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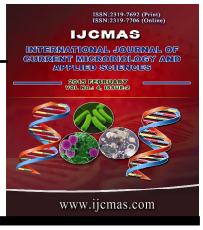

Original Research Article

Detection of various types of resistance patterns against Clindamycin among Staphylococcal isolates by phenotypic method

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Keywords

Constitutive resistance, D-Test, Inducible resistance, MRSA

A B S T R A C T

The macrolide lincosamide streptogramin B (MLS_B) group of antibiotics serves as an alternative for the treatment of various staphylococcal infections. However, increasing resistance to these drugs is frequently reported nowadays. The resistance is either constitutive ($cMLS_B$) or inducible ($iMLS_B$). This $iMLS_B$ can be easily missed if laboratory does not specifically look for it by performing 'D' Test. To demonstrate various patterns of clindamycin resistance in staphylococcal isolates and to determine their association with methicillin resistance. Antimicrobial susceptibility test were performed on 196 Staphylococcal isolates for erythromycin and methicillin resistance. Inducible clindamycin resistance was detected by using D-test on 104 erythromycin resistant isolates as per CLSI guidelines. In our study two distinct inducible phenotypes were noted. D phenotype (36 isolates) and D+ phenotype with isolated colonies inside the clindamycin-sensitive zone (16 isolates). Amongst non-inducible phenotypes, constitutive resistance was observed in 29 isolates, MS variety in 14 strains while Hazy 'D' (HD) phenotype was seen in 9 isolates. Both constitutive and inducible resistance were observed to be significantly higher in methicillin resistant isolates as compared to methicillin sensitive strains. D-Test is a simple test to detect $iMLS_B$ resistance avoiding treatment failure with clindamycin.

Introduction

Staphylococcus is one of the leading causes of pyogenic infections affecting mankind. Worldwide it is a primary cause of nosocomial and community acquired infections. Rapidly evolving multidrug resistance in these organisms has left the clinicians with very few therapeutic options. The macrolide-lincosamide-streptogramin B (MLS_B) group of antibiotics is one such alternative. These are structurally unrelated group of antibiotics with similar mechanism of action, Clindamycin, a lincosamide being the most preferred agent.

However, their widespread use has led to emergence of staphylococcal strains resistant to even these drugs.[1,2]

There are two different mechanisms causing resistance to MLS_B: first, an active efflux mechanism which is encoded by *msr A* gene (macrolides streptogramin resistance A) conferring MS phenotype. These strains appear clindamycin sensitive and erythromycin resistance in *vitro* and do not become clindamycin resistant during therapy. [3] The second mechanism being

the methylation of ribosomal target site, mediated by the *erm* gene (erythromycin ribosome methylase) encoding rRNA methylase enzyme. This mechanism can be either constitutive ($cMLS_B$), where thisenzyme is always produced, or inducible($iMLS_B$), where an inducing agent is required for its production.[3] Erythromycin is a strong inducer of methylase synthesis, while lincosamide such as clindamycin is comparatively a weak inducer.[4]

Antimicrobial Susceptibility Testing (AST) of staphylococcal isolates with constitutive phenotype ($cMLS_B$) reveals resistance to both erythromycin and clindamycin. On the contrary, those with inducible phenotype ($iMLS_B$) are resistant to erythromycin but appear susceptible to clindamycin if not placed adjacent to each other *in vitro*. However, administration of clindamycin in such patients results in emergence of constitutive *erm* mutants thereby rapidly developing clindamycin resistance causing clinical failure. [3,4]. These $iMLS_B$ phenotypes, when tested in the presence of a strong inducer (e.g. erythromycin), shows a'D' shape clindamycin zone of inhibition indicating its resistance which forms the basis of the *in vitro* induction test ('D' Test)[1,5]

Hence the present study was done to assess various patterns of clindamycin resistance in staphylococcal isolates and also to determine their association with methicillin resistance.

Materials and Methods

The study was conducted from April to December 2012 at SRTR Government Medical College, Ambajogai, Maharashtra.

Staphylococci isolated from pus/wound swab, urine, blood, respiratory secretions, aspirates etc were included in the study. Isolated microorganisms were identified as staphylococci by conventional methods such as colony morphology, Gram stain, catalase and slide/tube coagulase. Isolates were subjected to AST by modified Kirby-Bauer's disc diffusion method on Mueller Hinton agar (MHA) plates as per CLSI guidelines. Strains found resistant to erythromycin were further screened for inducible resistance by performing D-Test. Methicillin resistant isolates were detected by using 30µg cefoxitin disc.

