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Original Article

Inducible clindamycin resistance in clinical isolates of staphylococci in a rural hospital.

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ABSTRACT

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. 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. This study was undertaken to detect the presence of inducible clindamycin resistance among clinical isolates of staphylococci. Inducible clindamycin resistance was tested by the clindamycin disc induction test (D test) as per the CLSI recommendations. A total of 140 strains of staphylococci (*Staphylococcus aureus*-70 and CONS -70), isolated from various clinical samples at our institution, were included in this study. The isolates were identified using conventional methods. Of total 140 isolates included in this study 19(13.57%) were MRSA, 51(36.42%) were Methicillin Sensitive *Staphylococcus Aureus* (MSSA), 19(13.57%) were Methicillin Resistant Coagulase Negative *Staphylococci* (MRCONS) and 51(36.42%) were Methicillin Sensitive Coagulase Negative *Staphylococci* (MSCONS). Inducible clindamycin resistance was detected in 10.52% of 19 MRSA, 5.26% of 19 MRCONS and 3.92% of 51 MSCONS isolates and in none of the 51 MSSA. In our setting, clindamycin can be used for the treatment of infections due to staphylococci, but we recommend that staphylococci isolates, particularly MRSA, should be tested by the D-test before treatment so that the drug is used effectively and for maximum clinical utility.

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1. Introduction

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]. Macrolides, lincosamides and streptogramins (MLS) antibiotics are structurally unrelated; however they are related microbiologically because of their similar mode of action [2]. Resistance to MLSB can occur by two different mechanisms: an active efflux mechanism encoded by *msrA* gene

(macrolide, streptogramin resistance) and ribosomal target modification affecting macrolides, lincosamide and type B streptogramins coded by the *erm* gene (MLSB resistance). The *erm* genes encode enzymes that confer inducible and constitutive resistance to MLS agents via methylation of the 23S rRNA, thereby reducing the binding by MLS agents to ribosomes. The *msrA* gene confers the so called MS phenotype (resistance to erythromycin, inducible resistance to streptogramins and susceptibility to clindamycin) by efflux [3] Target site modification is the most common mechanism of acquired resistance to MLSB in staphylococci [4] MLSB resistance can be either constitutive (MLSBC) or inducible (MLSBI). In vitro MRSA isolates with constitutive resistance are resistant to erythromycin (ER) and clindamycin (CL), while isolates with inducible resistance are resistant to ER but appear susceptible to CL [5, 6, 7] Constitutive resistance to CL can be detected by standard susceptibility testing methods whereas, MLSBI is not recognized by using standard

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susceptibility test methods, including standard broth based or agar dilution susceptibility tests [8] or by size of inhibition zone. Failure to identify inducible CL resistance may lead to clinical failure of CL therapy (a frequent choice, particularly for staphylococcal skin and soft tissue infections) [7]

MLS_Bi can be detected by a disc induction test, a distorted 'D-shaped' zone of inhibition is observed around CL if an ER disc is placed nearby (15-20mm).

This study was undertaken to detect the presence of inducible clindamycin resistance among clinical isolates of staphylococci by disc diffusion induction test.

2. Materials and Methods

This study was undertaken to detect the presence of inducible clindamycin resistance among clinical isolates of staphylococci. A total of 140 strains of staphylococci (*Staphylococcus aureus*-70 and CONS -70), isolated from various clinical samples at our institution, were included in this study. The isolates were identified using conventional methods [9]

Inducible Clindamycin resistance was tested by the Clindamycin disc induction test (D test), [10] using erythromycin disc (15ug) and clindamycin disc (2 ug) procured from Hi media India ltd. The discs were placed at a distance of 15mm from centre to centre on Muller Hinton agar plates inoculated with test organism, as per the CLSI recommendations.

Flattening of the zone of Clindamycin towards the side facing the erythromycin disc showed the positive D test i.e. presence of inducible clindamycin resistance. Growth of organism till the edge of both the discs was taken as constitutive clindamycin resistance.

Quality control of erythromycin and clindamycin discs was performed with *Staphylococcus aureus* ATCC 25923 strain.

