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Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples

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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: 200 *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: 48 (41.02%) isolates showed inducible clindamycin resistance, 32 (27.35%) showed constitutive resistance while remaining 37 (31.62%) showed MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA (32.69%, 26.92% and 14.58%, 4.16% respectively).

Conclusion: Study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible Clindamycin resistance.

Keyword: Inducible, *Staphylococcus aureus*, Clindamycin, MRSA

1. 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, *Staphylococcus aureus* 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]

2. Materials and Methods

The study was conducted from October 2013 to July 2014. A total of 200 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 Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15mcg), penicillin (10U), ciprofloxacin (5mcg), vancomycin (30mcg) and ceftioxin (30 mcg) as per CLSI guidelines. Methicillin resistance was detected using ceftioxin (30 mcg) on Mueller Hinton agar by incubation at 37 °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 °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:

Fig:



1. MS Phenotype - Staphylococcal isolates 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 labelled as having this phenotype [Figure] a
2. Inducible MLS B Phenotype - Staphylococcal isolates 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 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 ≤ 13 mm) and clindamycin (zone size ≤ 14 mm) with circular shape of zone of inhibition if any around clindamycin [Figure] c.

3. Results

Two hundred Staphylococcal isolates were tested for susceptibility to erythromycin and other antibiotics of the panel by routine disc diffusion testing; 117 (58.5%) of them were erythromycin resistant. These isolates when subjected to D test showed 32 (27.35%) isolates resistant to both erythromycin and clindamycin indicating constitutive MLS B Phenotype, 48 (41.02%) isolates showed positive D test indicating inducible MLS B phenotype while 37 (31.62%) gave negative D test indicating MS phenotype [Table 1]. The overall percentage resistance for all three phenotypes were as follows.

Table 1: Distribution of isolates

Susceptibility pattern(Phenotype)	MRSA (%)	MSSA (%)	TOTAL (%)
cMLSB Resistance	28 (26.92)	4 (4.16)	32 (27.35)
iMLSB Resistance	34 (32.69)	14 (14.58)	48 (41.02)
MS Phenotype	17 (14.91)	20 (20.83)	37 (31.62)

cMLSB - Constitutive Macrolid Lincosamide Streptogramin B

iMLSB - Inducible Macrolid Lincosamide Streptogramin B

MS - Macrolid Streptogramin

Inducible clindamycin resistance - 41.02%

Constitutive clindamycin resistance - 27.35%

MS Phenotype - 31.62%

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

4. 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 [117 (58.5%)]. Amongst them 48 (41.02%) isolates tested positive for inducible clindamycin resistance by D test while rest of the isolates were negative for D test, out of which 32 (27.35%) were shown to have constitutive clindamycin resistance and 37 (31.62%) 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 (32.69% and 26.92% respectively) as compared to MSSA (14.58% and 4.16%). 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.

