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Original Research Article

Inducible and constitutive clindamycin resistance in *Staphylococcus aureus*: an experience from Western Nepal

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Abstract

Objective: This study aimed to determine prevalence of inducible and constitutive clindamycin resistance among clinical *S. aureus* isolates and also study their association with methicillin resistance.

Methods: A cross-sectional study including 140 non-duplicate isolates of *S. aureus* was done. Isolates were identified by standard microbiological methods and methicillin resistance was detected by cefoxitin disc diffusion method. Inducible clindamycin resistance was detected by D-test.

Results: Prevalence of inducible and constitutive clindamycin resistance was 12.1% and 7.9% respectively. Constitutive and inducible resistance was associated with MRSA. An unusual phenotype, erythromycin sensitive and clindamycin resistance, was detected in 2 MRSA isolates.

Conclusions: Inducible and constitutive clindamycin resistance is comparatively low in our setting. Constitutive and inducible resistance was higher among MRSA than MSSA. However the trends in resistance vary in different places. D-test reporting should be done routinely which will allow clinicians to opt for clindamycin judiciously and avoid potential treatment failure.

Keywords: Clindamycin resistance, MLSBi, D-test, MRSA, Nepal

1.Introduction

Antimicrobial resistance in *Staphylococcus aureus* has become an ever-increasing problem. Methicillin resistant *S. aureus* (MRSA) which are often multiply resistant to other classes of antibiotics in addition to β–lactams, often presents difficulties in therapy. The macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics is commonly used in the treatment of staphylococcal infections [1]. Clindamycin, a lincosamide, represents an attractive option for treatment of both methicillin-resistant and susceptible staphylococcal infections, especially skin and soft tissue infections, for various reasons: available in both oral and intravenous formulations; excellent tissue penetration; less costly; inhibits productions of certain toxins and virulence factors in staphylococci [2,3]. However, possible presence of inducible clindamycin resistance among staphylococcal isolates is a major concern in use of clindamycin [4].

Macrolide resistance arises either by an efflux mechanism or by target modification, the later resulting into resistance not only to macrolide but also to lincosamides and group B streptogramins [5]. An *erm* gene encodes methylation of the 23S rRNA-binding site that is shared by these drugs. Phenotypically, such resistance can be constitutive (MLSBc phenotype) or inducible (MLSBi phenotype) [6]. It is also possible for mutations to occur spontaneously that will transform MLSBi strains to MLSBc phenotype without the presence of a macrolide inducer, a concern being that this change might occur in the midst of therapy [7].

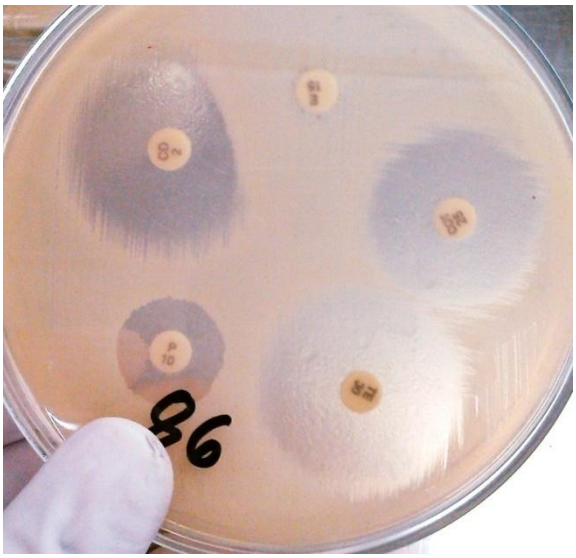
S. aureus isolates with constitutive resistance show resistance to erythromycin and clindamycin on in vitro testing, whereas isolates with inducible resistance show resistance to erythromycin but appear sensitive to clindamycin on disc diffusion testing. Inducible clindamycin resistance in

staphylococci can be detected by D test [8]. For erythromycin-resistant isolates, D test can help to determine whether clindamycin could be used as a therapeutic option. Reports on prevalence of inducible clindamycin resistance are scanty from Nepal. This study was undertaken to determine prevalence of inducible and constitutive clindamycin resistance among clinical *S. aureus* isolates and also study their association with MRSA.

