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Original Research Article

Prevalence of Inducible clindamycin resistance in *Staphylococcus aureus* at a tertiary care hospital: Implications for clinical therapy

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A B S T R A C T

The increase in incidence of MRSA has highlighted the need for better agents to treat such infections. Although macrolide-lincosamide-streptogramin B (MLS_B) groups of antibiotics are commonly used for such infections with clindamycin being the preferred choice but emergence of resistance to clindamycin has also been reported. This study reports the detection of inducible clindamycin resistance in erythromycin resistant strains of *Staphylococcus aureus and* coagulase negative *Staphylococcus* by D test. 150 *S. aureus* and 31 coagulase negative *Staphylococcus* isolates were isolated form different patient samples and were identified using standard microbiological techniques. These isolates were screened for MRSA, MSSA and clindamycin sensitivity. Among the *S. aureus* isolates, 25.4% were MRSA and 74.6% were MSSA. Constitutive resistance was seen in 37.3% of isolates. Of these 47.4% were MRSA and 33.9% were MSSA. Inducible resistance was seen in 16.6% of isolates, in which 28.9% were MRSA and 12.6% were MSSA. In coagulase negative *Staphylococcus* isolates, 32.3% showed resistance while 67.7% showed sensitivity to methicillin. Constitutive resistance was seen in 38.7% isolates. Of these 30% were methicillin resistant and 42.8% were methicillin sensitive. Out of 25.8% inducible resistance isolates, 30% were methicillin resistant and 23.8% were methicillin sensitive. Results indicate that the true percentage of clindamycin resistance is still underestimated, and the test should be performed more routinely to avoid therapeutic failure.

Introduction

The increase in the occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) and emergence of Vancomycin intermediate *S. aureus* (VISA)/ Vancomycin resistant *S. aureus* (VRSA) has left fewer options for the treatment of infections. The macrolide-lincosamide-streptogramin B (MLS_B) groups of antibiotics are commonly used in the treatment of *Staphylococcal* infections with clindamycin being the preferred agent. Good oral absorption

makes it an important option in outpatient settings [Prabhu et al., 2011]. However, resistance to MLS_B antibiotics can occur due to drug interaction, target site modification, or efflux mechanisms. Target site modification mediated by *erm* (erythromycin ribosome methylase) genes is the most common mechanism. The *erm* genes encode enzymes that cause methylation of A2058 residue located in the conserved domainV of 23s rRNA, which result in emergence of resistance pattern phenotype, known as MLS_B.

This resistance can be expressed either constitutively or inducible [Roberts et al., 1999; Fiebelkorn et al., 2003; Deotale et al., 2010]. Detection of inducible clindamycin resistance in these type of strains remains difficult since these strains may appear sensitive to clindamycin & resistant to erythromycin. However, therapeutic failures are common with the emergence of selective constitutive *erm* mutants during clindamycin therapy (Todd et al., 2005).

The detection of such inducible resistance requires the antimicrobial susceptibility testing to be carried out by placing erythromycin and clindamycin adjacent to each other. Standard disc diffusion method known as the D-test have been described for detection of inducible clindamycin resistance [Fiebelkorn et al., 2003; CLSI, 2007]. Since MLS_B is emerging at an alarming rate, therefore the screening of clinical isolates of *S. aureus* MLS_B is need of hour to select the appropriate therapeutic regime. Considering this, the present study was designed to detect the prevalence of inducible clindamycin resistance in clinical isolates of *Staphylococcus spp* isolated from patients sample in a tertiary care hospital.

Materials and Methods

Bacterial Isolates

150 erythromycin resistant and 31 erythromycin resistant coagulase negative *S. aureus* strains isolated from clinical samples (Dept. of Microbiology, Lady Harding's Medical College & Hospital, New Delhi, India) were used. Study protocol was duly approved by Institutional Ethics Committee. All the isolates were identified using conventional microbiological methods such as colony morphology, Gram stain, catalase and coagulase tests etc. For the use of isolates, ethical approval was granted from Institutional Ethical Committee.

