

Research Article

Inducible Clindamycin Resistance among Staphylococcus Aureus Isolated From Various Clinical Samples with Special Reference to MRSA

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Abstract: Staphylococcus aureus are increasingly being reported as multidrug resistant with high resistance to macrolides and lincosamides, leaving very few therapeutic options. The aim of the study is to find out the presence of Inducible Clindamycin Resistance among Staphylococcus aureus isolated from various Clinical Samples with special reference to Methicillin Resistant Staphylococcus aureus (MRSA). This is a prospective study and was done in the year 2013 at Govt. general hospital, Vijayawada. Various Clinical samples were collected on random basis to isolate Staphylococcus aureus by observing its characteristics on following standard methods. All erythromycin resistant isolates were processed for Inducible clindamycin resistance by D-test. Out of 164 Staphylococcus aureus isolates in different clinical samples, 119(72.5%) were Erythromycin resistant and 71(43.2%) were found to be MRSA. Among these, 34 (20.7%) were iMLSB (Erythromycin resistant Clindamycin sensitive by D test), 29(17.6%) were cMLSB and 56(34.1%) were MS Phenotype (Erythromycin resistance, Clindamycin sensitive). MRSA isolates were more than MSSA in MS Phenotype. We conclude that it is necessary to perform D-test for detection of inducible clindamycin resistance among Staphylococcus aureus in routine antibiotic sensitivity testing. Therapeutic failures may thus be avoided.

Keywords: Staphylococcus aureus, Inducible clindamycin resistance (iMLSB), Constitutive clindamycin resistance (cMLSB), MS Phenotype, D test, MRSA.

INTRODUCTION

Staphylococcus aureus is an important bacterial pathogen causing infection in both hospital and community settings. S.aureus 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 commonly used in treatment of staphylococcal infections. Clindamycin is a frequent choice for some staphylococcal infections, particularly skin and soft-tissue infections, and as an alternative in the penicillin-allergic patient. Inducible

MLSB resistance is not recognized by using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests. Failure to identify inducible MLSB resistance may lead to clinical failure of clindamycin therapy. Conversely, labeling all erythromycin-resistant staphylococci as clindamycin resistant prevents the use of clindamycin in infections caused by truly clindamycin-susceptible staphylococcal isolates.

Bacterial resistance to antimicrobial agents generally involves drug inactivation, target site modification, impermeability or efflux mechanisms. Macrolides antibiotic resistance in Staphylococcus aureus and coagulase-negative staphylococci (CNS) may be due to an active efflux mechanism encoded by *msrA* (conferring resistance to macrolides and type B streptogramins only) [6,7] or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins (MLSB resistance). *Erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, reducing binding by MLS agents to the ribosome [8]. Resistance

is induced by the binding of a macrolide to upstream translational attenuator sequences, leading to changes in mRNA secondary structure, exposure of the ribosomal binding site, and translation of the erm methylase. Alterations in these 5' upstream sequences, including deletions, duplications, and other mutations, lead to constitutive expression of the methylase gene and constitutive MLSB resistance [9, 10]. Strains with inducible MLSB resistance (iMLSB) strains demonstrate in vitro resistance to 14- and 15-member macrolides (e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins; strains with constitutive MLSB resistance (cMLSB strains) show in vitro resistance to all of these agents [8]. The resistance to antimicrobial agents among Methicillin resistant *Staphylococcus aureus* (MRSA) is also increasing problem worldwide.

Isolates with inducible clindamycin resistance are found to be resistant to erythromycin but susceptible to clindamycin when these discs are not placed adjacent to each other during antimicrobial sensitivity testing. These isolates can be detected by the D-test, a disc diffusion test in which induction of clindamycin resistance by erythromycin is tested [11]. Phenotypic detection of inducible resistance can be done by this double disc diffusion test (D-test). D-test is simple, reliable, inexpensive and easy to interpret with high sensitivity and specificity. Clindamycin is a good option but prevalence of inducible resistance should be known, as it varies by geographical location and bacterial species. The present study was also aimed to find out the percentage of inducible clindamycin resistance (iMLSB) among *Staphylococci* isolates in our geographical area using D-Test especially in MRSA.

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology at Siddhartha Medical College, Vijayawada in 2013. A total of 164 *Staphylococcus* spp. were isolated from various clinical

specimens like pus, urine, sputum, pleural/synovial fluid, blood, throat swab. All isolates were identified morphologically and biochemically by standard laboratory procedures [12].

