susceptibility testing. [13]

The disc diffusion test, based on the D test, showed four phenotypes.

The Inducible MLS_B phenotype ($iMLS_B$): Inducible resistance to clindamycin was manifested by flattening or blunting of the CL zone adjacent to the ER disc, giving a D shape.

The Constitutive MLS_B phenotype ($cMLS_B$): Isolates which were resistant to both erythromycin and clindamycin.

The MS phenotype: Isolates which were resistant to erythromycin and susceptible to clindamycin.

RESULTS

Between August 2010 and July 2011, 233 isolates of staphylococci were collected from various types of clinical samples. The isolates were identified as 50 coagulase negative Staphylococci (CoNS) and 183 *Staphylococcus aureus*, and of the latter 86 (36.96%) were methicillin resistant *Staphylococcus aureus* (MRSA) and 97 (41.63%) were methicillin sensitive *Staphylococcus aureus* (MSSA), as shown in Table 1.

The majority of staphylococci were found from pus samples. *S. aureus* was isolated more frequently from pus samples (49 of 86, 57%), followed by urine samples (15 of 86, 17.4%).

All staphylococci isolated from clinical samples were tested for inducible CL resistance using the D-test. A positive D-test, D-shaped zone around CL disk indicating an inducible CL resistance, was detected in 55 of 233 isolates (23.6%). Concerning the *S. aureus*, an inducible CL resistance was found in MRSA more than in MSSA, 28 of 86 (32.56%) and 15 of 97 (41.63%) respectively, whereas, 12 of 50 isolates of CoNS (21.45%) were inducible CL resistance as shown in Table 2.

All staphylococcal isolates showing inducible CL resistance were sensitive to vancomycin and linezolid.

The disk diffusion based on the D-test produced four of staphylococci, designated as D-positive, D-negative, resistant (R) and susceptible (S) (Table 3). A D-shaped zone around the CL disk ($iMLS_B$ phenotype), indicating an inducible CL resistance, was found in 32.56% of MRSA, 15.46% of MSSA, and 24% of CoNS. On the other hand, 23.25% of MRSA, 14.43% of MSSA, and none of CoNS were CL susceptible showing a circular shape zone around the CL disk (MS phenotype). The isolates of 17.44% of MRSA, 12.37% of MSSA, and none of CoNS were both CL and E resistant ($cMLS_B$ phenotype), whereas 26.74% of MRSA, 57.73% of MSSA, and 76% of CoNS were both CL and E susceptible (S phenotype).

Table 1:- *S. aureus* and coagulase-negative staphylococci isolated from various sources of clinical samples

Source of sample	*S. aureus*		Coagulase-negative staphylococci
	MRSA	*MSSA*	
Blood	13	17	6
Pus	49	51	7
Urine	15	13	21
Sputum	2	4	7
Throat	1	1	4
CSF	0	0	3
Others	6	11	2
Total	86	97	50

Table 2:- Detection of inducible clindamycin resistance of *S. aureus* and coagulase-negative staphylococci by D-test

Organism	No. of positive induction tests (%) (D-shaped zone)	No. of tested isolates
MRSA	28 (32.56)	86
MSSA	15 (15.46)	97
CoNS	12 (24)	50
Total	55	233

Table 3:- Clindamycin induction test phenotypes among S. *aureus* and coagulase-negative staphylococci

Organism	Total no. of isolates	iMLSB D-positive phenotype	cMLSB both CL and E resistant	MS phenotype CL susceptible and E resistant	Isolates sensitive to both CL and E susceptible
MRSA	86(36.90)	28(32.56)	15(17.44)	20(23.25)	23(26.74)
MSSA	97(41.63)	15(15.46)	12(12.37)	14(14.43)	56(57.73)
CoNS	50(21.45)	12(24.00)	--	--	38(76.00)
Total	233	55(23.60)	27(11.58)	34(14.59)	117(50.21)

DISCUSSION

Clindamycin is a useful drug in the treatment of both methicillin susceptible and resistant staphylococcal infections. [14] It is indicated for the treatment of soft tissue infections, pediatric infections caused by Staphylococci or the patients allergic to beta-lactam antibiotics. [15]

Inducible clindamycin resistant Staphylococci show susceptible results in conventional susceptibility tests, but can be converted to a constitutively resistant phenotype during clindamycin treatment. Reporting *Staphylococcus 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. Thus detection of inducible clindamycin resistance is necessary. [16, 17]

In our study, among 233 isolates of Staphylococci, inducible clindamycin resistance ($iMLS_B$ resistance) was observed in 23.6% (55/233) isolates similar to that reported by M R Angel et al [18] and Gadepalli et al [3] Some investigators have reported a higher incidence like Fiebelkorn et al [6] reported 28%, Delialioglu et al [2] reported 45% and Dizbay et al [19] reported that 90% of their Staphylococcus aureus strains were of the $iMLS_B$ phenotype. While others have indicated lower incidence of $iMLS_B$ resistance. [20-22]

It was observed that the percentage of inducible clindamycin resistance was higher in the MRSA (32.56%) as compared to the MSSA and CoNS, also reported by many authors previously [3,9,18,20-22] though one Korean study has reported higher $iMLS_B$ in CoNS. [23] Different studies from different parts of India have reported that 20% to 64% of their MRSA strains were of the $iMLS_B$ phenotype. [3, 18, 22, 24]

Though Angel et al [18] have not found any cMLSB resistance in Staphylococcus aureus strains, we found 27 (11.58%) Staphylococcus aureus strains with the cMLSB phenotype, out of which 15 (17.44%) were MRSA strains and 12 (12.37%) were MSSA strains. The incidence of the cMLSB phenotype is quite high outside India. [6 ,9]

Gadepalli et al [3] had reported 12% strains of the MSB phenotype among the Staphylococcus aureus strains comparable to our study, where 34 (14.59%) strains were of the MSB phenotype.

In summary, 32-35% of erythromycin non-susceptible and clindamycin-susceptible *S. aureus* and 90-94% of erythromycin non-susceptible and clindamycin-susceptible CNS showed inducible resistance to clindamycin. The results of this study represent Staphylococcal isolates from a single hospital and geographic area; the prevalence of $iMLS_B$ may differ in different regions.

CONCLUSION

We hereby conclude that if the D-zone test is not performed, Staphylococcal isolates with inducible clindamycin resistance would have been misclassified as Clindamycin sensitive, resulting in therapeutic failure. This is where the D-zone test becomes significant and important. Therefore clinical microbiology laboratory should report inducible clindamycin resistance routinely.

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