Antimicrobial Susceptibility Testing

Cefoxitin 30µg disc (Hi-Media Labs, India) was used to detect MRSA as per CLSI recommendations.[7] Susceptibility to clindamycin and erythromycin was determined by disc diffusion methods as per CLSI guidelines. For erythromycin, isolates exhibiting zone of inhibition <13mm were taken as resistant while those >21mm were taken as sensitive. For clindamycin, zone of inhibition <14mm were taken as resistant while >21mm were taken as sensitive. The isolates were further studied for inducible clindamycin resistance as per CLSI guidelines [CLSI, 2007].

D-Test

Suspension of organism equivalent to 0.5 McFarland was inoculated on Mueller Hinton agar plates. Clindamycin (2µg, Hi-Media, India) and Erythromycin (15µg, Hi-Media, India) discs were placed 15mm apart edge to edge. *S. aureus* ATCC 25923 was used as standard reference. Plates

were analyzed after 18 hours of incubation. Completely circular zone of inhibition was taken as negative D test, while blunting of clindamycin zone adjacent to erythromycin was interpreted as positive D test. Erythromycin resistant and clindamycin resistant strains were interpreted as constitutive MLS_B phenotypes, while erythromycin resistant, clindamycin sensitive and D test positive strains were interpreted as inducible MLS_B ($iMLS_B$) phenotypes. Erythromycin resistant, clindamycin sensitive and D test negative strains were interpreted as MS phenotypes (Deotale et al., 2010).

Results and Discussion

Out of 181 isolates, 150 were *S. aureus* & 31 were coagulase negative *Staphylococcus*. Among the *S. aureus* isolates, 38 (25.4%) were MRSA and 112 (74.6%) were methicillin sensitive (MSSA). Constitutive resistance was seen in 56 (37.3%) of isolates. Of these 18 (47.4%) were MRSA and 38 (33.9%) were MSSA. Inducible resistance was seen in 25 (16.6%) of isolates, in which 11 (28.9%) were MRSA & 14 (12.6%) were MSSA (Table 1). Among the coagulase negative *Staphylococcus* isolates, 10 (32.3%) showed methicillin resistance while 21 (67.7%) were methicillin sensitive. Constitutive resistance was seen in 12 (38.7%) isolates. Of these 3 (30%) were methicillin resistant and 9 (42.8%) were methicillin sensitive. 8 (25.8%) isolates exhibited inducible resistance, 3 (30%) were methicillin resistant and 5 (23.8%) were methicillin sensitive (Table 2).

Accurate detection of antimicrobial resistance in a microbe is an essential factor in determining appropriate therapeutic regimens. The increase in frequency of *Staphylococcal* infections among patients, and changes in antimicrobial resistance patterns have led to renewed interest in the use of clindamycin therapy (Yilmaz et al., 2007). Clindamycin is a useful drug in the treatment of skin and soft tissue infections and serious infections caused by *Staphylococcal* species and anaerobes. It is a good alternative for the treatment of both methicillin resistant and methicillin sensitive *Staphylococcal* infections [Dass et al., 2012]. It has excellent tissue penetration (except central nervous system) and accumulates in abscesses and no renal dosing adjustments are required. Good oral absorption makes it an important option in outpatient therapy or as follow up after intravenous therapy. It is of particular importance as an alternative antibiotic in penicillin allergic patients [Gadepalli et al., 2006]. But ignorance regarding inducible clindamycin resistance has led to therapeutic failures. However, inducible clindamycin resistance in *Staphylococci* cannot be detected simply by using *in vitro* susceptibility tests such as broth or agar dilution [Jorgensen et al., 2004]. Although, D test offers a reliable method for the detection of $iMLS_B$ phenotype but, if the discs of erythromycin & clindamycin are not placed in adjacent positions, the phenotype will be easily missed.