Antibiotic susceptibilities were studied by modified Kirby Bauer's disc diffusion method on Mueller Hinton Agar plates using Ampicillin (10 µg), Penicillin G (10 units), Cotrimoxazole (25 µg), Ciprofloxacin (5 µg), Vancomycin (30µg), Erythromycin (15 µg), Clindamycin (2 µg), Linezolid (30 µg) and Cefoxitin (30 µg) as per CLSI guidelines [13]. Quality Controls standards were maintained for every procedure, The isolates that were found to be erythromycin resistant (zone size < 13mm) were further studied for inducible clindamycin resistance.

Detection of Methicillin resistance: Methicillin resistance *Staphylococcus aureus* were identified by using cefoxitin (30µg) disc. An Inhibition zone diameter of ≤ 21 mm was reported as oxacillin resistant (MecA positive) and ≥ 22 mm was considered as oxacillin sensitive (MecA negative).

The detection of inducible clindamycin resistance was performed using the D-test. Briefly, an erythromycin disc (15µg) was placed 15 mm (edge to edge) from a clindamycin disc (2µg) in a standard disc diffusion test. A flattening of the zone of inhibition in the area between the discs where both drugs have diffused after 18-24 hours of incubation was considered to be inducible clindamycin resistance.

Three different phenotypes were appreciated after testing and then interpreted as follows [5]:

1. MS Phenotype - *Staphylococcal* isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥ 21mm) and giving circular zone of inhibition around clindamycin was labeled as having this phenotype [Fig.1].



Fig-1: Showing both erythromycin and Clindamycin Sensitive

2. Inducible MLS_B (iMLS_B) Phenotype - *Staphylococcal* isolates showing resistance to erythromycin (zone size ≤ 13mm) while being sensitive

to clindamycin (zone size ≥ 21mm) and giving D shaped zone of inhibition around clindamycin with

flattening towards erythromycin disc were labeled as having this phenotype [Fig.2 and Fig.3].

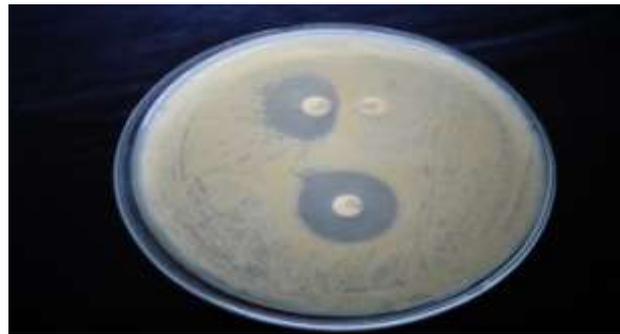


Fig- 2: Showing D test positive



Fig-3: Showing D test positive

3. Constitutive MLS_B ($cMLS_B$) Phenotype - this phenotype was labeled for those Staphylococcal isolates which showed resistance to both erythromycin (zone

size $\leq 13\text{mm}$) and clindamycin (zone size $\leq 14\text{mm}$) with circular shape of zone of inhibition if any around clindamycin [Fig 4].



Fig-4: showing both erythromycin and clindamycin resistance

Quality control of the erythromycin and clindamycin discs was performed with ATCC *Staphylococcus aureus* 25923[14].

RESULTS AND DISCUSSION:

The increasing prevalence of MRSA (Methicillin Resistance *Staphylococcus aureus*) infections especially with the spread of resistant strains in the community poses a challenge to physicians in terms of the use of alternative antibiotic agents. Although clindamycin has been considered an acceptable option for patients with community-acquired

MRSA infections, reports on high rates of clindamycin-resistant community-acquired MRSA strains are limiting its use [15].

In recent times, clindamycin has become an excellent drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients [16]. Clindamycin is a good substitute to treat soft tissue infections by both MRSA and MSSA infections. Its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments,

good tissue penetration and ability to directly inhibit toxin production are its advantages [17].

A 164 Staphylococcal isolates are obtained from various clinical samples such as pus, urine, sputum, pleural fluid/synovial fluid, blood, throat swab and the details are depicted in Table: 1

Table-1: Clinical samples vs Staphylococcal aureus isolates

S. No.	Samples	No. of Staphylococcal isolates	Percentage (%)
1	Pus	52	31.7
2	Urine	45	27.4
3	Sputum	35	21.3
4	Pleural/ Synovial fluid	16	9.7
5	Throat swabs	11	6.7
6	Blood	5	3
	Total	164	

In this study pus samples shown highest S.aureus isolation followed by urine and sputum. Kiran K *et al.*; [18] also observed that more percentage of Staphylococci in pus samples (56%) which was followed by blood(28%) and urine (12%). Among 164 Staphylococcus aureus isolates, 119 isolates were resistant to erythromycin. These erythromycin resistant isolates were subjected to D test to detect Clindamycin susceptibility pattern and Cefoxitin (30µg) disc test to detect MRSA as per CLSI Guidelines. Routine antibiotic Sensitivity testing by Kirby Bauer method

along with erythromycin and clindamycin discs for D test in the same plate can be performed.

