Inducible Clindamycin Resistance Among the *Staphylococcus aureus* Colonizing the Anterior Nares of School Children of Udupi Taluk

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Short Communication

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Abstract

Clindamycin is one of the preferred antibiotics in the treatment of *Staphylococcus aureus* (SA) skin and soft tissue infections. However the emergence of inducible clindamycin resistant SA is a major concern for clinicians in the management of such infections. Information about such resistant strains of SA colonizing the anterior nares is very important in planning infection control strategies. The objective of the current study was to assess the proportion of SA showing inducible clindamycin resistance and also to know their association with methicillin resistance. Among the isolates, 11.6% (44/378) strains were showing positive D test which indicates inducible clindamycin resistance and a highly significant 71% (12/17) inducible clindamycin resistance was also noticed in the case of MRSA. The nasal carriage of inducible clindamycin resistant SA showing a significant association with MRSA strains by the paediatric population from this area warrants the inclusion of D test in the routine antibiotic susceptibility testing of SA isolates. Information about the MLSBi status among the colonizing strains would also help the public health authorities to plan and implement infection control strategies at the community level.

Keywords: *Staphylococcus aureus*, D test, Inducible clindamycin resistance, MRSA

Background

Macrolides, lincosamides and streptogramin B (MLSB) antibiotics inhibit protein synthesis in the bacteria by binding to ribosomal subunits and by interfering the elongation of amino acids[1]. Lincosamides like clindamycin were successfully used in treating skin and soft tissue infections caused by MRSA. But emergence of clindamycin resistance during therapy discouraged many clinicians continuing this treatment regime[2].

Macrolide resistance in *Staphylococcus aureus* (SA) can be due to two reasons.[3] One mechanism is by the action of an energy dependent pump that expels macrolides from the cell of bacteria before it acts on drug target[3, 4]. This efflux pump is encoded by *msr*(A) gene[5]. This mechanism also results in resistance to group B streptogramins (eg. Quinupristin). This does not lead to resistance towards

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lincosamides (eg. Clindamycin). Second mechanism leads to resistance towards macrolides, lincosamides and streptogramins as well. This is commonly known as "MLSB resistance" [4, 6]. This type of resistance results from reduction of affinity due to N-6-dimethylation of an adenine residue in the 23S rRNA[4]. Methylation of this 23S rRNA binding site is due to a methylase encoded by genes *ermC* and *ermA*.

MLSB resistance can be expressed constitutively (MLSBc phenotype) or when induced into its production (MLSBi phenotype) [7]. A strain that possess *erm* gene if exposed to macrolides, the binding of the drug molecules to target results in alteration of secondary structure of mRNA, exposure of the binding site and translation of *erm* methylase. These changes in bacteria lead to constitutive resistance to MLSB drugs[7, 8]. But the mechanism of resistance in MLSBi phenotype is different. They are intrinsically resistant to majority of the macrolides but shows in vitro susceptibility to lincosamides and group B streptogramins. However a macrolide inducer can convert such strains to lincosamide and streptogramin resistant ones. Some spontaneous mutations can also result in change of MLSBi stains to MLSBc strains and if it happens in the middle of a therapy that may lead to treatment failure[3]. This property of SA can be detected in laboratory by a method called D test. The stain that shows MLSBi resistance will produce a D shaped zone of inhibition around clindamycin disc[2].

Nasal carriage is found to the major source for most of the community associated SA infections. So it is of great importance to know the antibiotic susceptibility pattern of such strains especially with respect to methicillin resistance and inducible clindamycin resistance. The present study was undertaken to know the level of inducible clindamycin resistance among the SA isolated from the anterior nares of school children. This study also assessed the association between inducible clindamycin resistance and methicillin resistant SA (MRSA).

Material and Methods

A total of 378 strains of SA isolated during June 2009 to December 2010 were used in the study. Originally those strains were isolated as a part of a prevalence study conducted by the same authors (unpublished). Institutional ethics committee clearance was obtained from Manipal University Ethics committee before the commencement of the study. Nasal swabs were collected from children of 6 – 15 years old going to various schools of Udupi taluk, Karnataka, India. The names of the schools from where the samples were obtained cannot be disclosed due to ethical considerations. Sterile cotton swabs (Hi-Media, Mumbai) were used to collect the specimen and standard methods were followed to isolate the SA[9].

Inducible clindamycin resistance was detected among the SA isolates by D test[2]. An erythromycin disk (15 µg) was placed 15 mm (edge to edge) from a clindamycin disk (2 µg) in a standard disk diffusion test (Figure 1). Muller Hinton agar medium was used for testing. A flattening of the zone of inhibition in the area between the disks where both drugs have diffused after 18-24 hours of incubation was considered to be inducible clindamycin resistance.

