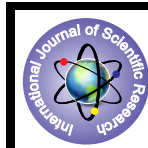


## Inducible Clindamycin Resistance in Staphylococcus Aureus Isolated from Clinical Samples



### Medical Science

**KEYWORDS :** Mobile phone marketing weather information and farmers

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### ABSTRACT

**Purpose:** Clindamycin is commonly used in the treatment of erythromycin resistant *Staphylococcus aureus* causing skin and soft tissue infections. 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 routine basis. **Materials and Method:** 234 *Staphylococcus aureus* isolates were subjected to routine antibiotic susceptibility testing including cefoxitin (30mcg) by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by D test, as per CLSI guidelines on erythromycin resistant isolates. **Results:** 59 (42.44%) isolates showed inducible clindamycin resistance, 39 (28.05%) showed constitutive resistance while remaining 41 (29.49%) showed MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA (35.83%, 28.33% and 14.03%, 4.38% respectively). **Conclusion:** Study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

### Introduction

Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives available to treat staphylococcal infections. The macrolide-lincosamide-streptogramin B (MLS B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due its excellent pharmacokinetic properties. [1]

However, widespread use of MLS B antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS B antibiotics. [2] 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 does not typically become clindamycin resistant during therapy. [3]

This study demonstrates a very simple method of detecting inducible resistance to clindamycin in erythromycin resistant staphylococcal isolates. i.e. D test as described by Fiebelkorn et al. [1],[4]

### Materials and Methods

The study was conducted from October 2010 to July 2011. A total of 234 isolates of *Staphylococcus aureus* isolated from various clinical specimens like pus, wound swab, aspirates, blood, and sterile fluids were tested. The isolates were first identified as *S. aureus* by standard biochemical techniques [5] and then subjected to susceptibility testing by Kirby Bauers disc diffusion method on Mueller Hinton agar plates using erythromycin (15mcg), penicillin (10U), ciprofloxacin (5 mcg), vancomycin (30mcg) and cefoxitin (30 mcg) as per CLSI guidelines. Methicillin resistance was detected using cefoxitin (30 mcg) on Mueller Hinton agar by incubation at 370 C. [4]

Those isolates which were erythromycin resistant were further subjected to 'D test' as per CLSI guidelines. Briefly, erythromycin (15 mcg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 mcg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at

37 0 C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance [4] [Figure ]b. Three different phenotypes were appreciated after testing and interpreted as follows: Figure :



a b c

1. MS Phenotype - *Staphylococcal* isolates exhibiting resistance to erythromycin (zone size  $\leq 13$ mm) while sensitive to clindamycin (zone size  $\geq 21$ mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype [Figure ]a
2. Inducible MLS B Phenotype - *Staphylococcal* isolates showing resistance to erythromycin (zone size  $\leq 13$ mm) while being sensitive to clindamycin (zone size  $\geq 21$ mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype [Figure ]b.
3. Constitutive MLSB Phenotype - this phenotype was labelled for those *Staphylococcal* isolates which showed resistance to both erythromycin (zone size  $\leq 13$ mm) and clindamycin (zone size  $\leq 14$ mm) with circular shape of zone of inhibition if any around clindamycin [Figure ]c.

### Results

Two hundred and thirty four *Staphylococcal* isolates were tested for susceptibility to erythromycin and other antibiotics of the panel by routine disc diffusion testing; 139 (59.40%) of them were erythromycin resistant. These isolates when subjected to D test showed nine 39 (28.05%) isolates resistant to both erythromycin and clindamycin indicating constitutive MLS B Phenotype; 100 isolates showed clindamycin sensitivity. Out of these, 59 isolates showed positive D test indicating inducible MLS B phenotype while 41 gave negative D test indicating MS phenotype [Table 1]. The overall percentage resistance for all three phenotypes was as follows.

**Table 1 :Distribution of isolates**

Susceptibility pattern(Phenotype)	MRSA (%)	MSSA (%)	TOTAL (%)
cMLSB Resistance	34 (28.33)	5 (4.38)	39 (28.05)
iMLSB Resistance	43 (35.83)	16 (14.03)	59 (42.44)
MS Phenotype	17 (14.16)	24 (21.05)	41 (29.49)

cMLSB- Constitutive Macrolid Lincosamide Streptogramin B  
 iMLSB- Inducible Macrolid Lincosamide Streptogramin B  
 MS- Macrolid Streptogramin

Inducible clindamycin resistance - 42.44%  
 constitutive clindamycin resistance - 28.05%  
 MS Phenotype - 29.49%

Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA [Table 1].

### Discussion

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms. There are many options available for treatment of MSSA and MRSA infections, with clindamycin being one of the good alternatives. [1] 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. [6] Reporting *Staphylococcus 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. [7]

In our study we found high percentage of erythromycin resistant isolates [139 (59.4%)]. Amongst them 59 (42.44%) isolates tested positive for inducible clindamycin resistance by D test while rest of the isolates were negative for D test, out of which 39 (28.05%) were shown to have constitutive clindamycin resistance and 41 (29.49%) showed true sensitivity to clindamycin

(MS phenotype). These observations suggest that had D test not been performed, nearly half 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 resistance were higher amongst MRSA (35.83% and 28.33% respectively) as compared to MSSA (14.03% and 4.38%). This was in concordance with a few of the studies reported before - Yilmaz et al.[6] found inducible resistance of 24.4% in MRSA and 14.8% in MSSA; Gadepalli et al.[2] showed it to be 30% in MRSA and 10% in MSSA, while Mohamed Rahabar et al.[8] reported 22.6% in MRSA and 4% in MSSA. Another study from India [9] showed very high frequency of inducible resistance (63%) in erythromycin -R, clindamycin sensitive isolates being 74% in MRSA and 45% in MSSA. On the contrary, Schreckenberger et al.[10] and Levin et al.[11] 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.

Constitutive resistance in our study was seen in 28.33% of MRSA isolates and 4.38% in MSSA isolates. Gadepalli et al reported higher constitutive resistance in MRSA (38%) than MSSA (15%)(2). Other studies from India showing similar results are of K.M.Mohansundaram 19% in MRSA and 10% in MSSA.(12)

In the light of the restricted range of antibiotics available for the treatment of methicillin-resistant staphylococcal infections and the known limitations of vancomycin, clindamycin should be considered for the management of serious soft tissue infections with methicillin-resistant staphylococci that are sensitive to clindamycin. [13] The true sensitivity to clindamycin can only be judged after performing D test on the erythromycin resistant isolates. The prevalence of inducible clindamycin resistance may vary from hospital to hospital. Although we did not study the prevalence of inducible clindamycin resistance in our area, from the current study, we can conclude that there is a fairly high percentage of inducible clindamycin resistance amongst the staphylococcal isolates which shows erythromycin resistance.

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.

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