2. Materials and Methods

A cross-sectional study was conducted (June 2013 to May 2014) which included 140 non-duplicate isolates of *S. aureus* from different clinical specimens such as pus (53), blood (56), urine (25), sputum (5), and body fluids (1) at Microbiology laboratory, Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Nepal. No consent was required as this study included routine clinical specimens which precluded any patient contact. The study was sanctioned by the department of Microbiology. Isolates were identified by standard microbiological methods and susceptibility testing was performed as per Clinical Laboratory and Standards Institute (CLSI) recommendations. Methicillin resistance was detected by using cefoxitin (30µg) disc diffusion method. Isolates with cefoxitin zone size ≥22mm were considered methicillin susceptible and those with ≤21mm were considered methicillin resistant [8]. Inducible clindamycin resistance was detected using D-test by placing erythromycin (15µg) and clindamycin (2 µg) disc at adjacent position, 15mm apart. Isolates resistant to erythromycin and having a clindamycin zone ≥21 mm with a D-shaped zone (Figure 1) were regarded as positive for inducible resistance (MLSBi phenotype) [8]. Isolates resistant to erythromycin and susceptible to clindamycin were considered negative for D-test (MS phenotype), and those resistant to both erythromycin and clindamycin were regarded as constitutive resistance phenotypes (MLSBc). Isolates susceptible to both erythromycin and clindamycin were regarded as susceptible strains. *S. aureus* ATCC 25923 was used to perform quality control of the erythromycin and clindamycin discs. Separate in-house selected *S. aureus* strains that demonstrated positive and negative D-test reactions were also used in quality control. Data was analyzed using SPSS 17.0. Chi-square test was used for analyzing categorical variables ($P<0.05$ was considered significant).

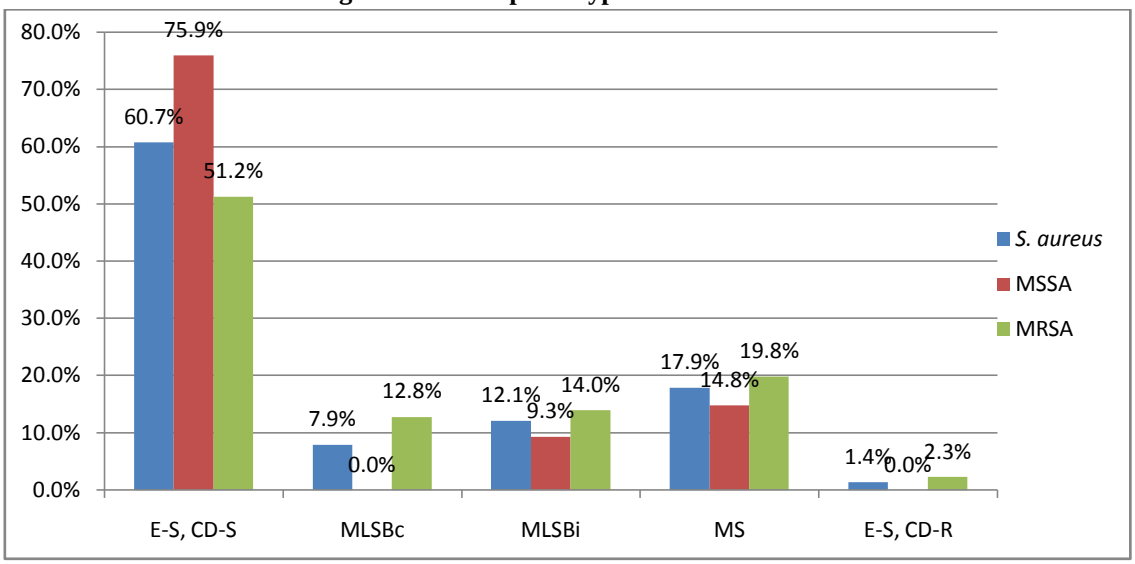
Figure 1: A positive D-test (flattening of clindamycin zone proximal to erythromycin) for detection of inducible clindamycin resistance



