

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

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Abstract

Background: *Staphylococcus aureus* is commensal flora but it can lead minor skin infections to life threatening conditions such as endocarditis, pneumonia and septicemia. Spread of multi drug resistance MRSA, therapeutic options become severely limited. Clindamycin can be an alternative option for use in the increasing drug resistance among the staphylococci. However, resistance to clindamycin can be missed in the laboratory, unless look specially thus necessitating the need to detect such resistance by a simple D test on routine basis.

Objectives: To find clindamycin resistance among clinical isolates of *S. aureus* by disk-approximation test (D-test) and to establish methicillin resistant *Staphylococcus aureus* (MRSA) isolates and its relationship with inducible clindamycin resistance.

Materials and Methods: *S. aureus* isolated from various clinical specimens in microbiology unit BP Koirala Institute of Health Sciences hospital, Nepal from 8th March 2012 to 10th September 2012 was studied. Isolation and identification of organism was done by standard microbiological technique. *S. aureus* resistant to erythromycin Kirby Baur disk diffusion test and phenotypic expression on inducible resistance was assessed using D-test.

Results: Among 300 *S. aureus* 41% were methicillin resistant. MRSA demonstrated 11.6% constitutive MLSBc. D test positive inducible resistance (MLSBi) found to be 24.59% and (22.4%) were MS type among MRSA isolates.

Conclusion: It was found 15.2% of isolates were inducible resistance to clindamycin, thus highlighting, whenever clindamycin is intended to be used for *S. aureus* infections, D-test should be performed to facilitate the appropriate treatment of patients.

Keywords: *Staphylococcus aureus*, inducible resistance, D-test.

1. Introduction

Staphylococcus aureus is commensal flora but it can lead minor skin infections to life threatening conditions such as endocarditis, pneumonia and septicemia. Emergence of methicillin resistant *Staphylococcus aureus* its changing pattern of antimicrobial resistance reduced to susceptibility to vancomycin is of great challenge [1]. With the appearance and spread of multi drug resistance MRSA therapeutic options become severely limited. Clindamycin can be an alternative option for use in the increasing drug resistance among the staphylococci. Vancomycin as a drug of choice for treatment of MRSA infections, it is

considerable high cost and side effects. Overuse of vancomycin, the strains of staphylococci is now emerged has the reduced susceptibility to vancomycin [2].

Clindamycin (CL) is a good alternative to treat soft tissue infections by both MRSA and MSSA infections [3]. Its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production are its advantages. However development of resistance especially inducible resistance is a major barrier in its usage [3-6].

CL belongs to the macrolide, lincosamide and streptogramin (MLS) family that act through inhibition of protein synthesis. Bacterial resistance to this group may be expressed through different mechanisms including target site modification, macrolide efflux pump and enzymatic antibiotic inactivation [3-6]. Modification of the ribosomal target is encoded by the *erm* genes that cause production of methylase enzymes which reduce binding of the drug to the rRNA target. This resistance can be either constitutive or inducible. If the *erm* genes are consistently expressed, isolates show in vitro resistance to erythromycin (ER), CL, and to other members of MLS, known as constitutive resistance phenotype. In case of inducible resistance, the *erm* genes require an inducing agent to express resistance to CL. ER can act as a strong inducer of methylase synthesis. These isolates known as inducible resistance phenotype show in vitro resistance to ER and susceptibility to CL. CL therapy in this phenotype can lead to clinical failure [7, 9, 10] *S. aureus* can also develop isolated macrolide resistance based on presence of an efflux pump, encoded by the *msrA* gene which leads to resistance to macrolides and type B streptogramins but not to lincosamides. These isolates known as MS phenotype also show in vitro resistance to ER and susceptibility to CL same as in inducible resistance phenotype, but CL therapy can be safely given in infections with this phenotype and there is no risk of clinical failure [3]. Therefore, it is important to differentiate these two mechanisms of resistance.

Phenotypic detection of inducible resistance can be done by double disk diffusion test (D-test). D-test is simple, reliable, inexpensive and easy to interpret with high sensitivity and specificity. Molecular markers for the *erm* genes are available, but they are costly and inconvenient for everyday use. Clindamycin is a good option but prevalence of inducible resistance should be known, as it varies by geographical location and bacterial species. So the aim of this study was to assess the frequency of phenotypic expression of inducible *erm* gene expression in clinical isolates of *S. aureus* by D-test.

2. Materials and Methods

A total of 300 of *S. aureus*, isolated during a period 8th March 2012 to 10th September 2012 from various clinical specimens. *S. aureus* were identified on colony morphology, gram stain, catalase test and coagulase test as per microbiological guidelines.

Methicillin resistance was confirmed by agar screen test using Mueller-Hinton agar plate supplemented with 4% NaCl and oxacillin (6µg/ml) incubated at 35°C for full 24 hour. *S. aureus* ATCC 25923 was used as a control and tested daily along with the test strains [11].

