

Original article

Inducible Clindamycin resistance in Staphylococcus aureus in a tertiary care Rural Hospital

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Abstract :

Introduction: Macrolide (MLS_B) resistance is the most widespread and clinically important mechanism of resistance encountered with Gram-positive organisms. Resistance may be constitutive (cMLS_B phenotype) or inducible (iMLS_B phenotype). The iMLS_B phenotypes are not differentiated by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disk approximation test (D-test) and demonstration of resistance genes by molecular methods. The present study was planned to demonstrate in vitro inducible clindamycin resistance (iMLS_B) in erythromycin-resistant (ER) clinical isolates of S.aureusto guide therapy. And to find out the relationship of MRSA with inducible clindamycin resistance

Materials and Methods: 256 Staphylococcusaureus isolates were examined for inducible clindamycin resistance by using D-test at 15 mm disk separation as per CLSI guidelines on erythromycin resistant isolates.

Results: 142(55.46%) clinical isolates showed erythromycin resistance.76(53.52%) isolates were found to exhibit the constitutive resistance,30(21.12%) the inducible MLS_B resistance phenotype and non-inducible(MS) in 36(25.35%). Two distinct induction phenotypes (18.30%) and D⁺ (2.81%) were observed. In MRSA isolates, 39.43% had the constitutive, 13.38% had the iMLS_B resistance and 16.19% had MS phenotype. In MSSA, 14.08% and 7.7% isolates were found to have the constitutive and inducible MLS_B resistance phenotypes respectively while 9.15% exhibited the MS phenotype. Thus, both the constitutive and inducible resistance phenotypes werefound to be significantly higher in MRSA isolates as compared to MSSA (39.43%, 13.38 % and 14.08% and 7.7% and constitutive MLS_B was predominant(53.52%)

Conclusion: Study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

Keywords: Clindamycin resistance, constitutive MLS_B phenotype, inducible MLS_B phenotype

Introduction:

Staphylococcus aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections in every region of the world. The increasing prevalence of methicillin resistance among Staphylococci is an increasing 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 and good penetration into various tissues including bones, except cerebrospinal fluid. ⁽²⁾ However, widespread use of MLS_B antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLS_B antibiotics. ⁽³⁾

The common mechanism of resistance to MLS_B in of Staphylococcal strains is of three types. The first mechanism is target site modification mediated by erm genes usually erm C or erm A which can be expressed either constitutively ($cMLS_B$) where rRNA methylase is always produced or inducible ($iMLS_B$) where methylase is produced in the presence of inducer like erythromycin.⁽⁴⁾

Another mechanism of resistance is specific efflux of antibiotic mediated through msrA gene (MS phenotype). This energy dependant pump effectively expels macrolide from bacterial cell before they can bind to their target site on the ribosomes.⁽⁵⁾ The third mechanism is by inactivation of lincosamide by chemical modification mediated by inuA gene and this is rare.^(6,7)

Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive in vitro when not placed adjacent to each other. In such cases, in vivo therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. On other hand, infections due to MS phenotype do not typically become clindamycin resistant during therapy.⁽⁸⁾ The MS phenotype and $iMLS_B$ phenotype are indistinguishable by using standard Susceptibility test method but can be distinguished by a simple invitro Disk approximation test- D test as described by Fiebelkorn.^(2,9)

With this background in mind the present study was planned to find out the percentage of *S. aureus* having constitutive (MLS_B phenotype) inducible clindamycin resistance ($iMLS_B$) using D-test. & to

find out the relationship between methicillin-resistant *S. aureus* (MRSA) and inducible clindamycin resistance.

Materials and Methods:

The study was carried out in the department of Microbiology, MIMER Medical College, Talegaon Dabhade, Pune from the period of July 2012 to January 2013. The study was approved by the Ethical committee, MIMER Medical College, Talegaon Dabhade, Pune. Total of 256 *Staphylococcus aureus* was isolated from various clinical specimens like Pus, Blood, Urine, fluids by standard biochemical techniques⁽¹⁰⁾ The isolates were then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15 μ g), clindamycin (2 μ g), cefoxitin (30 μ g) and oxacillin (1 μ g) disc as per CLSI guidelines.⁽⁹⁾ (Discs were procured from Hi-media Laboratories, Mumbai, India), An inhibition zone of 10 mm or less around oxacillin disc and 19 mm or less around cefoxitin indicates Methicillin resistant.

