Research Article

Detection of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*

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ABSTRACT

Introduction and Background: The resistance to antibiotic employed against staphylococcal isolates is an alarming problem. This has led to the interest in using macrolid-lincosamide streptogramin B (MLSB) antibiotics to treat infection produce by *Staphylococcus aureus*. *In vitro* test for detecting clindamycin suspectibility may fail to detect inducible clindamycin resistance due to presence of erm gene which results in treatment failure. **Aim:** This study aims to develop a simple method for detection of inducible resistance to clindamycin in staphylococcal isolates exhibiting resistance to erythromycin. **Methodology:** A total of 20 clinical isolates of *S. aureus* were subjected to antibiotics suspectibility testing by disc diffusion method. Inducible resistance to clindamycin was detected by D-test as per CLSI guideline. **Result:** 20% isolates of were positive for iMLB phenotype, while 15% isolates were belong to MS phenotypes.

KEY WORDS: Clindamycin resistance, Constitutive macrolide-lincosamide-streptogramin, Inducible macrolide-lincosamide-streptogramin B phenotype, Methicillin-resistant *Staphylococcus aureus*, Multiple sclerosis phenotype, Phenotype

INTRODUCTION

A very few therapeutic alternatives available to treat staphylococcal infections with the emergence of methicillin- and erythromycin-resistant strains. Staphylococcal strains acquiring resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics had a huge increase since the widespread use of MLSB antibiotics.[1,2]

There is a growing concern over erm gene-mediated antibiotic resistance against by mutated staphylococci. The first line antibiotics, namely, vancomycin, quinupristin-dalfpristin, linezolid, and tobramycin reserved for use under critical conditions are also becoming less effective as the pathogens are rapidly developing resistance to them. Clindamycin can be used as the antibiotic of choice in such situations. However, mutational changes against clindamycin effectiveness are also reported, and it may be very difficult to detect using the conventional methods as there could be masking effect during *in vitro* testing. Administration of clindamycin without detection of this phenomenon may be ineffective.[3,4] In such cases, *in vivo* therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure.[5,6]

This study elucidates a simple method for detection of inducible resistance to clindamycin in staphylococcal isolates exhibiting resistance to erythromycin.

Access this article online

Website: jprsolutions.info | ISSN: 0974-6943

MATERIALS AND METHODS

Clinical Isolates

A total of 20 clinical strains of *Staphylococcus aureus* were collected from different clinical specimens and included in the study. They were characterized by standard biochemical tests and identified as *S. aureus*.

Antibiotic Susceptibility Testing

Susceptibility to various routinely used antibiotics against *S. aureus* has been performed by Kirby–Bauer disc diffusion method (Clinical and Laboratory Standards Institute [CLSI] 2015). The antibiotic

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Received on: 26-09-2017; Revised on: 18-10-2017; Accepted on: 27-11-2017

includes erythromycin (50 mcg), norfloxacin (5 mcg), linezolid (30 mcg), vancomycin (30 mcg), clindamycin (2 mcg), oxacillin (1 mcg), and cefoxitin (30 mcg).[7]

Detection of Inducible Clindamycin Resistance

Isolates which were resistant to erythromycin were further subjected to "D test" as per the CLSI guidelines. *S. aureus* isolates were made into suspension and turbidity has been matched with 0.5 McFarland standard. These bacterial suspensions were lawn cultured on Mueller-Hinton agar. After a brief drying erythromycin (15 mcg), disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 mcg) disc and was incubated at 37°C overnight. D-shaped zone flattening around clindamycin in the area between the two discs signifies inducible resistance to clindamycin. Three varied phenotypes were observed after testing and interpreted as follows:

1. Multiple sclerosis (MS) phenotype - staphylococcal isolates demonstrating erythromycin resistance (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and showing a circular zone of inhibition around clindamycin were considered as MS phenotype.
2. Inducible MLSB phenotype - staphylococcal isolates demonstrating erythromycin resistance (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and showing D-shaped zone of inhibition around clindamycin with flattening toward erythromycin discs were considered as having this phenotype [Figure 1].
3. Constitutive MLSB phenotype - staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of the zone of inhibition if any around clindamycin were considered as having this phenotype.[8]

RESULTS

Antibiotic Susceptibility Pattern

We have observed a varied pattern of sensitivity among one *S. aureus* isolates. There was complete resistance observed for penicillin (100%), 9/20 (45%) isolates were shown to the resistant to erythromycin, 6/20 (30%) were to cotrimoxazole, 4/20 (20%) were to linezolid, followed by 3/20 (15%) were resistant to ciprofloxacin and clindamycin, respectively [Table 1].

S. aureus isolates were subjected for susceptibility to erythromycin and other group of antibiotics by the Kirby-Bauer disc diffusion method of 20 isolates. 12 (60%) of them were erythromycin resistance. Results of D-test were projected in Table 2. The percentage of both inducible and constitutive resistances was found to be more among the methicillin-resistant *S. aureus* (MRSA) strains in compare to methicillin-susceptible *S. aureus* (MSSA).