In the present study, 181 clinical isolates of *S. aureus* were screened for $iMLS_B$ phenotype. It was observed that 16% *S. aureus* & 26% coagulase negative *Staphylococcus* possess inducible clindamycin resistance. Amongst *S. aureus*, 29% of MRSA & 12% of MSSA showed this phenotype. These findings correlate well with the previous results where MRSA and MSSA, both were showed to have clindamycin resistance

Table.1 Table showing the spectrum of *Staphylococcus aureus* resistance and susceptibility to erythromycin and clindamycin and results of D test.

Phenotype	MRSA (%)	MSSA (%)	Total (%)
ER-R, CL-R	18(47.4)	38(33.9)	56(37.3)
ER-R, CL-S, D+	11(28.9)	14(12.6)	25(16.6)
ER-R, CL-S, D-	9(23.7)	60(53.5)	69(46)
	38(25.4)	112(74.6)	150

Key: ER-R: erythromycin resistant, CL-R: clindamycin resistant, CL-S: Clindamycin sensitive, D+: D test positive, D-: D test negative

Table.2 Table showing the spectrum of coagulase negative *Staphylococcus aureus* resistance and susceptibility to erythromycin and clindamycin and results of D test

Phenotype	MRSA (%)	MSSA (%)	Total (%)
ER-R, CL-R	3(30)	9(42.8)	12(38.7)
ER-R, CL-S, D+	3(30)	5(23.8)	8(25.8)
ER-R, CL-S, D-	4(40)	7(33.4)	11(35.5)
	10(32.3)	21(67.7)	31

Key: ER-R: erythromycin resistant, CL-R: clindamycin resistant, CL-S: Clindamycin sensitive, D+: D test positive, D-: D test negative

[Gadepalli et al., 2006; Ciraj et al., 2009; Deotale et al., 2010]. Prevalence of inducible clindamycin among MRSA and MSSA has also been reported, previously [Paul et al., 2004; Yilmaz et al., 2007]. Various workers have reported similar findings on this aspect from different parts of world [Hamilton et al., 2004; Delialioglu et al., 2005]. Although multiple reports have shown the inducible clindamycin resistance in *S. aureus* but reports from different regions have shown different pattern of resistance. On Indian scenario, very few reports exist on emergence of inducible clindamycin resistance. The emergence of inducible clindamycin resistance has been reported from different parts of India [Gadepalli et al., 2006; Vandana et al., 2009; Prabhu et al., 2011; Dass et al., 2012]. The existing data on inducible clindamycin resistance suggest that the incidence of resistance is highly variable with regard to geographic distribution, and hence the local data pertaining to inducible clindamycin resistance in a particular geographical area, can be helpful in guiding and deciding the anti-*Staphylococcal* therapy to avoid the therapeutic failures [Ciraj et al., 2009].

Implications for therapy

One of the major concerns with the usage of clindamycin therapy is the possible presence of inducible clindamycin resistance. Uncertainty regarding reliability of susceptibility reports and suspicion over inducible resistance can lead to avoidance of clindamycin therapy by clinicians. The clindamycin therapy can be safely given in non-MLSbi infections [Delialioglu et al., 2005]. In addition, it has been reported that constitutive

resistance to clindamycin prevents inhibition of toxin production and fails to inhibit growth of *S. aureus*. However, the same is not clear for inducible resistance. Inducible resistance phenotypes may appear to be resistant to erythromycin but susceptible to clindamycin, if both discs are not placed adjacent to each other [Coyle et al., 2003]. CLSI also recommends that clinical laboratories must consider routine testing and reporting of inducible clindamycin resistance using D test, where MRSA is routinely encountered [NCCLS, 2004]. Moreover, close follow up and monitoring for failure or relapse is needed in case of MLSBi producing isolates. Study suggest that non-reporting of MRSA cases treated with clindamycin is needed to better understanding for the role of this compound in organisms with varying MLS_B resistant phenotypes.

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