3. Results

Of 140 *S. aureus* isolates 61.4% (86/140) were MRSA. Erythromycin and clindamycin resistance was seen in 37.9% (53/140) and 9.3% (13/140) isolates respectively. Both erythromycin resistance (46.5% vs. 24.1%) and clindamycin resistance (15.1% vs. nil) was significantly higher in MRSA than among MSSA (P=0.008 and P=0.003 respectively).A total of 42 isolates showed erythromycin resistance and clindamycin sensitive phenotype out of which 40.5% (17/42) were positive for MLSBi phenotype. The overall prevalence of MLSBi and MLSBc phenotype was 12.1% (17/140) and 7.9% (11/140) respectively. An unusual phenotype showing erythromycin sensitive and clindamycin resistant was seen in 2 isolates both of which were MRSA. Both MLSBi and MLSBc phenotypes predominated in MRSA strains (P=0.014) (figure 2).

Figure 2: MLSB phenotypes of *S. aureus* isolates



E: Erythromycin, CD: Clindamycin, S: Sensitive, R: Resistant, MLSBc: Constitutive Macrolide-Lincosamide-Streptogramin B resistance, MLSBi: Inducible Macrolide-Lincosamide-Streptogramin B resistance, MS: Macrolide-Streptogramin B resistance

4. Discussion

Recent trends in epidemiology of MRSA indicate that these strains are no longer limited to healthcare facilities and new strains have appeared in community. The changing pattern of antimicrobial resistance of MRSA strains has led to renewed interest in the use of clindamycin. Clindamycin is often used for treatment of skin and soft tissue infections [7]. However therapeutic failures due to inducible clindamycin resistant strains have been reported.

In this study the prevalence of MLSBi among *S. aureus* was found to be 12.1% which is similar to that reported by Ansari *et al.* from Nepal (12.4%) [9] and Van der Heijden *et al.* (11.3%) from Brazil [10]. Varying prevalence rates of MLSBi have been reported in different other studies; 18.2%from Nepal [11], 19.8%from Turkey [12], 20.3% [13] and 8.4% [14] from India. Higher MLSBi prevalence of 45% from Germany [15] and 62% from US [16] has also been reported. Constitutive resistance (7.9%) was lower than that reported elsewhere [11-13]. A comparatively low prevalence of inducible and constitutive resistance in this study indicates a greater utility of clindamycin in our setting.

An unusual phenotype showing erythromycin sensitivity and clindamycin resistance was detected in 2 MRSA isolates. Tests were repeated for these strains exhibiting unusual phenotype and same result was obtained. Such phenotype has been reported in MRSA isolates at a French hospital [17] and also in *Streptococcus agalactiae* isolates from New Zealand [18]. The biochemical and genetic basis for this new phenotype of resistance remains obscure [18]. Clindamycin resistance may be misidentified in strains with such phenotype if only erythromycin is tested.

This study shows a significantly higher prevalence of MLSBi as well as MLSBc in MRSA strains than MSSA which is consistent with other reports [11-13]. Molecular studies have shown that some SCC*mec* elements carry transposon Tn*554* which contains the gene *erm*A mediating MLS resistance [19]. However, Schreckenberger *et al.* [20] and Levin *et al.* [21] reported a higher incidence of MLSBi among MSSA.

Clindamycin therapy for staphylococcal isolates with the inducible phenotype has been somewhat hampered by possibility of emergence of constitutively resistant mutant strains during therapy. The available data on clinical efficacy of clindamycin therapy in infections with MSLBi strains are limited and present conflicting results [22-25]. Uncertainty about the reliability of susceptibility reports for clindamycin when D-test results are not available, as well as confusion over the clinical importance of this inducible resistance, has led some clinicians to avoid use of clindamycin for staphylococcal infections whenever erythromycin resistance is noted [7]. Clinical microbiology laboratories should consider performing routine testing and reporting for inducible clindamycin resistance in staphylococcal isolates so that use of clindamycin is judiciously undertaken especially for treatment of MRSA infections before switching over to vancomycin.

5. Conclusions

Inducible and constitutive clindamycin resistance is comparatively low in our setting. Constitutive and inducible resistance was higher among MRSA than MSSA. However the trends in

resistance vary in different places. D-test reporting should be done routinely which will allow clinicians to opt for clindamycin judiciously.

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