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RESEARCH ARTICLE

Phenotypic detection of inducible Clindamycin resistance among *Staphylococcus* species

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Abstract

Background: Clindamycin is commonly used for the treatment of Staphylococcal infection for its excellent pharmacokinetic properties. The *Staphylococcus species* that are resistant to erythromycin and susceptible to clindamycin may show inducible resistance due to expression of *erm* gene resulting in treatment failure. The study was undertaken to detect the inducible clindamycin resistance by in vitro D-test on the Staphylococcal isolates obtained from samples of indoor and outdoor patients, attending Shri Mahant Indiresh Hospital, Dehradun. **Materials and methods:** A total of 168 *Staphylococcus species* were isolated from different clinical samples. Erythromycin resistant strains were subjected to 'D' test for the detection of inducible clindamycin resistance as per CLSI guidelines. Results were analyzed statistically. **Result:** Four different phenotypes ($iMLS_B$, $cMLS_B$, MS, sensitive both erythromycin and clindamycin) were observed among MRSA, MSSA and coagulase negative *S. aureus*. 42.8% methicillin resistant *S.aureus* and 16.4% methicillin sensitive *S.aureus* isolates showed inducible clindamycin resistant phenotype. MRSA isolates showed significantly higher $iMLS_B$ phenotype (P= 0.0001). **Conclusion:** This study strongly proposes routine in vitro D-test of *Staphylococcus species*, which in turn will help in taking appropriate therapeutic decisions.

Introduction

Clindamycin is used in the treatment of skin and soft-tissue infections, caused by the *Staphylococcal species*. Good oral absorption makes this drug an important option in outpatient therapy or as a follow up after intravenous therapy. Clindamycin is also used as an alternative for patients who are allergic to penicillin (Fiebelkorn et al., 2003). Methicillin resistant (MR) strains of *Staphylococcus aureus* and CoNS are considered as main organisms causing nosocomial and community acquired infections. Macrolide, lincosamide and streptogramin B (MLS_B) antimicrobial agents are commonly used for the treatment (Chelae, 2009). The bacteria resist these groups of antibiotics in 3 ways: (a) through target site modification by *erm* (erythromycin ribosome methylation) gene, (b) through efflux mechanism by the *msrA* gene (macrolides streptogramins resistance) and (c) by drug inactivation (Chelae, 2009; Laclercq, 2002). Expression of MLS_B resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$). Macrolide induces the production of methylase and cause inducible resistance to clindamycin, on the other hand mutation on promoter region of *erm* allows production of methylase without an inducer and these strains are stably resistant to both erythromycin and clindamycin (Laclercq, 2002; Levin et al., 2005). Failure to identify $iMLS_B$ may cause failure of therapy with clindamycin. When the disc diffusion test is used to determine susceptibility, a distorted 'D-shaped' zone of inhibition is observed around clindamycin (Cc) if erythromycin (E) disc is placed nearby (Paul et al., 2004). Detection of $iMLS_B$ can be accomplished by agar disc diffusion method in accordance with the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2012). Therefore there is an increasing interest in assessing the frequency or prevalence of $iMLS_B$ because these strains have the genetic potential (presence of *erm* gene) to develop constitutive resistance to clindamycin during therapy (Chelae, 2009). The aim of the present study was to detect $iMLS_B$ in *Staphylococcus species* isolated from the indoor and outdoor patients of our hospital.

Materials and methods

A total of 168 *Staphylococcus species* were isolated from different clinical specimens like pus, wound swab, aspirates, blood and sterile body fluids obtained from various outpatient and inpatient departments at Shri Mahant Indiresh Hospital, Dehradun. The study was conducted from May to December 2012. The isolates were identified by standard biochemical techniques (Baird, 2007). Antibiotic susceptibility test was performed by the Kirby-Bauer disc diffusion method. *Staphylococcus* ATCC 25923 was used as control strain. All coagulase positive Staphylococcal isolates were confirmed by culturing on the mannitol salt agar, DNAase agar. Methicillin resistance was detected using oxacillin disc (1µg) diffusion on Muller Hinton agar (MHA) supplemented with 2% NaCl followed by incubation at 35°C and cefoxitin (30µg) disc diffusion test.

Erythromycin resistant strains were subjected to 'D' test for the detection of inducible clindamycin resistance as per CLSI guidelines (CLSI, 2012). The test was performed using 2µg clindamycin (Cc) disc and 15µg erythromycin (E) disc procured from Himedia, India Ltd. using the bacterial suspension of 0.5 McFarland on MHA plate as lawn culture, E (15µg) disc was placed at a distance of 15mm (edge to edge) from Cc (2µg) disc. Following overnight incubation at 37°C flattening of zone around clindamycin in the area between two discs indicate inducible clindamycin resistance (Fig 1). Four different phenotypes were identified are as follows:

Inducible MLS_B phenotype (D+): Staphylococcal isolates showing D shaped zone of inhibition around the clindamycin disc (zone size ≥ 21mm) and resistant to erythromycin (zone size ≤13mm) (Fig.1a).

Constitutive MLS_B phenotype (D-): The isolates of *Staphylococcus* which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) (Fig.1b).

MS phenotype: This phenotype showed resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥ 21mm). (Fig.1c).

