Original Article

Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolated from Clinical Samples

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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 (MLS_B) antibiotics to treat *Staphylococcus aureus* (*S. aureus*) infections. 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 MLS_B antibiotics. *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.

Materials and Methods: One hundred and ninety *S. aureus* isolates were subjected to routine antibiotic susceptibility testing including oxacillin (1 µg) and cefoxitin (30 µg) by modified Kirby Bauer disc diffusion method. Inducible resistance to clindamycin in *S. aureus* was tested by 'D test' as per CLSI guidelines.

Results: Twenty (10%) isolates showed inducible clindamycin resistance, 18 (9%) showed constitutive resistance while remaining 16 (8%) showed MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA (20%, 16% and 6%, 6%, respectively).

Conclusion: Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infections depending upon the antimicrobial susceptibility results. This study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in *Staphylococci* for the optimum treatment of patients.

Keywords: Clindamycin resistance, constitutive MLSB phenotype, inducible MLSB phenotype, MRSA, MS phenotype

INTRODUCTION

Staphylococcus aureus (*S. 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.[2,3] However, widespread use of MLS_B antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to MLS_B antibiotics.[3,4]

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Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible.[5] The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype). 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. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, *Staphylococcal* isolates appear erythromycin-resistant and clindamycin-sensitive both *in vivo* and *in vitro* and the strain do not typically become clindamycin resistant during therapy.[3]

The present study was aimed to find out the percentage of *S. aureus* having inducible clindamycin resistance ($iMLS_B$) in our geographic area using D-test. Also, we tried to

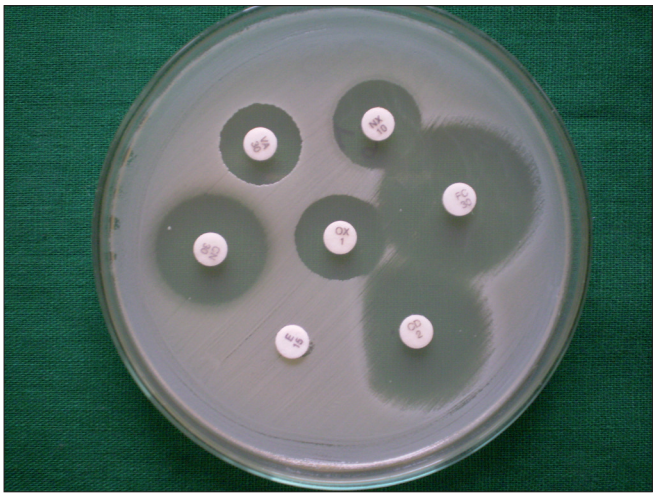

Figure 1: D-test showing inducible clindamycin resistance

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

MATERIALS AND METHODS

This prospective study was conducted from April 2010 to July 2010. A total of 190 *S. aureus* were isolated from various clinical specimens like pus, wound swab, aspirates, blood, and sterile fluids and tested. The isolates were first identified as *S. aureus* by standard biochemical techniques[6] and then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15 µg), norfloxacin (5 µg), fusidic acid (10 µg), vancomycin (30 µg), clindamycin (2 µg), oxacillin (1 µg), and cefoxitin (30 µg) as per CLSI guidelines.[7] An inhibition zone of 10 mm or less around oxacillin disc and 19 mm or less around cefoxitin disc indicates MRSA.

Inducible resistance to clindamycin was tested by 'D test' as per CLSI guidelines.[7] Briefly, erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) disc on a Mueller–Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D-shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance [Figure 1].

Three different phenotypes were appreciated after testing and then interpreted. This interpretation was done only for erythromycin-resistant *S. aureus* strains. All the erythromycin-sensitive strains were excluded.

1. MS phenotype - *Staphylococcal* isolate exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin was labeled as having this phenotype.
2. Inducible MLS_B ($iMLS_B$) phenotype - *Staphylococcal* isolate showing 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 was labeled as having this phenotype.
3. Constitutive MLS_B phenotype - this phenotype was labeled for those *Staphylococcal* isolates, which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around clindamycin.

