

Detection of inducible Clindamycin resistance in clinical isolates of *Staphylococcus aureus* by the Disc Diffusion Induction Test

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ABSTRACT

Therapeutic failure to Clindamycin has been reported due to mechanisms which confer resistance constitutively, or by the presence of low level inducers which can lead to therapeutic failure. Therefore, this study was undertaken to identify the strains that have the potential to become resistant during therapy. Inducible Clindamycin resistance was tested by the Clindamycin disc induction test (D test) as per the CLSI recommendations.

The study showed 54.78% MRSA isolates and 32.5% inducible Clindamycin resistant isolates among them as compared to 15.53% in MSSA isolates. We concluded that routine screening for inducible resistance to Clindamycin must be performed so that the drug is used effectively and for maximum clinical utility.

Key Words: inducible clindamycin resistance, staphylococcus

MAIN ARTICLE

The increasing frequency of Methicillin resistant *Staphylococcus aureus* (MRSA) infections and the changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat such infections [1]. However, their widespread use has led to an increase in the number of *Staphylococcal* strains which are resistant to MLSB antibiotics [2]. Resistance to MLSB can occur by two different mechanisms: an active efflux mechanism encoded by the *msr A* gene (macrolides streptogramin resistance) and the ribosomal target modification affecting macrolides, lincosamides and type B streptogramins coded by the *erm* gene (MLSB resistance). *erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, thereby reducing the binding by MLS agents to the ribosome. The *msr A* gene confers the so called MS phenotype (resistance to erythromycin, inducible resistance to streptogramins and susceptibility to clindamycin) by efflux [3]. MLSB resistance can be either constitutive (cMLSB) or inducible (iMLSB). In vitro *Staphylococci* isolates with constitutive resistance are resistant to both erythromycin (E) and clindamycin (CL), while isolates with inducible resistance are resistant to, but appear to be susceptible to clindamycin CL [1]. Inducible MLSB resistance cannot be determined by using standard susceptibility test methods including standard broth based or agar dilutions susceptibility tests. Low levels of ER are the most effective inducers of inducible MLSB resistance [2].

Uncertainty about the reliability of the susceptibility reports for clindamycin, as well as confusion over the clinical importance of this inducible resistance, has led some clinicians to avoid the use of clindamycin for *Staphylococcal* infections where erythromycin resistance is noted. Failure to identify MLSB may lead to the clinical failure of the CL therapy. iMLSB can be detected by a disc induction test in which a distorted 'D-Shaped' zone of inhibition is observed around CL if an ER disc is placed nearby (15mm) [2].

Thus, the aim of the present study was to detect the inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus* by the disc diffusion induction test.

MATERIALS AND METHODS

The present study was conducted for a period of 10 months from March-2009 to December-2009 and included a total of 230 non duplicate, consecutive isolates of *S.aureus* from samples of pus/wound swab, respiratory tract infections, blood, urine, body fluids, etc. The *Staphylococcus aureus* strains were identified by using standard microbiological procedures [4]. Antibiotic susceptibility

tests were performed by the Kirby-Bauer disc diffusion method. Methicillin resistance was detected by using a 30 µg cefoxitin disc. *Staphylococcus ATCC 25923* was used as the control strain for the disc diffusion method.

To identify the MLSBi phenotype, the D-test was performed. 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 [5]. The disc diffusion test, based on the D test, showed four phenotypes.