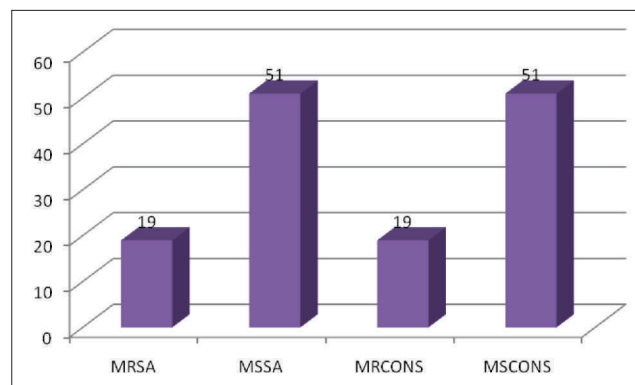
3.Results:

Of total 140 isolates included in this study 19(13.57%) were MRSA, 51(36.42%) were Methicillin Sensitive *Staphylococcus Aureus* (MSSA), 19(13.57%) were Methicillin Resistant Coagulase Negative *Staphylococci* (MRCONS) and 51(36.42%) were Methicillin Sensitive Coagulase Negative *Staphylococci* (MSCONS). (Table 1, Fig 1)

Table: 1 - Methicillin resistant and Methicillin susceptible *S. aureus* and CONS

Type of isolate	No. of isolates (%)
MRSA	19 (13.57)
MSSA	51 (36.42)
MRCONS	19 (13.57)
MSCONS	51 (36.42)
TOTAL	140 (100)

Fig: 1 Methicillin resistance in *Staphylococcus aureus* and CONS isolates.



Inducible Clindamycin resistance was detected in 10.52% of the 19 MRSA, 5.26% of the 19 MRCONS and 3.92% of the 51 MSCONS isolates and in none of the 51 MSSA isolates. Constitutive resistance to MLS_B was detected in 37 (26.43%) of the total isolates. It was more observed in MRSA 10(52.63%) and MRCONS 15(78.95%). (Table 2)

Table: 2 – Resistance phenotypes of isolates

Sensitivity pattern	MRSA n (%)	MSSA n (%)	MRCONS n (%)	MSCONS n (%)	Total
E-S CL-S	4(21.05)	44(86.27)	3(15.79)	33(64.71)	84(60)
E-R CL-R (Constitutive)	10(52.63)	4(7.84)	15(78.95)	8(15.69)	37(26.43)
E-R CL-S (D test positive - MLS _B i)	2(10.53)	0		2(3.92)	5(3.57)
E-R CL-S (D test Negative- MS Phenotype)	3(15.79)	3(5.88)	1(5.26)	8(15.69)	14(10)
Total	19(100)	51(100)	19(100)	51(100)	140(100)

Fig 2: *Staphylococcal* isolate sensitive to both Erythromycin and clindamycin suggestive of E-S CL-S Phenotype

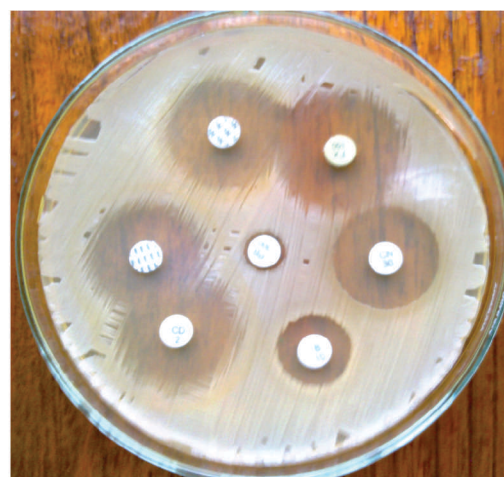


Fig: 3 Erythromycin resistant and Clindamycin sensitive Staphylococcal isolate with circular zone of inhibition around Clindamycin suggestive of MS Phenotype

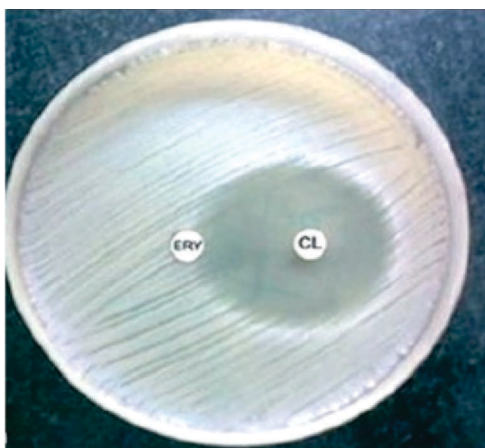


Fig: 4 Erythromycin resistant and Clindamycin sensitive Staphylococcal isolate giving D shaped zone of inhibition around Clindamycin with flattening toward Erythromycin disc suggestive of Inducible MLSB phenotype



Fig: 5 Staphylococcal isolate resistant to both Erythromycin and clindamycin suggestive of constitutive MLSB Phenotype



