

Original Research Article

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**Inducible Clindamycin resistance in *Staphylococcus aureus*
isolated from Clinical Samples at C. U. Shah Medical College and
Hospital, Surendranagar, Gujarat, India**

Sanyogita Jain*, Atul Rukadikar and Anil Kumar Jain

Department of Microbiology, Chirayu Medical College and Hospital, Bhopal, MP, India

**Corresponding author*

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Clindamycin is one of the important antibiotics for the therapy of skin and soft tissue infections caused by *Staphylococcus aureus* particularly MRSA (methicillin-resistant *S. aureus*). *In vitro* routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes. Use of clindamycin in such cases would result in treatment failure. D-test (Disk approximation test) is an accurate and simple test which can be used for detection of clindamycin resistance in laboratory. The present study was carried out to determine incidence of inducible clindamycin resistance among clinical isolates of *S. aureus* by D-test. The study was conducted from April 2010 to May 2015. Total 108 *Staphylococcus aureus* strains were isolated and identified from various clinical specimens. Antibiotic susceptibility testing including detection of methicillin resistance was done by disk diffusion method as per standard guidelines. All erythromycin resistant *S. aureus* isolates were subjected to D-test to detect the inducible clindamycin resistance as per CLSI guidelines. Out of total 108 *S. aureus* isolates, 50 were erythromycin resistant. Out of 50 erythromycin resistant isolates, 13 (12%) showed inducible clindamycin resistance, 4 (3.7%) showed constitutive resistance while remaining 33 (30.55%) showed MS phenotype in D-test. Inducible resistance was found to be higher in MRSA (23.8%). The incidence of inducible clindamycin resistance was 12% in present study. D-test is simple and handy test to detect inducible clindamycin resistance in *S. aureus* isolates. 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.

Introduction

Antibiotic resistance in *Staphylococcus aureus* has become an ever-increasing problem. In *Staphylococcus*, penicillin resistance was recognized first in 1944 and methicillin resistance was recognized first in 1961. ^[1]

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 to its excellent pharmacokinetic properties.^[2]

However, widespread use of MLS_B antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics.^[3] 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 via efflux of antibiotic, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* and *in vitro* and the strain do not typically become clindamycin resistant during therapy.^[4] The chemical structures of macrolides, lincosamides and streptogramin B are very different, but their mechanism of action is identical. All 3 antibiotics block protein synthesis by inhibiting peptidyl transferase. Bacteria develop cross resistance quite often to MLS_B due to overlapping binding sites in 23S rRNA.^[5]

In inducible MLS_B resistance, the bacteria produce inactive mRNA which is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. Bacterial strains having an inducible *erm* gene are resistant to the inducer but appear to be susceptible to clindamycin by the disc diffusion method, so when reported as 'Clindamycin sensitive', clinicians have only two options,

either to avoid prescribing a useful drug such as clindamycin or to lead to a therapeutic failure by using it. Inducible clindamycin resistance is not detected by standard broth microdilution testing, automated susceptibility testing devices, the standard disc diffusion test or the E-test^[6].

The present study was carried out to determine the incidence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* by D-test (In accordance with CLSI Guideline).

Materials and Methods

The study was conducted from April 2010 to May 2015 at the Department of Microbiology, C.U.Shah Medical College and Hospital, Surendranagar, Gujarat. The study was approved by Institutional Ethics Committee (Human Research). A total of 108 *Staphylococcus aureus* were isolated from various clinical specimens like pus, wound swab, aspirates, blood and body fluids.

Antibiotic susceptibility testing was performed for all *S. aureus* strain by disk diffusion method. Detection of methicillin resistance was done by Cefoxitin (30 µg) disk diffusion method. Inducible clindamycin resistance was detected by D-test, as per CLSI guidelines on erythromycin resistant isolates.^[7]

Screening for inducible clindamycin resistance

Mueller Hinton agar plate was inoculated with 0.5 McFarland bacterial suspension, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc and after overnight incubation at 37 °C, zone of inhibition around clindamycin in the area between the

two discs were observed^[7] [Figure1].

Three different phenotypes were appreciated after testing and interpreted as follows:

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 [Figure 1a].
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 [Figure 1 b].
3. Constitutive MLS_B Phenotype: 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 1c].