D Positive (iMLSB Phenotype): Inducible resistance to Clindamycin was manifested by flattening or blunting of the CL zone adjacent to the ER disc, giving a D shape. [Table/Fig 1]



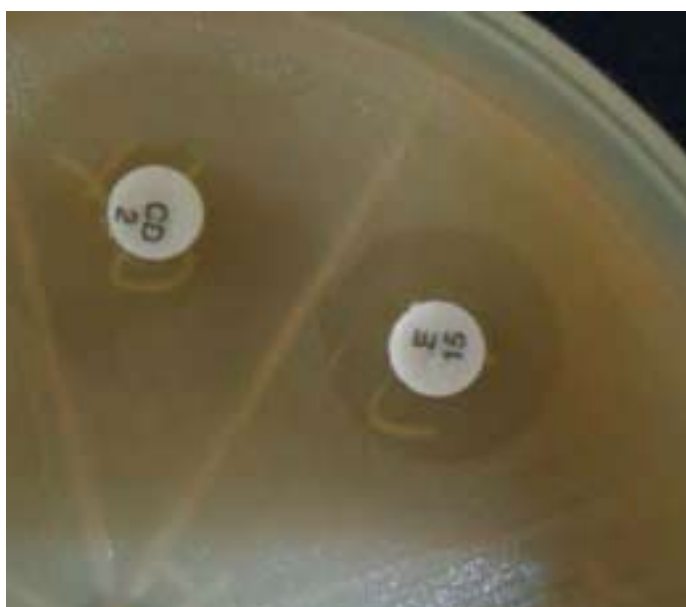
[Table/Fig: 1] D test positive isolate, inducible resistance to Clindamycin



[Table/Fig: 2] D test negative isolate. Resistant to erythromycin and sensitive to clindamycin



[Table/Fig: 3] Constitutive resistance to clindamycin



[Table/Fig: 4] Sensitive isolate. E-erythromycin 15 µg disc; CL-clindamycin 2 µg disc

D Negative (MSB Phenotype): No flattening of the CL zone; Resistant to ER but susceptible to CL. [Table/Fig 2]

Constitutive Resistance (cMLS_B Phenotype): Resistant to both ER and CL. [Table/Fig 3]

[Sensitive (Phenotype): Sensitive to both ER and CL. [Table/Fig 4]

RESULTS

Among the 230 clinical isolates of Staphylococcus aureus, 54.78% (126) were Methicillin resistant Staphylococcus aureus (MRSA) and 45.21 (104) were Methicillin sensitive Staphylococcus aureus (MSSA) [Table/Fig 5]

Total Samples	MRSA		MSSA	
	No	%	No	%
230	126	54.78	103	45.21%

[Table/Fig: 5]: Occurrence of MRSA & MSSA isolates in Staphylococcus aureus

Of the 230 Staphylococcus aureus isolates, 41.3% (95) had the E-S and CL-S phenotype, 18.26 % (42) had a constitutive resistance phenotype, 15.65 % (36) had an MSB phenotype and 24.89% (57) had the inducible resistance phenotype. [Table/Fig 6]

Phenotype	MRSA(%)	MSSA(%)	Total
ER-S, CL-S	34(26.98%)	61(58.65%)	95(41-30)
ER-R, CL-R(MLS _B c)	32(25.39%)	10(9.61%)	42(18.26%)
ER-R, CL-S,D (MSB)	19(15.07)	17(16.34)	36(15.65%)
ER,CL-S,D+(MLS _B i)	41(32.53%)	16(15.38%)	57(24.89)
Total	126	104	230

[Table/Fig 6]: Susceptibility to Erythromycin(ER) and Clindamycin (CL) among all Staphylococcus aureus isolates

Among the MRSA isolates, the inducible resistant phenotype (32.53%) predominated over the ER-S and CL-S phenotype (26.98%, 25.39% and 15.07% respectively). The ER-S and CL-S phenotype (58.65%) predominated over the MSB phenotype and the inducible resistance and the constitutive resistance phenotypes (16.34%, 15.38% and 9.61% respectively) among the MSSA isolates.

While in Methicillin resistant Staphylococcus aureus isolates, the constitutive CL-R phenotype level was 25.39% and the inducible resistance phenotype was 32.53%; in Methicillin sensitive Staphylococcal isolates, the constitutive CL-R phenotype was 9.61% and the inducible resistant phenotype level was 15.38%.