Isolates were initially screened for erythromycin resistance. The isolates those were found to be erythromycin resistant were further studied for inducible clindamycin resistance by 'D test' as per CLSI guidelines.⁽⁹⁾

In this, erythromycin (15 μ g) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 μ g) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions. Plates were incubated at 37⁰C for overnight and zone diameters were recorded. Induction test categories were interpreted as given in Table 1

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Table 1: Characteristics of clindamycin induction test phenotypes as tested by disk approximation test⁽⁸⁾

Induction test phenotype	Resistance phenotype	CLI Result	ERY Result	Induction test description
D	Inducible MLS _B	S	R	Blunted, D- shaped clear zone around CLI disc proximal to the ERY disc.
D ⁺	Inducible MLS _B	S	R	Blunted, D- shaped clear zone around CLI disc proximal to the ERY disc and small colonies growing to/near CLI disc in otherwise clear zone.
Negative	MS _B	S	R	Clear zone around CLI disc without any D zone.
HD	Constitutive MLS _B	R	R	Two zones of growth around the CLI disc. One is a light, hazy growth extending to the CLI disc. Second zone where the growth is much heavier and Blunted proximal to the ERY disc as in Phenotype D.
R	Constitutive MLS _B	R	R	No hazy zone. Growth up to CLI and ERY discs.
S	No Resistance	S	S	Clear, susceptible zone diameter.

Zone diameter around –ERY (Erythromycin) disc ≤13mm(R); 14-22mm (I); ≥23mm (S) and CLI (Clindamycin) disc ≤14mm(R); 15-20mm (I); ≥21mm (S)

Result:

A total of 256 S.aureus were isolated from various clinical specimens.165 were found to be Methicillin resistant and 91 were MSSA.One hundred and forty-

two (55.46 %) clinical isolates which showed erythromycin resistance were tested for inducible resistance by D test.(Table 2)

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Table 2: MLS_B resistance phenotypes in S. aureus

	Erythromycin resistance n (142)			MS phenotype	Erythromycin susceptible n (114)	Total 256
	Constitutive MLS _B resistance	Inducible MLS _B resistance				
		D	D ⁺			
MRSA	56	16	3	23	67	165
MSSA	20	10	1	13	47	91
Total	76	26	4	36	114	256
	53.52%	18.30%	2.81%	25.35%		

(MRSA: Methicillin resistant S.aureus, MSSA: Methicillin susceptible S.aureus)

Out of 142 erythromycin resistant strains, Inducible MLS_B phenotypes were seen in 30(21.12 %) - (63.33% in MRSA (19/30) & 36.66% in MSSA (11 /30) . 19(63.33 %) Inducible MLS_B phenotypes were observed to be Methicillin resistant . D-test yielded two distinct induction phenotypes, D-zone phenotype (figure 1) was observed in 26 (18.30%) and D⁺ phenotype (figure 2) in 4(2.81%) isolates. Both D and D⁺ results were considered positive for CLI induction - iMLS_B phenotypes. MS phenotype (Figure 3) was seen in Thirty-six (25.35%) isolates. cMLS_B phenotype (Figure 4) were seen in seventy-six isolates (53.52%) of which 56 were MRSA, 20 MSSA. No hazy D zone (HD) phenotype was observed.

Discussion:

Clindamycin is a useful drug in the treatment of skin and soft tissue infections and serious infections caused by staphylococcal species as well as anaerobes. It has excellent tissue penetration (except for the central nervous system) and accumulates in

abscesses, and no renal dosing adjustments are needed.⁽¹¹⁾ Good oral absorption makes it an important option in outpatient therapy or as follow up after intravenous therapy. Clindamycin is also of particular importance as an alternative antibiotic in the penicillin allergic patient.