For detection of inducible clindamycin resistance ($iMLS_B$), 15µg erythromycin disc and 2µg clindamycin disc were placed 15 mm apart (edge to edge) over a MHA plate previously swabbed with 0.5 McFarland suspension of erythromycin resistant isolate. It was incubated overnight at 37˚C. If $iMLS_B$ is present, diffusion of erythromycin through the agar will induce clindamycin resistance, resulting in a flattened 'D shaped' clindamycin zone of inhibition towards the side of erythromycin disc. It is the area between two discs where two drugs have diffused after 18-24 hours of incubation.[1,6] *S.aureus* ATCC 25923 was used as the quality control.

There are two distinct inducible phenotypes namely, **D** and **D+**:[3,5]

1) **D Phenotype**:In which there is D-shaped blunting in the inter-disc area with otherwise a clear zone around clindamycin disc.

2) **D+ Phenotype**: BluntedD-shapedzone of clindamycin with small colonies present between the edge of the zone of inhibition and the clindamycin disc.

The non inducible phenotypes[3] are:

1)MS Phenotype: Isolates showing resistance to erythromycin and sensitive to

clindamycin without any D zone.

2) Constitutive phenotype (R phenotype): Resistant to both clindamycin and erythromycin with confluent growth around discs.

3) HD phenotype: (Hazy D zone), showing two zones of growth around clindamycin disc, inner zone of a light, hazy growth up to clindamycin disc while the outer zone with heavy growth showing "D" shape.

4) S phenotype: Sensitive to both clindamycin and erythromycin.

Result and Discussion

A total number of 196 isolates were studied over a period of nine months. Majority of them were *S.aureus*(134) with 40%being MRSA. On subjecting all these 196 isolates to erythromycin sensitivity, 104(53%) were found to be erythromycin resistant. (Table.1). D test was performed on these erythromycin resistant strains for detection of various resistance patterns.

Amongst 52 inducible phenotypes, 36 isolates showed a 'D' shaped clear zone of inhibition around clindamycin with flattening towards erythromycin disc (D Phenotype), while 16 isolates showed blunted zone along with small colonies between the edge of the zone of inhibition and the clindamycin disc(D+ Phenotype). (Fig. 1 and 2)

As shown inFig. 3 and 4, non inducible strains demonstrated either a double zone of inhibition with an inner ring of reduced growth up to the edge of the disks (HD phenotype; 9 isolates) or confluent growth around the clindamycin and erythromycin disks (R phenotype; 29 isolates).

MS Phenotype(Fig. 5) was seen in 13.5% isolates.

Finally, 92 isolates were susceptible to both clindamycin and erythromycin(S phenotype). Majority of these isolates were methicillin sensitive (86 out of 92) and were not screened further.

Percentage of both inducible and constitutive resistance was found to be higher in methicillin resistant isolates as compared to methicillin sensitive staphylococcal strains. (Table 2)

Accurate AST for the clinical isolate is important to avoid indiscrimina te usage of antibiotics which has led to the emergence of multidrug resistant organisms.Production of methylase and efflux proteins is the most widespread and important mechanism of resistance among staphylococci, conferring resistance to MLS_B group of antibiotics.

Clindamycin is an excellent drug for treating majority of infections caused by staphylococci. It gets widely distributed in tissues owing to its good tissue penetration except for the central nervous system. Its efficacy is not affected by high bacterial load at the site of infection and dose-adjustment is not required even in severe hepatic or renal dysfunction. It is less expensive than some of the newer agents with good oral absorption, making it an attractive substitute for outpatient therapy.[7,8]

However, many recent reports have indicated treatment failure with this drug due to inducible clindamycin resistance despite of its*in vitro* susceptibility. [8] As $iMLS_B$ resistance cannot be determined using standard susceptibility methods and also as the prevalence of this resistance varies with different geographical locations, D-test becomes an important part of routine AST for all *Staphylococcal* isolates.[9]

Two distinct inducible phenotypes were noted in our study, one with clear zone of inhibition (D Phenotype) and other with smaller colonies inside the zone of inhibition (D+ Phenotype). These smaller colonies inside the D+ zone were found to have constitutive resistance on re-testing.

Although there is no clinical significance of these two inducible phenotypes, it is crucial to note that both are considered positive for induction of clindamycin resistance. They can also provide information to characterize the isolates for epidemiological studies in community. Studies using PCR have shown that D phenotype contained *erm*A gene while those with D+ has *erm*C gene, with or without *erm*A. [3,5,10] This, however, was not possible in the present study due to the unavailability of PCR at our institute.

