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Original Article

Phenotypic study of Macrolide-Lincosamide-Streptogramin B Resistance in *Staphylococcus aureus* and their relationship with Methicillin-Resistant Staphylococcus aureus (MRSA) at Tertiary Care in Eastern Nepal

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Abstract

Background

Resistance to antimicrobial agents is prevalent among *Staphylococci*. This has led to wide uses of macrolide-lincosamide-streptogramin B (MLS_B) antibiotics to treat *Staphylococcus aureus (S. aureus)* infections. MLS_B though chemically distinct, have similar target site and mode of action. The multiple mechanisms are responsible for resistance to MLS_B antibiotics which can lead to clinical failure. The aim of the study was to investigate the frequency of inducible and constitutive clindamycin resistance among clinical isolates of *S. aureus* and their relationship with Methicillin-resistant Staphylococcus aureus (MRSA).

Material & Methods

A total of 336 unique *Staphylococcus aureus* isolates from different clinical samples obtained from patients were studied. Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method. "D test" was performed to detect inducible clindamycin resistance as per CLSI guidelines. MRSA was detected using Cefoxitin (30μg) and results were interpreted according to CLSI criteria.

Results

Inducible clindamycin resistance was seen in 45 (13.39%), constitutive clindamycin resistance was seen among 58 (17.26%) while MS phenotype was observed among 38(11.30%) of isolates. Inducible resistance as well as constitutive resistance was higher among MRSA as compared to MSSA (21.11%, 4.48% and 21.11%, 12.82%respectively).

Conclusion

The Successful use of clindamycin for the treatment of infection caused by *S. aureus* can be predicted based on the result of simple and inexpensive D test.

Key Words: *Clindamycin resistance, iMLSB, MRSA, Nepal*

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most frequent microorganisms responsible for both community and hospital acquired infections. Methicillin resistance Staphylococcus aureus (MRSA) which are resistance to multiple classes of antibiotics often pose problems in therapy. This has renewed concern for the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat *S. aureus* infections [1]. Clindamycin, a lincosamide,

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is a preferred option to treat infections, especially skin and soft tissue caused by both methicillin resistant and methicillin susceptible *S. aureus* because of the various reasons [2]. However, because of extensive use of MLS_B antibiotics high incidence MLS_B resistant Staphylococcal strains are reported [3, 4]. The resistance to macrolide is either due to active efflux of antibiotics mediated by protein encoded by *msrA* gene or due to modification of ribosome by r-RNA methylase enzymes encoded by *erm* genes which confer inducible or constitutive resistance to MLS_B antibiotics. In constitutive resistance ($cMLS_B$), the enzyme r-RNA methylase is constitutively produced while in inducible resistance ($iMLS_B$) it is produced only in the presence of inducible agent [5]. Low level erythromycin is the most efficient inducer of $iMLS_B$ resistance. *In vitro*, constitutively resistance *Staphylococcus aureus* are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin [6]. If clindamycin is used to treat patients harbouring $iMLS_B$ *Staphylococcus*, selection for constitutive *erm* mutants occur leading to therapeutic failure [7]. The objective of the present study was to investigate the prevalence of inducible clindamycin resistance among *Staphylococcus aureus* isolated from our teaching hospital and to detect their distribution among Methicillin-resistant Staphylococcus aureus (MRSA).

Material and Methods

This study was a prospective study conducted from 1st January 2015 to 30th June 2015. A total of 336 non-duplicate *Staphylococcus aureus* isolates from different clinical samples obtained from patients attending Nobel Medical College and Teaching Hospital, Biratnagar, Nepal were studied. The isolates were identified as *Staphylococcus aureus* using standard microbiological procedures [8]. Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method on Muller Hinton agar using various antimicrobial agents: penicillin (5μg), cefoxitin (30μg), amikacin (30μg), erythromycin (15μg), cotrimoxazole (1.25/23.75μg), chloramphenicol (30μg), clindamycin (2μg), teicoplanin (30μg), linezolid (30μg) as per CLSI guidelines [9].

MRSA was detected by Kirby Bauer disc diffusion method using 30μg cefoxitin disc on Muller Hinton Agar seeded with 0.5 McFarland bacterial suspensions. After overnight incubation at 35^0C, the results were interpreted according to CLSI guidelines [9]. The strains were confirmed as Methicillin resistance by agar dilution method using Muller Hinton medium containing 4% NaCl and 6μg/mL oxacillin. *Staphylococcus aureus* NCTC 6571 and S. *aureus* NCTC 12493 were used as a control strain for methicillin-sensitive and methicillin-resistant strain respectively. Test to detect inducible clindamycin resistance was performed by placing erythromycin (15μg) disc and clindamycin (2μg) spaced 15mm from edge-to-edge on a Mueller–Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions. Following overnight incubation at 35^0C the results were read as per CLSI guidelines [9].

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

1. MS Phenotype - Staphylococcal isolates resistance to erythromycin (zone size $\leq$13mm) and sensitive to clindamycin (zone size $\geq$21mm) giving circular zone of inhibition around clindamycin.

2. Inducible MLSB ($iMLS_B$) Phenotype - Staphylococcal isolates resistance to erythromycin and sensitive to clindamycin with D – shaped zone of inhibition adjacent to erythromycin disc.

3. Constitutive MLSB ($cMLS_B$) Phenotype - Staphylococcal isolates resistance to both

erythromycin and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin. *S. aureus* ATCC 25923 was used for routine quality control of the erythromycin and clindamycin discs. Also an in-house chosen *S. aureus* with confirmed positive and negative D-test were used as additional quality control.