4. Discussion

The macrolide- lincosamide- streptogramin B family of antibiotics is commonly used in treatment of staphylococcal infections. However one important issue in Clindamycin treatment is the risk of clinical failure during therapy. Therapeutic failure caused by MLSB inducible resistance is being more commonly reported [11]. MLSB resistance can be either constitutive (MLSBc) or inducible (MLSBi). The erythromycin resistance methylase (erm) genes encode enzymes that confer inducible or constitutive resistance to MLSB agents. Constitutively resistant isolates are resistant to all MLSB antibiotics and are detected readily by standard susceptibility testing methods. Inducible resistance is expressed in presence of strong inducers of methylase synthesis, such as 14 membered (e.g. erythromycin), and 15 membered (e.g. azithromycin) macrolides. The 16 membered macrolides (e.g. spiramycin), lincosamide (e.g. Clindamycin) and streptogramin B antibiotics may appear active when susceptibility is tested by standard method as they are only weak inducers of methylase synthesis, but inducible resistance can be detected by the disc diffusion induction test (D test) [10]. The strains that are resistant to both erythromycin and clindamycin are defined as showing constitutive MLSB resistance (Fig 5). The strains that show flattening of Clindamycin zone adjacent to erythromycin disc are defined as having inducible MLSB resistance (Fig 4). The strains that are resistant to erythromycin and sensitive to clindamycin (with no induction) are defined as MS phenotype (Fig 3) (8,12).

Reporting *Staphylococcus aureus* as susceptible to Clindamycin without checking for inducible clindamycin resistance may thus 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. To avoid false in vitro results routine testing of staphylococcal isolates for inducible clindamycin resistance is recommended by 2005 CLSI guidelines.

In the present study out of total of 140 isolates tested according to these guidelines, it was found that 5(3.57 %) isolates showed inducible clindamycin resistance. The inducible clindamycin resistance was more observed in MRSA, 2 (10.53%).

Characteristically, reports from different regions have shown a different pattern of resistance. Some reports have indicated a higher prevalence of inducible phenotype while others have indicated the frequency of incidence shifting from inducible to constitutive type.

In our study we found that, out of total 140 isolates 37 (26.43%) showed constitutive resistance, which were more frequently observed in MRSA 10(52.63%) and MRCONS 15 (78.95%). The total 14 (10%) isolates which were erythromycin resistant and clindamycin susceptible (with no inducible

were susceptible to clindamycin and erythromycin respectively in SS phenotype. (Table)

Most of the studies have indicated higher incidence of constitutive MLSB (MLSBc) resistance than inducible MLSB resistance (MLSBi). Shantala G B et al [1] also found higher incidence of MLSBc in *Staphylococcus aureus* 18.26% and it was observed to be more in MRSA 25.39%. Gupta et al [13] have reported MLSBc resistance in 19% of total isolates of which 46% were MRSA type and 10% were MSSA type.

We also recorded higher incidence of MLSBc in CONS 32.86%. It was more in MRCONS 78.95% as compared to MSCONS 15.69%. In a study by Shreckenberger et al [6] which had covered 2 hospitals in their study, at the university of Illinois medical centre constitutive resistance among CONS was found to be 37%, and inducible MLSB resistance in CONS was 14%. Delialioglu et al [3] also reported higher constitutive resistance 40.2% among CONS.

There is a higher variation for constitutive clindamycin resistance between various studies, because it depends on overuse of the drug and conversion of inducible phenotype to constitutive phenotype during treatment [14].

Inducible clindamycin resistance was detected in 10.52% of MRSA and 5.26% of MRCONS in the present study. Delialioglu et al [3] in their study have also reported only 5.4% inducible MLSB resistance among MRSA isolates. Whereas Deotale et al [15] reported 45% of isolates of *Staphylococcus aureus* to be MLSBi. The true incidence of MLSBc and MLSBi depends on patient population studied, the methicillin susceptibility and hospital characteristics.

In our study prevalence of inducible clindamycin was not very high.

Use of D test in a routine laboratory will enable us in guiding clinicians about judicious use of clindamycin; as clindamycin is not a suitable drug for D test positive isolates while it can definitively prove to be a drug of choice in case of D test negative isolates. We conclude that it is important for laboratories to be aware of the local prevalence of MLS Bi isolates. On the basis of their data they can choose whether or not to perform the D-test routinely. The D-test is an easy, sensitive, and reliable means for detection of MLSBi strains in a clinical laboratory setting without specialized testing facilities. This prevalence of MLSBi may change over time with the emergence of strains with different sensitivity patterns, so periodic surveys should be performed if testing is not a routine.

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