Results and Discussion

108 *S. aureus* isolates isolated from clinical specimens were tested for susceptibility to erythromycin and other antibiotics including cefoxitin by disc diffusion testing. 50 (32.4%) isolates were found to be erythromycin resistant. Erythromycin resistant isolates were subjected to D-test. Out of 50 isolates, 4 (3.7%) were resistant to both erythromycin and clindamycin indicating constitutive MLS_B Phenotype, 46 isolates were clindamycin sensitive. Out of 46, 13 (12%) isolates showed positive D-test indicating inducible MLS_B phenotype, while 33 (30.55%) showed negative D-test indicating MS phenotype.

Out of 108 *S. aureus* isolates 42 (%) isolates were found to be cefoxitin resistant (MRSA). 27 MRSA isolates were found to be erythromycin resistant. Out of 27 MRSA isolates, 3 (7.14%) isolates were constitutive MLS_B Phenotype, 10 (23.80%) isolates were inducible MLS_B phenotype, while 14 (33.33%) isolates were MS phenotype. (Table 1)

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms. Clindamycin is one of the important antibiotics for the therapy of skin and soft tissue infections caused by *Staphylococcus aureus* particularly MRSA.^[2]

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.^[8] Reporting *Staphylococcus aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other side negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.^[8]

In present study out of 108 *S. aureus* isolates 50 (32.4%) were erythromycin resistant. Amongst them 13 (12%) isolates were inducible clindamycin resistant, 4 (3.7%) were constitutive clindamycin resistant and 33 (30.55%) were clindamycin sensitive (MS phenotype) as per D-test. The percentages of inducible resistance (23.80%) and MS phenotype (33.33%) were higher amongst MRSA.

Table.1 Distribution of isolates-

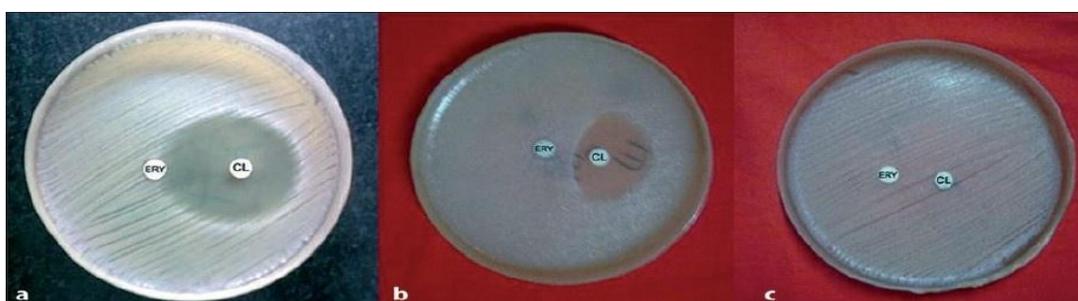
Susceptibility pattern	<i>S. aureus</i>	MRSA
ERY-S, CL-S	58 (53.7%)	15 (35.71%)
Constitutive MLS _B Resistance (ERY-R,CL-R)	4 (3.7%)	3 (7.14%)
Inducible MLS _B Resistance (ERY-R,CL-S, positive D-test)	13 (12%)	10 (23.80%)
MS phenotype (ERY-R,CL-S, negative D-test)	33 (30.55%)	14 (33.33%)
Total	108	42

ERY- Erythromycin, CL- Clindamycin, S-sensitive, R-resistant,

Table.2 Inducible clindamycin resistance in MRSA

Authors	Inducible clindamycin resistance in MRSA (Percentage)
Yilmaz <i>et al</i> ⁸	24.40%
Gadepalli <i>et al.</i> ²	30%
Ajantha GS <i>et al</i> ⁹	74%
Rahabar M <i>et al.</i> ¹⁰	22.60%
Present study	23.80%

Fig.1 Inducible clindamycin resistance



a- MS Phenotype

b- Inducible MLS_B Phenotype

c-Constitutive MLS_B Phenotype

These observations suggest that if D-test had not been performed, nearly one fourth of the erythromycin resistant isolates would have

been misidentified as clindamycin sensitive and resulting in therapeutic failure. Various authors have reported inducible clindamycin

resistance ranging from 22.60% to 74% as shown in Table-2.

In conclusion, out of 108 *S. aureus* isolates 50 (32.4%) were erythromycin resistant isolates and 42 (%) isolates were found to be cefoxitin resistant (MRSA), Out of 42 MRSA, 27 were found to be erythromycin resistant. Incidence of Inducible clindamycin resistance was 12% observed in all *S.aureus* and 23.80% in MRSA isolates. Sensitivity to clindamycin can be judged after performing D test on the erythromycin resistant isolates.

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