

A Study to Detect Inducible Clindamycin Resistance among *Staphylococcus Aureus* Isolates and Explore Its Relationship with Methicillin Resistance in a Tertiary Care Hospital of West Bengal

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Abstract

Background: Among the treatment options available for MSSA and MRSA, clindamycin is no doubt an important one. However, staphylococcal isolates can develop inducible clindamycin resistance, and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro testing and in vivo, during clindamycin therapy. Reporting of clindamycin susceptible *Staphylococcus aureus* without testing for inducible resistance may result in treatment failure.

Aim: This study was conducted to detect inducible clindamycin resistance among *Staphylococcus aureus* isolates and to explore its relationship with methicillin resistance.

Methodology: *Staphylococcus aureus* was identified using standard methods from various clinical samples collected over a period three months. Then, methicillin-resistant strains were identified by using screening technique i.e. cefoxitin disc (30µg) diffusion testing method. Then, all erythromycin resistant isolates were tested by double disc diffusion assay (D test) using erythromycin and clindamycin according to CLSI.

Result: Out of total 78 *Staphylococcus aureus* isolates, 48 (61.54%) of them were erythromycin resistant and 58 (74.36%) were found to be methicillin resistant. Overall, 28.21% MS phenotype (D test negative), 23.08% inducible MLS_B phenotype (D test positive) and 10.26% constitutive MLS_B phenotype were detected. Inducible resistance and MS phenotype were found to be higher in MSSA as compared to MRSA (30%, 40% and 20.69%, 24.14% respectively).

Conclusion: It is mandatory to consider the D test in routine antibiotic susceptibility testing (by disc diffusion method) for *Staphylococcus aureus* both for MRSA and MSSA.

Key words: Clindamycin resistance, constitutive MLS_B phenotype, inducible MLS_B phenotype, MRSA, MS phenotype

Date of Submission: 07-11-2019

Date of Acceptance: 23-11-2019

I. Introduction

Emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an ever-increasing problem raising real concern regarding viable treatment options for such infections.¹ High resistance to macrolides (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin) and emerging resistance to newer antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin which have been recommended in the management of such isolates, created a serious therapeutic challenge.²⁻⁴ This has led to renewed interest in the usage of macrolide lincosamide- streptogramin B (MLS_B) family of antibiotics with clindamycin being the preferred agent due to its excellent pharmacokinetic properties particularly for skin and soft tissue infections.^{1,5}

Although, MLS_B antibiotics have structural variation, they have similar mechanism of action. They inhibit bacterial protein synthesis by binding to 23s rRNA, which is a part of large ribosomal subunit. They also have a similar spectrum of activity against gram-positive cocci, gram negative cocci and intracellular bacteria such as chlamydiae and rickettsiae.⁶ But, widespread use of MLS_B antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics.⁷ Among diverse mechanisms of macrolide resistance, the *msr(A)* gene coding for efflux mechanism and *erm* gene encoding for enzymes that confer inducible or constitutive resistance to MLS_B antibiotics are important. The r-RNA methylase is always produced in constitutive resistance (cMLS_B), but presence of an inducing agent triggers its production in inducible

resistance (iMLS_B).⁸ As an inducing agent, erythromycin is more effective than clindamycin. *In vitro*, *S. aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (iMLS_B).⁹ Thus in vivo therapy with clindamycin in patients harboring iMLS_B staphylococci may select constitutive mutants leading to clinical therapeutic failure.¹⁰ That is why, early detection of staphylococcal isolates with inducible resistance to clindamycin is of paramount importance which can be done by D test as described by Fiebelkorn *et al.*^{11,12}

The present study attempted to evaluate the prevalence of inducible clindamycin resistance by D test among *S. aureus* isolates and to explore any relationship with methicillin resistance.

II. Material & Methods

The present study was conducted in the Department of Microbiology, Burdwan Medical College, Purba Bardhaman, India, over 3 months from April 2019 to June 2019. After collection of various samples, first, direct smear was prepared and stained with gram stain and then the samples were cultured on various culture media, like Blood agar and MacConkey's agar. And after 24hrs of incubation, colony morphology was noted and from the colony; gram stain was performed again. Then, only samples showing gram positive cocci arranged in irregular grape-like clusters were taken into account. After that catalase test and slide and tube coagulase tests were performed to identify *Staphylococcus aureus*.

Detection of MRSA

Cefoxitin disc (30 µg) diffusion testing method was used to screen MRSA from all of the isolates as per CLSI guideline. From each strain, a suspension equivalent to 0.5 McFarland was prepared. After that, a swab was dipped in it and streaked on the surface of a Mueller–Hinton agar and Cefoxitin disc (30 µg) was placed onto it and incubated for 24 h at 35°C. The isolate was considered as MRSA if the zone of inhibition was ≤21 mm in diameter.¹²

Detection of inducible clindamycin resistance of *Staphylococcus aureus*

Erythromycin resistant isolates were further subjected to 'D test' as per CLSI guidelines. In this test, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. After overnight incubation at 37°C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance.¹²

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

1. MS Phenotype – Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype and this suggest negative D-test.
2. Inducible MLSB Phenotype – Staphylococcal isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype and this suggest positive D-test.
3. Constitutive MLSB Phenotype – This phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin suggesting negative D-test.

III. Results

In this present study, a total of 774 samples were collected in the Microbiology department, Burdwan Medical College, over a period of three months from April 2019 to June 2019. Of these, *Staphylococcus aureus* was isolated in seventy-eight (78) samples. Amongst all *Staphylococcus aureus* isolates, we observed high percentage of erythromycin resistance 48(61.54%). These isolates when subjected to D test showed 8 (10.26%) isolates resistant to both erythromycin and clindamycin indicating constitutive MLS_B Phenotype; 40 isolates showed clindamycin sensitivity. Out of these, 18 isolates showed positive D test indicating inducible MLS_B phenotype while 22 gave negative D test indicating MS phenotype [Table 1].