The resistance to macrolide can be mediated by msrA gene or via erm gene encoding for enzymes that confer inducible or constitutive resistance to macrolide, lincosamide and Type B streptogramin . For the clinical laboratory, the differentiation of erm-mediated iMLS_B(D and D⁺) phenotypes from msrA-mediated (Neg-phenotype) resistance is the critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant S. aureus isolate. However, differentiating D from D⁺ phenotypes could also provide information to characterize isolates for epidemiologic studies in healthcare and community settings.⁽⁸⁾

Since the iMLS_B resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location and even from hospital to hospital, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of *S. aureus*.⁽¹²⁾

Some reports have indicated a higher prevalence of inducible phenotypes, while others have indicated the frequency of incidence shifting from inducible to constitutive type in *S. aureus*.⁽¹³⁾ Indian reports on inducible clindamycin resistance are scanty.^(3,14) Sensitivity of D-test performed at 15-20 mm disk spacing was 100% when correlated with detection of *erm* and *msr* genes by polymerase chain reaction (PCR).^(2,8)

In our study, of 256 *S. aureus* studied over a period of time, Erythromycin resistant was seen in 142(55.46 %). Among the Erythromycin resistant *S. aureus*, iMLS_B resistance was observed in 21.12 % (30/142) similar to that reported by Gadepalliet al (21%), Fiebelkorn et al (28%) and Pal et al (24.63%)^(3,2,15) Some investigators have reported a lower incidence of inducible clindamycin resistance. Prabhu K et al (10%), AM Ciraj et al (13.1%), Deotale et al (14.5%), Janssen et al (7.2%)^(16, 17, 18, 13) while others reported higher incidence of iMLS_B resistance.^(14,19) Ajantha et al showed very high frequency of inducible resistance (63%) in erythromycin resistance clindamycin sensitive isolates being 74% in MRSA and 45% in MSSA.⁽²⁰⁾ On the contrary, Schreckenberger et al and Levin et al showed higher percentage of inducible resistance in MSSA as compared to MRSA, 7-12% in MRSA and 19-20% in MSSA; 12.5% MRSA and 68% MSSA respectively.^(21,22)

Our study found inducible resistance of 13.38 % in MRSA and 7.7% in MSSA. Yilmaz et al reported

inducible resistance of 24.4% in MRSA and 14.8% in MSSA; Gadepalliet al 30% in MRSA and 10% in MSSA, Deotale et al reported 27.6%, in MRSA and 1.6% in MSSA; Saikia et al reported 9.38% in MRSA and 3.33% in MSSA.^(1, 3, 18, 23)

As observed in studies by Steward et al and Schreckenberger et al our CLI induction results also showed two phenotypes, D (18.30%) and D⁺ (2.81%) phenotypes and both are considered to be positive D-zone test.^(8,21)

In our study Constitutive MLS_B resistance was noted 53.52%. Similar were the findings of Pal et al (46.97%), Fokaset et al found 3.5 per cent *S. aureus* isolates had inducible, 60 per cent had constitutive MLS resistance. Ciraj et al found MLS_{Bi} and MLS_{Bc} phenotypes were 5.4% and 43.7%.^(15, 24, 17) Interestingly, in a study by Janssen et al & Angel et al there has been no constitutive MLS_B resistance.^(13,14) Deotale et al found Constitutive resistance in 7.3% of MRSA isolates. Ciraj found 15.3% were cMLS_B phenotype among the MRSA strains. Saikia L et al reported 50% in MRSA isolates and 5% in MSSA.^(18, 17, 23) In our study constitutive phenotype predominated over the inducible phenotype among both in MRSA (39.43% vs. 13.38 %) and MSSA isolates (14.08% vs. 7.7%) similar is the finding of Gadepalli et al (38% vs 30%) in MSSA (15% vs 10 %)⁽³⁾