Quality control (QC) of the erythromycin and clindamycin discs was performed with *S. aureus* ATCC25923, according to the standard disc diffusion QC procedure. Additional QC was performed with separate in-house selected *S. aureus* strains that demonstrated positive and negative D-test reactions.

Results were tabulated and analysed statistically.

RESULTS

One hundred and ninety *S. aureus* strains were tested for susceptibility to erythromycin and other antibiotics by routine disc diffusion testing; 54 (28.42%) of them were erythromycin resistant. Result of D-test analysis was shown in Table 1. Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA [Table 2].

DISCUSSION

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.[8] Also, clindamycin has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics.[9] However, clindamycin resistance can develop in *Staphylococcal* isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both *in vitro* testing and *in vivo* during clindamycin therapy.[1] 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.[3] Since the $iMLS_B$ resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an imperative part of routine antimicrobial susceptibility

Table 1: Susceptibility to erythromycin (ERY) and clindamycin (CL) among all *S. aureus* isolates

Susceptibility pattern (Phenotype)	Number of isolates	Percentage
ERY-S, CL-S	136	71.57%
ERY-R, CL-R (Constitutive MLS_B)	18	9.47%
ERY-R, CL-S (D-test positive, $iMLS_B$)	20	10.52%
ERY-R, CL-S (D-test negative, MS)	16	8.42%
Total	190	100

ERY=Erythromycin, CL=Clindamycin, S=Sensitive, R=Resistant, Constitutive MLS_B=Constitutive MLS_B phenotype, $iMLS_B$=inducible MLS_B phenotype, MS=MS phenotype

Table 2: Association of clindamycin resistance with methicillin resistance

Clindamycin resistance	Methicillin Resistance		Total (n = 190)
	MRSA (n = 60)	MSSA (n = 130)	
ERY-S, CL-S	30 (50)	106 (81.64)	136 (71.57)
ERY-R,CL-R (Constitutive MLS_B)	10 (16.66)	8 (6.15)	18 (9.47)
ERY-R, CL-S (D-test positive, $iMLS_B$)	12 (20)	8 (6.15)	20 (10.52)
ERY-R, CL-S (D-test negative, MS)	8 (13.33)	8 (6.15)	16 (8.42)

ERY=Erythromycin, CL=Clindamycin, S=Sensitive, R=Resistant, Constitutive MLS_B=Constitutive MLS_B phenotype, $iMLS_B$=inducible MLS_B phenotype, MS=MS phenotype, MRSA=Methicillin-resistant *Staphylococcus aureus*, MSSA=Methicillin sensitive *Staphylococcus aureus*, Figures in parenthesis are in percentage

test for all clinical isolates of *S. aureus*.[10]

In our study we found high percentage of erythromycin-resistant *S. aureus* isolates (28.42%). Among them 20 (37.52%) isolates tested positive for inducible clindamycin resistance by D-test, while rest of the isolates were negative for D-test, out of which 18 (16.66%) were shown to have constitutive clindamycin resistance and 16 (29.62%) showed true sensitivity to clindamycin (MS phenotype). The findings are consistent with the previous studies,[11] and these observations suggest that had D-test not been performed, one-third of the erythromycin-resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

It was also 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). This was in concordance with few of the studies reported before.[1] Some studies have shown a very high frequency of inducible resistance MRSA.[4] On the contrary, few studies have showed higher percentage of inducible resistance in MSSA as compared to MRSA.[12,13]

Accurate susceptibility data are important for appropriate therapy decisions. The pattern of macrolide resistance in *S. aureus* varies in different regions. Depending upon this the prescription rate will not be uniform in different regions. There is no substantial data regarding clindamycin prescription from India. It is kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending upon the antimicrobial susceptibility results. Further, by using clindamycin, use of vancomycin can be avoided.[10] However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug.[14] So, clinical microbiology laboratories should report inducible clindamycin resistance in *S. aureus*, and D-test can be used as a simple, auxiliary and reliable method to delineate inducible and constitutive clindamycin resistance in routine clinical laboratories.

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