On performing D test, the result shown, among 119 erythromycin resistant isolates 34(28.5%) shown inducible clindamycin resistance (iMLS_B), 29(24.3%) isolates shown Constitutive resistance (cMLS_B) and 56(47%) isolates shown MS phenotype resistance. Similarly we analysed the susceptibility patterns to erythromycin and clindamycin as shown in Fig: 5.

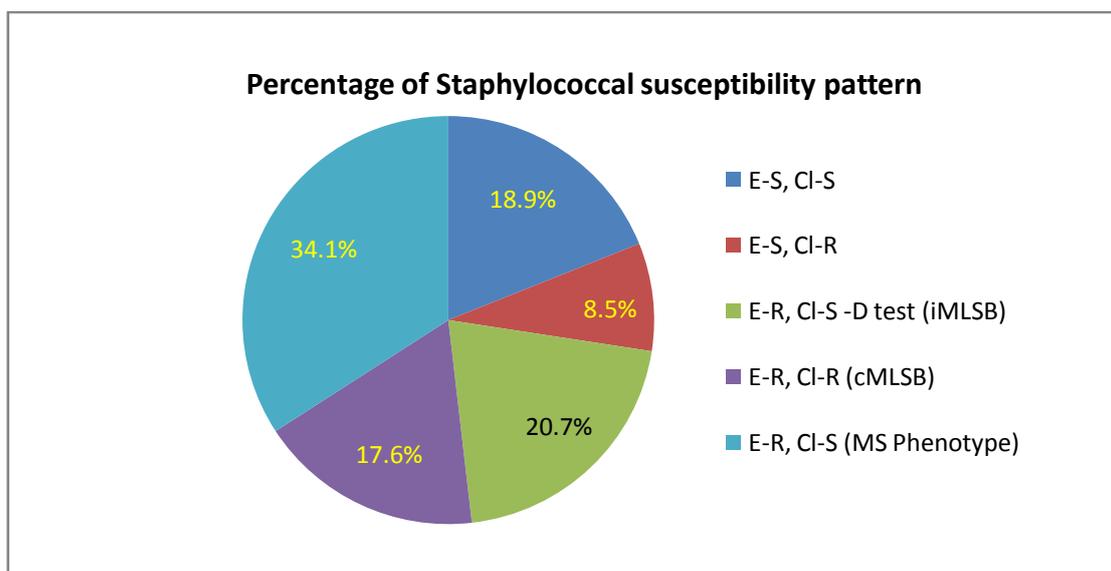


Fig-5: Staphylococcus aureus isolates (164) with variable erythromycin and clindamycin susceptibility patterns

In the present study 72.5% of Staphylococcal isolates are erythromycin resistant. Among which 28.5% are iMLS_B, 24.3% cMLS_B and 47% MS Phenotype. iMLS_B shown more percentage than cMLS_B. This study is in line with Deotale *et al.*; [5], Pal *et al.*; [19], Mittal *et al.*; [20] and Lall *et al.*; [21]

which reported that iMLS_B percentage greater than cMLS_B.

Likewise 164 Staphylococcus aureus isolates, 71(43.2%) isolates were MRSA and among 119 erythromycin resistance isolates, 57(47.8%) isolates were MRSA.

Table-2: Clindamycin Susceptibility patterns among MRSA and MSSA in all isolates

S.No.	Susceptibility pattern	MRSA	MSSA	Total
1	E-S, CI-S	3	28	31 (18.9%)
2	E-S, CI-R	11	3	14 (8.5%)
3	E-R, CI-S - D test positive (iMLSB _B)	14	20	34 (20.7%)
4	E-R, CI-R (cMLSB _B)	21	8	29 (17.6%)
5	E-R, CI-S (MS Phenotype)	22	34	56 (34.1%)
	Total	71	93	164

E-Erythromycin, CI-Clindamycin, R-Resistance, S-Sensitive, MRSA - Methicillin Resistant Staphylococcus aureus, MSSA - Methicillin Sensitive Staphylococcus aureus

As per this study among 119 erythromycin resistant isolates, 11.7% iMLSB has shown MRSA, 17.6% cMLSB and 18.4% MS Phenotype shown MRSA respectively. Other studies such as Prabhu *et al.*; [16] shown iMLSB 20% MRSA, Ajap *et al.*; [22] and Pal *et al.*; [19] reported 5% and 43.5% iMLSB MRSA respectively.