Hazy D phenotype should not be confused with $iMLS_B$ resistance as growth extends up to the edge of the disc indicating clindamycin resistance. Studies have found them to have a variety of*ermA, ermC,* and *ermA* plus *msrA*gene. [3]

Studies on MLS_B resistance of staphylococci from various geographical regions have shown an array of resistance patterns. In our study we observed 26.5% of $iMLS_B$ phenotypes (52 out of 196) which is in comparable with the studies done by Fibelkorn *et al* and Angel *et al* who observed 28% and 23.2% of $iMLS_B$ phenotypes, respectively.[11,12]. It is also evident from our study that from all the 196 staphylococci screened, incidence of both inducible and constitutive resistance was higher in methicillin resistant isolates as compared to methicillin sensitive which is quite similar to most of the other studies. [12,13,14]

Slight variations are rather pertinent as clindamycin resistance is known to vary from one geographical area to another and also from one timeperiod to another even in the same city.[15,16] In our study we observed that nearly 50% erythromycin resistant isolates were having $iMLS_B$ resistance. Without performing D-test these isolates would have been wrongly reported as clindamycin susceptible causing therapeutic failure in patients. On the other hand $iMLS_B$ negative report confirms clindamycin susceptibility providing an excellent therapeutic option.

Table.1 Clindamycin resistance pattern

Erythromycin Resistance (n=104)						
	Constitutive 'R'-Phenotype	'HD' Phenotype	Inducible Phenotype (52)		MS Phenotype	**Total**
			'D' Phenotype	'D+' Phenotype		
MRSA	16	6	14	8	5	**49 (47.2%)**
MSSA	4	3	8	3	2	**20 (19.2%)**
MRCNS	8	-	12	5	6	**31 (29.8%)**
MSCNS	1	-	2	-	1	**4 (3.8%)**
Total	**29 (27.8%)**	**9 (8.7%)**	**36 (34.6%)**	**16 (15.4%)**	**14 (13.5%)**	**104**

MRSA- Methicillin Resistant *Staphylococcus aureus*; **MSSA-** Methicillin sensitive *Staphylococcus aureus*; **MRCNS-** Methicillin Resistant Coagulase negative *Staphylococci*; **MSCNS**- Methicillin Sensitive Coagulase negative *Staphylococci.* **HD**- Hazy D, **R-** Reistance to both erythromycin and clindamycin.

Table.2 Distribution of methicillin resistance amongst various phenotypes

Total staphylococcal isolates (n=196)			
Phenotype	**Methicillin resistant (%)**	**Methicillin sensitive (%)**	**Total(%)**
Inducible	39(75%)	13(25%)	**52**
Constitutive 'R'	24(82.75%)	5(17.25%)	**29**
HD	6(66.67%)	3 (33.33%)	**9**
MS	11(78.57%)	3 (21.43%)	**14**
S	6(6.52%)	86 (93.48%)	**92**
Total	**86**	**110**	**196**

HD- Hazy D, **R**- Reistance to both erythromycin and clindamycin, **S**- sensitive to both erythromycin and clindamycin