Statistical analysis to study the relationship between MRSA and inducible clindamycin resistance was carried out using SPSS version 16.

Results

Out of the 336 *S. aureus* isolates tested, 180 (53.57%) strains were found to be MRSA. Results of D-test analysis showed that out of 336 *S. aureus* 45 (13.39%) were positive for D test. Constitutive clindamycin resistance was observed in 58 (17.26%) isolates **[Table 1].** Prevalence of inducible as well as constitutive resistance was higher among MRSA as compared to MSSA (Chi-square test, $p < 0.001$) **[Table 2].** All the isolates showing inducible clindamycin resistance were susceptible to chloramphenicol, linezolid, and teicoplanin **[Table 3].**

Table:1 Susceptibility pattern against Erythromycin and Clindamycin among total S. aureus isolates

Susceptibility pattern (Phenotype)	No. of isolates	Percentage
Sensitive to both erythromycin and clindamycin	195	58.03
Resistant to both erythromycin and clindamycin ($cMLS_B$)	58	17.26
Erythromycin resistant and clindamycin sensitive (D test positive, $iMLS_B$)	45	13.39
Erythromycin resistant and clindamycin sensitive (D test negative, MS)	38	11.30
Total	**336**	**100**

Discussion

Testing for antimicrobial susceptibility among the clinical isolates of microorganisms is crucial for the optimum outcome of the treatment. This is particularly important as the number of resistance is increasing day by day.

Table:2 Susceptibility pattern against Erythromycin and Clindamycin among Methicillin Resistant S. aureus(MRSA) isolates

Isolate	E-S, CD-S	E-R, CD-R ($cMLS_B$)	E-R,CD-S, (D test positive, $iMLS_B$)	E-R, CD-S (D test negative, MS)
MRSA (180)	87 (48.33)	38 (21.11)	38 (21.11)	17 (9.44)
MSSA (156)	108 (69.23)	20 (12.82)	7 (4.48)	21 (13.46)
Total (336)	**195 (58.03)**	**58 (17.26)**	**45 (13.39)**	**38 (11.30)**

E = erythromycin, CD = clindamycin, S = sensitive, R = resistant, $cMLS_B$ = constitutive MLS_B phenotype, $iMLS_B$ = inducible MLS_B phenotype, MS = MS phenotype

Table:3 Susceptibility pattern of inducible resistant($iMLS_B$) phenotype

Antimicrobial agents	No. of sensitive strain	No. of resistant strain
Methicillin	7	38
Penicillin	0	45
Amikacin	41	4
Chloramphenicol	45	0
Cotrimoxazole	32	13
Linezolid	45	0
Teicoplanin	45	0

Recently clindamycin has become an excellent drug for the treatment of infections especially skin and soft tissues infections caused by *Staphylococcus aureus* [6]. However, Staphylococcal isolates with inducible phenotypes develops resistance to clindamycin and from such phenotypes mutants with constitutive resistance can arise spontaneously during clindamycin therapy [10]. Therefore, Staphylococcal isolates must be checked for inducible resistance before they are reported as susceptible to clindamycin to prevent therapeutic failure because isolates that demonstrate negative result for inducible clindamycin resistance confirms susceptibility to clindamycin and provide better therapeutic option [11].

In our study overall prevalence of inducible clindamycin resistance ($iMLS_B$) among the *Staphylococcus aureus* was 13.39%. Such an occurrence is similar to that reported by Ansari *et al* (12.4%) *and* Sah *et al* (12.1%) from Nepal [12, 13]. In contrary this

finding was low as compared to other reports from Nepal and other part of the world [10, 14-18]. Constitutive resistance ($cMLS_B$) (17.26%) obtained in present study was low as compared another reports [10, 14-16]. Such variations could be because of differences in period of study, patient group and geographical locations.

The present study demonstrated higher prevalence of $iMLS_B$ and $cMLS_B$ among the MRSA as compared to MSSA. This finding is in concordance with other reports [13-16]. On the contrary, certain reports suggest a remarkably greater occurrence of $iMLS_B$ among MSSA [19-21].

Clindamycin, by virtue of its excellent bone and tissue penetration and accumulation in abscesses, has become a useful antibiotic for the treatment of serious infections caused by methicillin sensitive as well as methicillin resistant *Staphylococcus aureus*. Further clindamycin is an alternative for penicillin-allergic patients. Better oral absorption and lack of need for renal adjustment makes it an important therapeutic agent [5]. However major risk with the use of clindamycin as a therapeutic agent is existence of $iMLS_B$ and $cMLS_B$ among *S. aureus* and its use for the treatment of patients harboring $iMLS_B$ phenotype will lead to therapeutic failure. However there are reports which states that infections caused by *S. aureus* expressing $iMLS_B$ resistance can successfully be treated with clindamycin [6]. Hence, limiting the use of clindamycin for the treatment of *S. aureus* is not desirable [22]. Therefore D test should be performed routinely and the clinician should be informed regarding the possible failure of clindamycin therapy in infections caused by *S. aureus* harboring $iMLS_B$ resistance.

Conclusion

The high incidence of stphylococccal infections all over the world and emergence of multi drug resistance has led use of clindamycin for the treatment of infections caused by *S. aureus* [15]. As clindamycin is not a drug of choice for D – test positive isolates while it can definitely be a suitable drug in D - test negative isolates, performance of D - test in a routine laboratory will enable us to guide the clinicians in judicious use of clindamycin.

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