Among 164 staphylococcal isolates, Methicillin resistant was more in MS Phenotype about 13.4% followed by Constitutive clindamycin resistance and Inducible clindamycin resistance, were about 12.8% and 8.5% respectively. Among 119 erythromycin isolates, Constitutive Clindamycin resistance shown higher MRSA (17.6%) than MSSA (4.8%). Inducible clindamycin resistance and MS Phenotype was higher in MSSA than in MRSA.

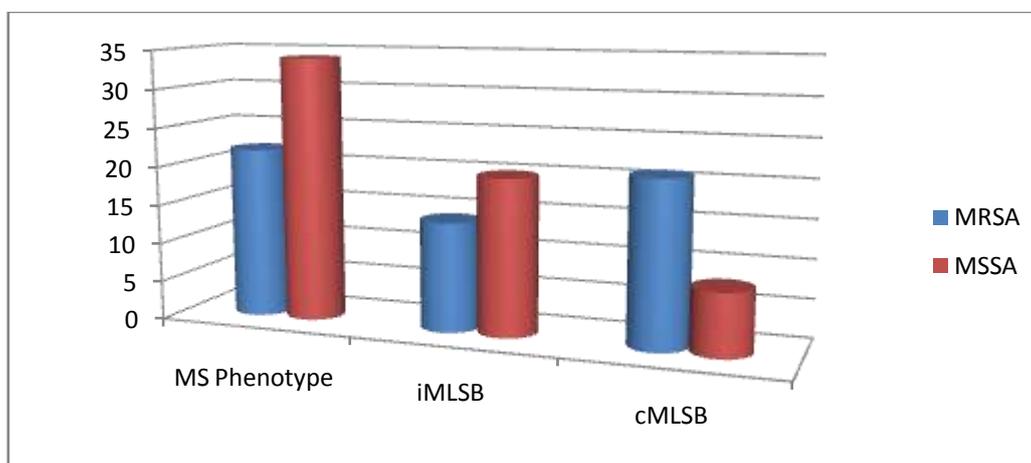


Fig- 6: showing Clindamycin susceptibility pattern among erythromycin resistant isolates

The difference in various resistant phenotypes in literature can be due to the difference in bacterial susceptibility in different geographical regions and also due to varying antimicrobial prescribing patterns of

clinicians. Prevalence of clindamycin resistance among clinical isolates of S.aureus in various Indian studies is shown in [Table: 3].

Table-3: Studies showing percentage of clindamycin susceptibility among Staphylococcal isolates

Authors name	MRSA			MSSA		
	iMLSB Phenotype (%)	cMLSB Phenotype (%)	MS Phenotype (%)	iMLSB Phenotype (%)	cMLSB Phenotype (%)	MS Phenotype (%)
Gadepalli <i>et al.</i> ; [23]	30	30	12	10	15	12
Gupta <i>et al.</i> ; [24]	20	46	16	17.3	10	37.3
Deotale <i>et al.</i> ; [5]	27.6	7.3	24.3	1.6	0	4
Pal <i>et al.</i> ; [19]	43.6	38.8	18.7	6.9	7.3	10.9
Debdas <i>et al.</i> ; [25]	18	23	48	2	3	6
Mittal <i>et al.</i> ; [20]	44.8	8.6	13.3	8.4	4.5	16.1
Lall <i>et al.</i> ; [21]	37.1	16.6	22.8	6	4.8	13.5
Present study	8.5	12.8	13.4	12.1	4.8	20.7

So keeping in view of this relative high frequency of iMLS_B resistance among MRSA isolates, D-test should be performed in the laboratory as a routine procedure. The D-test is an easy, sensitive and reliable test to perform along with routine susceptibility testing in clinical laboratory settings without specialized testing facilities to detect iMLS_B resistance among staphylococcal isolates which in turn help in proper effective treatment. The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible clindamycin resistance is helpful in guiding anti-staphylococcal therapy [12].

CONCLUSION:

Accurate susceptibility data are important for appropriate therapy decisions. Inducible resistance can be reliably detected on a routine basis by D test in clinically significant isolates; clindamycin can be safely and effectively used in those patients with true clindamycin-susceptible strains. In this study, we have described a simple, reliable method to detect inducible resistance to clindamycin in erythromycin-resistant isolates of *S. aureus*.

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