DISCUSSION

Clindamycin is a drug which is useful for treating skin and soft tissue infections which are caused by Staphylococcus aureus. It has excellent tissue penetration, it accumulates in abscesses, it is not impeded by high bacterial burden at the infection site and no renal dose adjustments are needed. Good oral absorption makes it an important option in outpatient therapy as follow up after intravenous therapy. CL is also a good alternative antibiotic for the penicillin allergic patients and in infections due to MRSA. CL is less costly than some of the newer agents that might be considered for these infections. However, recent reports indicate that treatment failure may occur in the case of inducible MLS_B resistance, in spite of invitro susceptibility to CL [6].

Accurate susceptibility data are important for appropriate therapy decisions. However, false in vitro susceptibility results may be

obtained by the microdilution method and disc diffusion testing with erythromycin and clindamycin disc is nonadjacent positions. Hence, the routine testing of Staphylococcal isolates for inducible clindamycin resistance is recommended by the 2005 CLSI guidelines [7].

The prevalence of the MRSA strains was high (54.78%) in the present study, which is similar to the results obtained by Gadepalli et al (52%), Mallick SR et al (51.6%) and S. Anuprabha et al (54.8%). [7],[8],[9]

Of the total 230 isolates, 57(24.78%) were of the iMLSB phenotype in the present study, which is similar to that reported by Angel et al from CMC Vellore (23.2%) and Fiebelkorn et al who have reported 28% iMLSB[10],[11]. Various authors have highlighted the relationship of MRSA and MSSA with different phenotypes of S.aureus isolates. 41 (32.5%) of the 126 MRSA isolates were found to be of the iMLSB phenotype in the present study. Studies from different parts of India have reported 30% to 64% of the MRSA isolates to be of the iMLSB phenotype [8].

In the present study, 15.53% of the MSSA isolates showed iMLSB resistance, which correlates with the findings by Delialioğlu et al, who reported 10.7% iMLSB resistance in the MSSA isolates and Gupta et al who reported 17.3% iMLSB resistance in the MSSA isolates [3],[12].

In the present study, 42 (18.26%) S.aureus strains were of the cMLSB type, of which 25.39% were MRSA and 9.6% were MSSA. Angel et al have not found any cMLSB resistance in the S.aureus strains. However, Gupta et al have reported 19% cMLB resistance, 46% of which were of the MRSA type and 10% were of the MSSA type [10],[12]. 15.65% S.aureus strains showed the MSB phenotype in the present study. Gadepalli et al have reported 12% strains of the MSB phenotype among the S.aureus strains[7].

Most of the studies have indicated a higher prevalence of constitutive resistance than inducible resistance in Staphylococcus aureus. The true incidence depends on the patient population studied, the geographical region, the hospital characteristics and Methicillin susceptibility. The present study showed a higher incidence of iMLSB (24.89%) as compared to cMLSB (18.26%) in the S.aureus isolates, which was similar to the findings of a study conducted by Mallick et al, who reported 18.6% strains of iMLSB and 3.8% strains of cMLSB [8].

Furthermore, the D-test was performed as a routine test on all Staphylococcal strains which were isolated in the laboratory, whereas most of the published D-test studies select only isolates that are ER resistant and CL susceptible for testing. The authors were concerned that if the D-test was delayed until ER resistance

and CL susceptibility were noted in the isolate, the results may not be available for maximum clinical utility.

In conclusion, the implementation of the D-test or the Disc induction test, a simple, auxiliary method with routine antibiotic susceptibility testing, delineates inducible and constitutive clindamycin resistance. The high rates of occurrence of inducible resistance in both the MSSA and MRSA strains raise concerns that clindamycin treatment failures may occur with MSSA as well as with MRSA infection. Consequently, early detection helps in the use of CL only in infections caused by truly CL susceptible S.aureus and thus helps to avoid treatment failures.

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