And also both the constitutive and inducible resistance phenotypes were found to be significantly higher in MRSA isolates as compared to MSSA (39.43%, 13.38 % and 14.08%, 7.7% per cent respectively). Prabhu K et al observed that percentages of inducible resistance and constitutive clindamycin resistance were higher amongst MRSA as compared to MSSA ((20%, 16.66% and 6.15%, 6.15%, respectively)⁽¹⁶⁾ Sireesha reported the

prevalence of constitutive MLS_B , $iMLS_B$ and MS phenotype in *S.aureus* isolates as 10%, 18% and 4% respectively.⁽²⁵⁾

In the present study, 25.35% of erythromycin-resistant *Staphylococcal* isolates showed true clindamycin susceptibility (MS phenotype). Patients with infections caused by such isolates can be treated with clindamycin without emergence of resistance during therapy.

The high frequency of methicillin-resistance isolates (63.33 %) with in-vitro inducible clindamycin resistance at our institute raises concern of clindamycin treatment failures with methicillin-resistant infections.

In the light of the restricted range of antibiotics available for the treatment of methicillin-resistant staphylococcal infections and the known limitations of vancomycin, accurate susceptibility data are

important for appropriate therapy decisions. Clindamycin should be considered for the management of serious soft tissue infections with methicillin-resistant staphylococci that are sensitive to clindamycin.⁽²⁶⁾ The true sensitivity to clindamycin can only be judged after performing D test on the erythromycin resistant isolates. However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug.⁽²⁷⁾ So, clinical microbiology laboratories should report inducible clindamycin resistance in *S. aureus*.

Conclusions:

Use of D test in a routine laboratory will enable us in guiding the clinicians regarding judicious use of clindamycin in skin and soft tissue infections; 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.

Figure No 1: D Phenotype

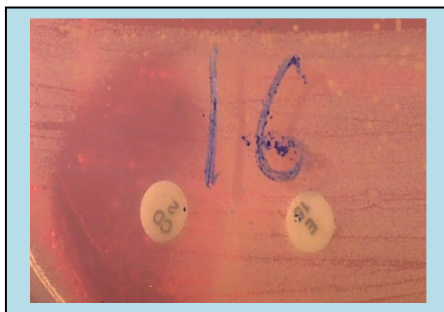


Figure No 2: D⁺Phenotype

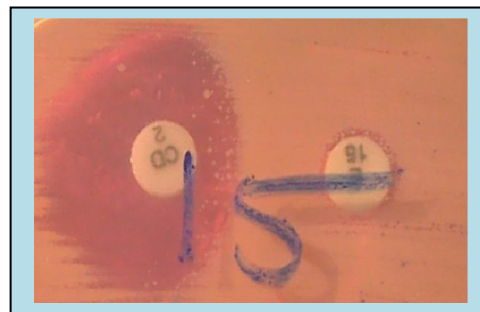


Figure No 3 : MS Phenotype

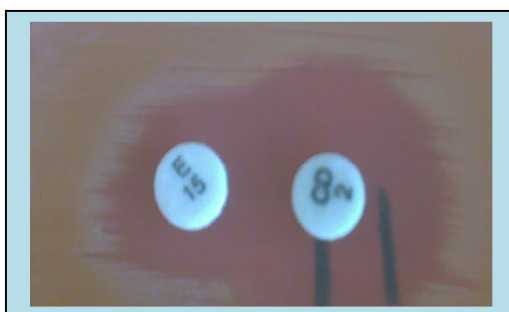
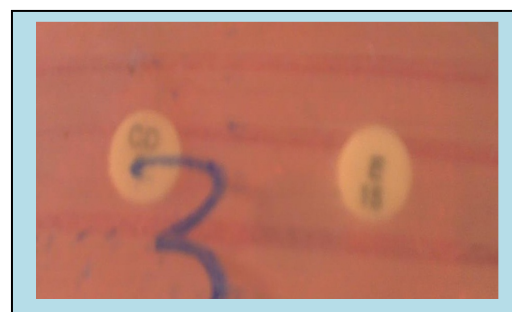


Figure No 4 : cMLS_BPhenotype



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