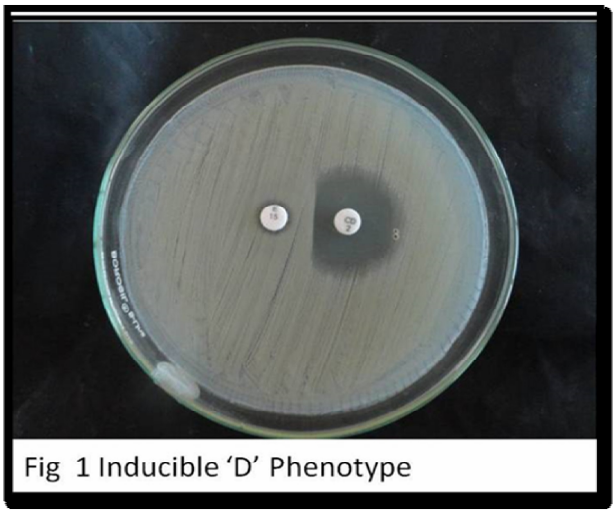

Fig 1 Inducible 'D' Phenotype

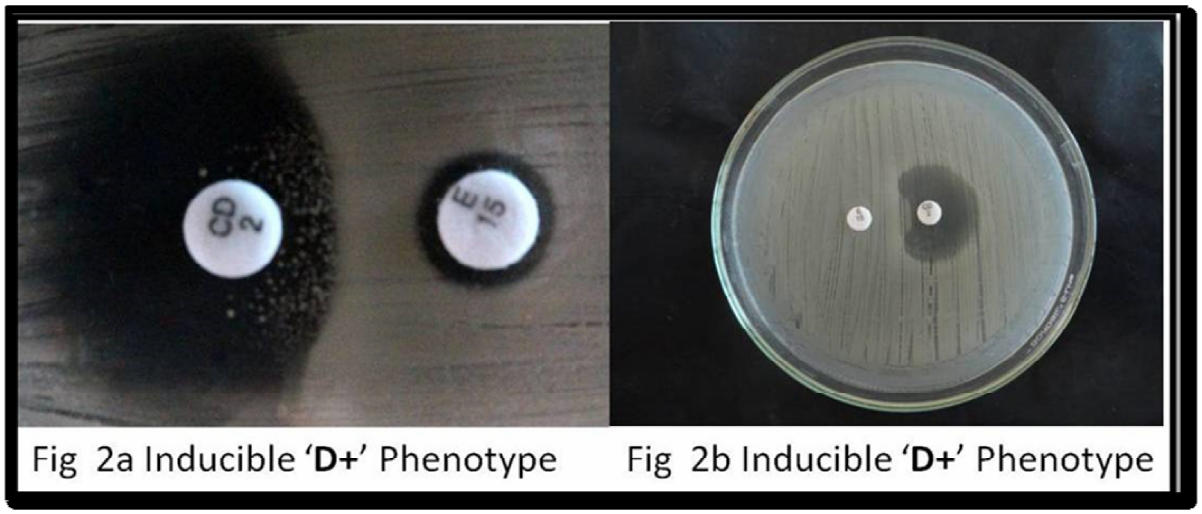

Fig 2a Inducible '**D+**' Phenotype Fig 2b Inducible '**D+**' Phenotype

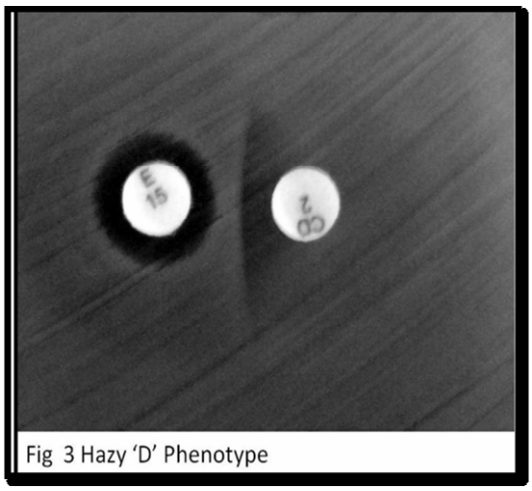
Fig 3 Hazy 'D' Phenotype


Fig 4 Constitutive 'R' Phenotype

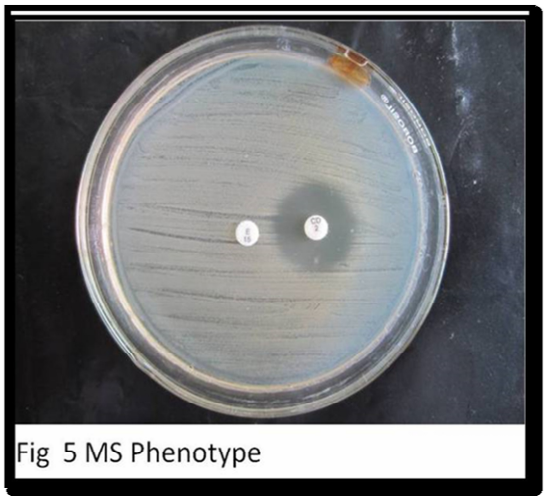
Fig 5 MS Phenotype

The present concluded in the clinical scenario with limited range of antibiotics available for MRSAtreatment and many known adverse drug reactions of vancomycin,clindamycin remains a good alternative for management of skin and soft tissue infections. [14]

True susceptibility of clindamycin can be confirmed only after performing D-test on erythromycin resistant isolates. With higher number of inducible clindamycin resistance in the present study, we recommend that D-test should be performed along with routine susceptibility testing for correct interpretation of various resistance patterns. This simple test will enable us in the judicious use of clindamycin for various infections.

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