All the strains of SA were inoculated onto Muller Hinton Agar (MHA) containing 4% sodium chloride and 6µg/ml of oxacillin. Growth of one or more colonies of Staphylococcus aureus in this medium was considered as MRSA. During antibiotic susceptibility testing, susceptibility to 30µg cefoxitin was also tested. A zone diameter of ≤ 19 mm was considered as resistance and confirmed as MRSA[10]. Statistical analysis to study the association between MRSA and inducible clindamycin resistance was carried out using SPPS version 16.

Results

Out of the 378 isolates tested, 17 strains (4.1%) were found to be resistant to cefoxitin in disk diffusion test and all those strains were grown on salted Muller Hinton agar (MHA) containing 6µg/ml of oxacillin.

Among the SA, 11.6% (44/378) strains were showing inducible clindamycin resistance and other 88.4% strains were not showing positive D test.Among the MRSA, 71% (12/17) strains were MLSBi phenotype. When cross tabulation was done by Chi square test to know the significance of inducible clindamycin resistance among MRSA isolates, there was a statistically significant association noticed with $p < 0.05$ (Table 1).

Table 1: MRSA and MLSBi resistance

	MLSBi resistance		Total
	Negative	Positive	
MSSA	329	32	361
MRSA	5	12	17
Total	334	44	378

Discussion

The most widespread and clinically important resistance mechanisms encountered with gram-positive organisms are the production of methylases and efflux proteins. Resistance to MLSB antibiotics are the best examples. The clinical failure of clindamycin therapy has been reported before.Lewis JS in 2005 cited about the case report of Mc Geheeet al. as they found that poor prognosis in a case of erythromycin resistant SA infection after treating with lincomycin and clindamycin. This particular strain was susceptible to lincomycin and clindamycin in vitro testing but developed resistance during treatment[11].

Such emerging strains that shows inducible clindamycin resistance among SA isolates is one of the major concerns for clinicians while choosing the appropriate antibiotic

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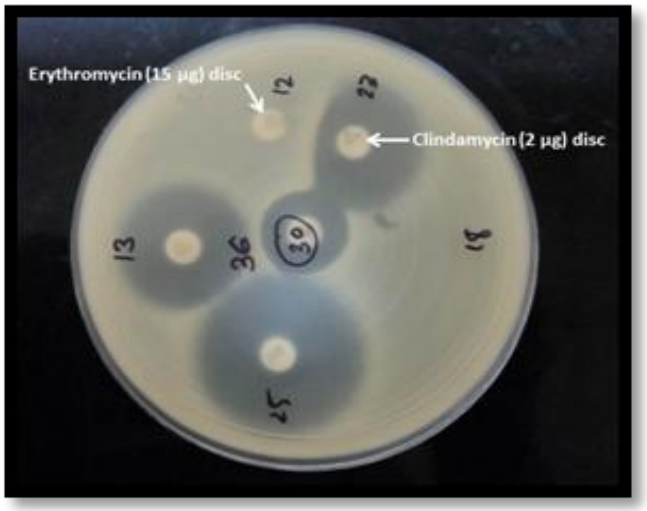
regimen. Hence, there is a need to identify the mechanisms that confer resistance to MLS antibiotics with regard to clindamycin therapy of staphylococcal infections.

Figure 1: Detection of inducible clindamycin resistance by D test



Conclusion

Routine investigations of MLSBi status would, therefore, be of great significance in the choice of antibiotics. The same holds good in the case of the colonizing strains as well. It needs to be emphasized here that, in the population investigated, a high incidence of inducible clindamycin resistance (71%) was noticed among colonizing strains of MRSA. When the inducible clindamycin resistance crops up, a roughly 25 fold increase is registered in MRSA when compared with MSSA. This information about the MLSBi status among the colonizing strains would also help the public health authorities to plan and implement infection control strategies at the community level.

Author's Contribution:

All the authors were involved in designing the study, collecting and compiling the data; SG did the analysis of data, were involved in the data interpretation, and drafted the manuscript. Manuscript was revised by SG, CAM, IB. All the authors approved the final document.

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Conflict of interest:

There is no conflict of interest among authors arising from the study.

List of Abbreviations

SA – Staphylococcus aureus
MRSA – Methicillin resistant Staphylococcus aureus
MLSB – Macrolides, lincosamides and streptogramin B
MHA – MullerHinton Agar
OR – odds ratio

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