Table 1: Results of antibiotic susceptibility pattern of *S. aureus*

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin	0	0	20 (100)
Erythromycin	10 (50)	1 (5)	9 (45)
Clindamycin	15 (75)	2 (10)	3 (15)
Ciprofloxacin	9 (45)	8 (40)	3 (15)
Tetracycline	14 (70)	4 (20)	2 (10)
Cotrimoxazole	10 (50)	4 (20)	6 (30)
Linezolid	10 (50)	6 (30)	4 (20)

S. aureus: Staphylococcus aureus

Table 2: Results of clindamycin resistance among *S. aureus* isolates

Clindamycin resistance	Methicillin resistance		Total *n*=20 (%)
	MRSA *n*=12 (%)	MSSA *n*=8 (%)	
ERY-S, CL-S	3 (25)	5 (64)	8 (40)
ERY-R, CL-R	4 (33)	1 (12)	5 (25)
ERY-R, CL-S (D-test+ve, iMLS)	3 (25)	1 (12)	4 (20)
ERY-R, CL-S (D-test−ve, MS)	2 (17)	1 (12)	3 (15)

S. aureus: Staphylococcus aureus, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

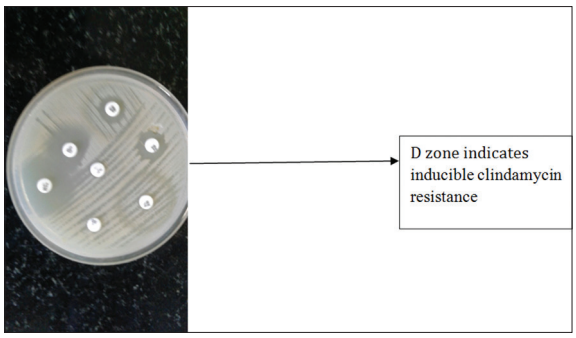

Figure 1: Representive picture showing D zone of inducible clindamycin resistance

DISCUSSION

S. aureus is recognized as a major pathogen inducing nosocomial and community-acquired infection in many parts of the world. *S. aureus* has developed adequate resistance to several antimicrobial agents and is posing as a serious threat to the diseased patients. In our study, we have observed equal number of isolates which found to be erythromycin-resistant *S. aureus*. 11 (55%) and 9 (45%) of the isolates were erythromycin and clindamycin sensitive. The present study shows 12 isolates of MRSA and 8 isolates of MSSA. Some of the study shown very high percentage of inducible resistance mRSa (4).On contrary, few studies have shown more percentage of inducible resistance in MSSA when compared to MRSA.[9,10]

Yilmaz *et al*. did a study on detection and prevalence of inducible clindamycin resistance in Staphylococci and concluded clindamycin should not be used in patients with infections caused by inducible-resistant *S. aureus*.

Delialioglu *et al*. studied about inducible clindamycin resistance in staphylococci isolates and concluded inducible clindamycin-resistant staphylococci cannot be detected using *vitro* susceptibility tests such as broth dilution and agar dilution tests. By applying proper disc placement on a routine basis to detect inducible clindamycin resistance, clindamycin can be used effectively on staphylococcal infections.

Deotale *et al*. did a study on inducible clindamycin resistance in *S. aureus* isolated from the infected clinical samples and concluded the D-test should be used as a definitive process in routine disc diffusion testing to identify inducible clindamycin resistance.

Kloos and Banerman extensively studied the clinical significance of coagulase-negative staphylococci and reported that vancomycin was very effective against the *Staphylococcus* species which are commonly resistant to antibiotics. Lim also confirmed the importance of the D-zone test in detecting inducible clindamycin resistance in *Staphylococcus* to aid in the optimal treatment of patients.

Gupta *et al*. have stated that clindamycin should be kept as a reserve drug and it usually advocated in severe methicillin-resistant *S. aureus* infection depending on the anti-microbial susceptibility results. Schreckenberger *et al*. have suggested a policy of performing a D-test only on coagulase-negative *S. aureus* isolates based on the existing prevalence in the region. Levin *et al.* have stated that erm gene product confers clindamycin resistance on *S. aureus*. They have documented a clindamycin failure when resistance developed in a D-test positive MSSA isolates.

Siberry *et al.* reported surgical site infected situation caused by clindamycin-susceptible, erythromycin-resistant MRSA unresponsive to treatment was resistant to clindamycin. The MRSA isolate obtained after treatment was resistant to clindamycin but was observed to be identical by pulsed-field gel electrophoresis to the clindamycin-susceptible isolate gathered before treatment. A follow-up erythromycin-induction test (D-test) validated the presence of *in vitro* inducible macrolide-lincosamide-streptogramin B resistance (iMLS) in the before treatment isolate.

In recent scenario, clindamycin plays an excellent role in treating some staphylococcal infection, especially skin, soft tissue infection, and as an alternative to penicillin allergy individuals.[11,12] Clindamycin has a better oral availability making this as a good option for outpatients and change over after intravenous antibiotics.[13] However, the acquisition of clindamycin resistance in staphylococcal strains with inducible phenotypes and from such isolates, unprompted, and constitutively resistant mutations have emerged in both *vitro* and *vivo* during clindamycin therapy, and D-test can be used to detect them to facilitate better treatment and care to the patients suffering from severe infections.[14]

CONCLUSION

The clinician must have a wide knowledge of inducible clindamycin resistance and report to laboratory immediately for prompt treatment. The D-test is a simple and reliable method to detect inducible and constitutive clindamycin resistance in routine clinical diagnosis setting.

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