Thus, the percentage resistance for all three phenotypes of clindamycin susceptibility of *Staphylococcus aureus* isolates was as follows:

Inducible clindamycin resistance –23.08%

Constitutive clindamycin resistance –10.26%

MS Phenotype –28.21%

It was further noticed that percentages of inducible resistance and MS phenotype were higher amongst MSSA (30% and 40%) as compared to MRSA (20.69% and 24.14% respectively) [Table 1].

Table 1: Distribution of *Staphylococcus aureus* isolates according to methicillin resistance and three different phenotypes of clindamycin resistance. (N=78)

	MRSA	MSSA	Total
ERY- S CL- S	24 (41.38%)	6 (30%)	30 (38.14%)
ERY- R CL- R (Constitutive MLS _B)	8 (13.79%)	-	8 (10.26%)
ERY- R CL- S (Inducible MLS _B)	12 (20.69%)	6 (30%)	18 (23.08%)
D test positive			
ERY- R CL- S (MS)	14 (24.14%)	8 (40%)	22 (28.21%)
D test negative			
Total	58 (74.36%)	20 (25.64%)	78

ERY – Erythromycin, CL- Clindamycin, S- Sensitive, R-Resistant, Constitutive MLS_B - Constitutive resistance to clindamycin, Inducible MLS_B -Inducible resistance to clindamycin, MS -MS phenotype

IV. Discussion

Clindamycin is one of the important alternative antibiotics in the therapy of *Staphylococcus aureus* (both MRSA and MSSA) particularly in the treatment of skin and soft-tissue infections.¹¹ But staphylococcal isolates can develop inducible clindamycin resistance (iMLS_B) and from such isolates, spontaneous constitutively resistant mutants have arisen both *in vitro* testing and *in vivo* during clindamycin therapy.¹³ Reporting of clindamycin susceptible *Staphylococcus aureus* without testing for inducible resistance may result in treatment failure. On the contrary, negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.¹⁴

In this study, amongst all *Staphylococcus aureus* isolates, we observed high percentage of erythromycin resistance 48 (61.54%) which is higher (15.7%,¹⁵ 28.4%,¹⁶ 32.4%¹) than reported in literature but remains comparable only to Lyall *et al.*¹⁷ (51.7%).

Moreover, in our study, inducible clindamycin resistance was observed in 23.08% isolates which was comparable to the study by Lyall *et al.*¹⁷ (33.3%) but as compared to our study higher rate of inducible clindamycin resistance (50.6%,¹⁸ 49%,¹⁵ 45%¹) and lower rate of inducible clindamycin resistance (10.5%,¹⁶ 13.1%⁶) was reported by others.

Amongst the erythromycin resistant *Staphylococcus aureus* isolates, inducible clindamycin resistance was detected by positive D test in 18(37.5%) isolates and rest of the isolates were negative for D test. Of these, 8(16.67%) isolates were found to have constitutive clindamycin resistance and 22(45.83%) isolates demonstrated true sensitivity to clindamycin (MS phenotype). These observations prove that, testing of all erythromycin resistant *Staphylococcus aureus* isolates for inducible clindamycin resistance by D test is mandatory, otherwise we would have been misidentified as clindamycin sensitive in at least half of the erythromycin resistant isolates ultimately triggering treatment failure.

It was further noticed that percentages of inducible resistance and MS phenotype were higher amongst MSSA (30% and 40%) as compared to MRSA (20.69% and 24.14% respectively). This was supported by the studies by Schreckenberger *et al.*¹⁹, Levin *et al.*²⁰ and Patel *et al.*²¹ where they 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; 50% MRSA and 60% MSSA respectively. However, on the contrary, Deotale *et al.*¹ reported inducible resistance of 27.6% in MRSA and 1.6% in MSSA; Yilmaz *et al.*¹³ observed inducible resistance of 24.4% in MRSA and 14.8% in MSSA; Gadepalli *et al.*⁷ noticed it to be 30% in MRSA and 10% in MSSA, while Mohamed Rahabar *et al.*²² found 22.6% in MRSA and 4% in MSSA. In another Indian study by Ajantha *et al.*,¹⁵ very high frequency of inducible resistance (63%) was noticed in erythromycin -R, clindamycin sensitive *Staphylococcus aureus* isolates and also in MRSA(74%) and in MSSA (45%). But interestingly, Lyall *et al.*¹⁷ observed almost similar frequency of inducible resistance both in MRSA (33.2%) and MSSA (34.6%). This proved that D test is mandatory in routine antibiotic susceptibility testing (by disc diffusion method) for all *Staphylococcus aureus* irrespective of MRSA or MSSA.

Moreover, constitutive resistance in our study was seen in 13.79 % of MRSA isolates, which is contrary to the only study from India, by Angel *et al.*,²³ which failed to find it in any of the strains.

V. Conclusion

In view of limited therapeutic options for MRSA infections and the known limitations of vancomycin, clindamycin could be an important choice particularly for serious soft tissue infections but testing of the isolates (Both MRSA and MSSA) beforehand by D test is must to avoid treatment failure.²⁴

Acknowledgement

I express my heartfelt thanks and gratitude to my institution, Burdwan Medical College & Hospital for allowing me to conduct the study.

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Tapajyoti Mukherjee. "A Study to Detect Inducible Clindamycin Resistance among Staphylococcus Aureus Isolates and Explore Its Relationship with Methicillin Resistance in a Tertiary Care Hospital of West Bengal." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 11, 2019, pp 23-26.