Each isolate was subjected to the disk diffusion test for detection of MRSA as recommended by the CLSI [11]. For detection of inducible clindamycin resistance, the D-test was performed. Briefly, on a lawn of *Staphylococcus aureus* isolate on Mueller-Hinton agar plate, standard discs of erythromycin (15 µg) and clindamycin (2 µg) were placed. Inter-disc edge-to-edge distance between E and CL discs was fixed at 15mm [3]. All media and discs were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. After 18 hours of incubation at 35°C, the zones of inhibition around the discs were measured and compared with the CLSI standard charts and classified as sensitive (≥ 23 mm) or resistant (≤ 13 mm) to erythromycin. Around those isolates of *Staphylococcus aureus* which were resistant to E, the zone of inhibition around CL was carefully examined. Three types of patterns were noted:

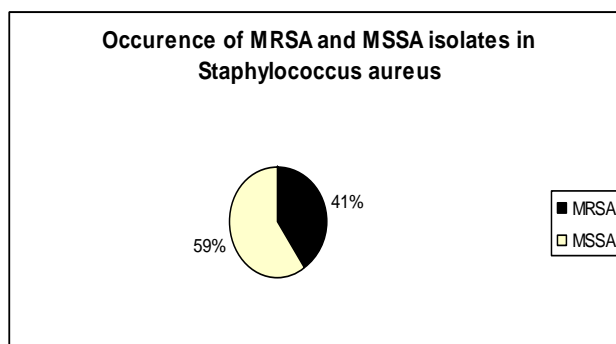
- 1) No zone of inhibition (CD-resistant; constitutively expressed MLSb resistance or cMLSb type of resistance).
- 2) Clindamycin sensitive with no blunting of the inhibition zone facing the E disc (the MS phenotype) and,
- 3) Flattening of the zone of inhibition around the CD disc facing the E disc, in the form of 'D'. These isolates were declared D-test positive (D +) and were inducible clindamycin resistant (iMLSb type of resistance).

Knowledge of the extent of staphylococcal infections and the antimicrobial susceptibility plays an important role in choice of therapy. Available alternative drugs are often prohibitively costly or not readily available in Nepal. Detection of early inducible clindamycin resistance in *Staphylococcus aureus* will not only make right choice of antibiotic therapy but also recommend this as a routine bench work to prevent antimicrobial resistance in our local isolates.

3. Results

Among the 300 clinical isolates of *Staphylococcus aureus* 41% (122) were MRSA and 59% were MSSA (Figure 1)

Figure 1: Frequency of distribution of *Staphylococcus aureus*



MRSA- Methicillin resistant *Staphylococcus aureus*; MSSA- Methicillin susceptible *Staphylococcus aureus*

Out of 300 *S aureus* 216 (72%) had the ERY -S and Cl-S phenotype, 50 (40.9%) were MRSA isolates and 166 (93.24%) were MSSA. Among MRSA isolates 14 (11.2%) were constitutive resistance cMLSB. Total of 33 (15.2%) of *S. aureus* exhibited Inducible resistance iMLSB (D test positive) where 30 (24.59%) were MRSA and 1.68% were MSSA and 22.4% of MRSA and 5.14% of MSSA were found to be MS type D test negative. (Table 1)

Table 1: Distribution of isolates

Phenotype	MRSA%	MSSA%	Total
ERY-S, Cl-S	51 (40.8%)	163 (93.14%)	214 (71.3%)
ERY-R, Cl-R Constitutive MLSBc	14 (11.2%)	--	14 (4.6%)
ERY-R, Cl-S D positive Inducible MLSBi	30 (24.59%)	3 (1.68%)	35 (15.2%)
ERY-R, Cl-S D-test negative MS	28 (22.4%)	9 (5.14%)	36 (16.6%)

ERY- Erythromycin; CL- Clindamycin; S- Sensitive, R- Resistant; Constitutive MLSBc - Constitutive resistance to clindamycin; Inducible MLSBi- inducible resistance to clindamycin; MS- MS phenotype

ERY-S and Cl-S type is predominant among the MSSA isolates i.e. (93.14%) in comparison to MRSA (40.8%). It was found that MRSA isolates had higher inducible resistance (24.59%) than MSSA isolates (1.68%)

In MRSA isolates, the constitutive CL-R phenotype level was 11.2 % where as there was no isolate of this type in MSSA.

Figure 2: A positive D-test (flattening of clindamycin zone proximal to erythromycin) for detection of inducible clindamycin resistance



4. Discussion

CL is a good alternative to treat soft tissue infections by both MRSA and MSSA infections. Its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production are its advantages [7-9].

Increasing trend of antimicrobial resistance in *Staphylococcus aureus* has led to use of clindamycin in skin and soft tissues infections. Tremendous use of clindamycin in infections may develop therapeutic failure in inducible resistant phenotype.

In our study, in comparison to MSSA, MRSA has higher rate of resistance to erythromycin. In various studies conducted in Nepal, Constitutive resistance MLSB and inducible D positive among MRSA isolates were found to be higher 37.8% Thapa *et al* [12], 21.1% Kumar S *et al* [13] where as a study conducted by Sah *et al* showed but lower (14%) which is lower findings than ours[14].

Similarly in the present study, out of 122 MRSA, 14 (11.2%) were constitutive MLSBc and 30(24.59%) was inducible D test positive and 28(22.4%) were D test negative which is concordance to the findings reported by Vikek *et al* from India where 24.3% of MRSA were inducible resistant [15]. Upadhaya A *et al* from India showed (35.33%) MLSBi [16], 61% by Suveerat C *et al* [17] from Thailand, 53.4% by Hazendarogh T *et al* from Turkey [18] and 70% by Fasih *et al* from Pakistan [19] had higher rate of MLSBi finding than our study. This is pertinent to note because, clindamycin resistance is known to vary from one geographic area to another and also from one time period to another.

D test implention is the laboratory is simple and easy method with routine antimicrobial susceptibility testing. 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 suitable antibiotic for D test positive isolates while it can definitely prove to a drug of choice in a case of D test negative isolates. Routine and consistently performing the D test in the diagnostic bench adds the early detection of its phenotypic resistance pattern that ultimately guide the clinician to avoid the treatment failure.

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