Fourth phenotype are those isolates which were sensitive to erythromycin (zone size ≥ 23mm) as well as clindamycin (zone size ≥ 21mm) (Fig.1d).

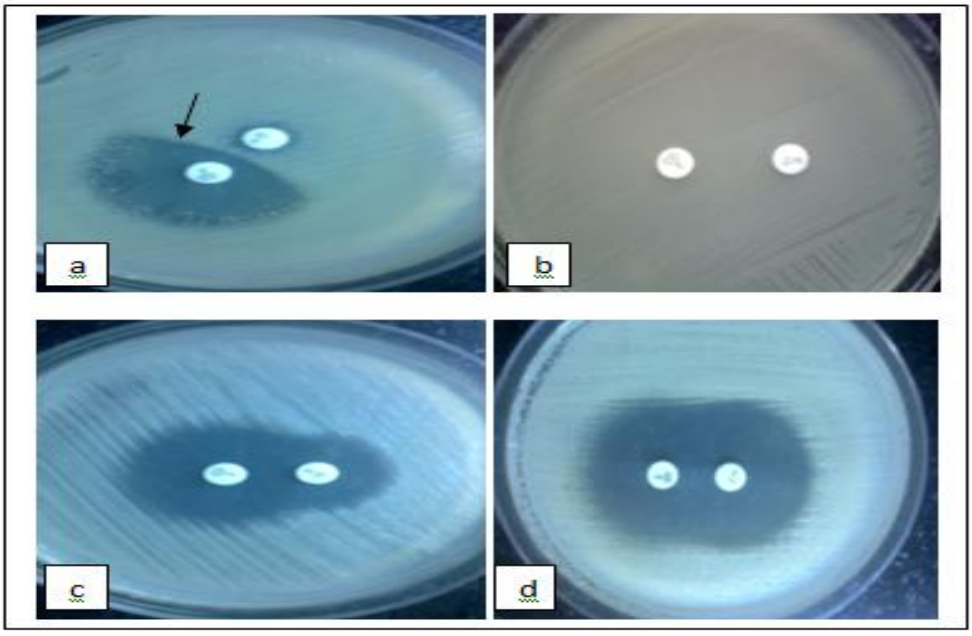
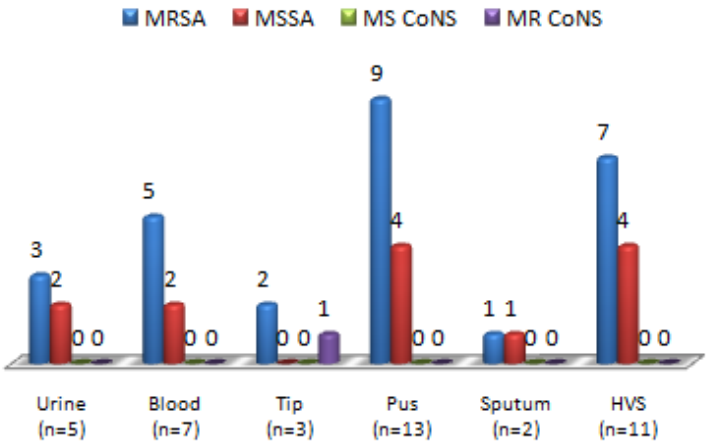


Figure: 1. a) $iMLS_B$ phenotype: induction of clindamycin resistance by erythromycin shown as blunting of zone of inhibition around the clindamycin disc, Positive D-test (arrow). b) $cMLS_B$ phenotype: Staphyococcal isolate resistant to both erythromycin and clindamycin. c) MS phenotype: Erythromycin resistant and clindamycin sensitive Staphyococcal isolate with circular zone of inhibition around clindamycin. d) Isolates sensitive to both erythromycin as well as clindamycin.

Table:1. Distribution of different phenotypes among *Staphylococcus* species.

		$iMLS_B$ (D+)	$cMLS_B$ (D-)	MS	E=S, Cc=S
MRSA	**(n=63)**	27	20	07	09
MSSA	**(n=79)**	13	19	30	17
MRCoNS	**(n=18)**	01	05	05	07
MSCoNS	**(n=08)**	0	05	0	03
Total	**168**	**41**	**49**	**42**	**36**

Figure:2. Specimen wise distribution of $iMLS_B$ phenotypes of *S. aureus* and CoNS.



Results

Out of one sixty eight isolates, 142 were *Staphylococcus aureus* and rest were coagulase negative *Staphylococcus* (CoNS). Among 142 *S. aureus*, 81.69% (116/142) were resistant to erythromycin, of which 34.48% (40/116) expressed inducible clindamycin resistance and 33.62% (39/116) had constitutive resistance, where as 31.89% (37/116) were MS phenotypes (Table:1). Twenty six strains of *S. aureus* were sensitive to both erythromycin and clidamycin (Table:1). The percentage of inducible clindamycin resistance was higher among MRSA isolates compared to MSSA isolates and found to be statistically significant (χ^2=0.0001, P< 0.01).

Similarly among CoNS, 61.54% (16/26) isolates were erythromycin resistant and 6.25% (1/16) were $iMLS_B$ phenotype (Table:1).

Discussion

Increasing frequency of MRSA infections and antimicrobial resistance have led to the renewed interest in the use of clindamycin. MRSA strains that are resistant to erythromycin but susceptible to clindamycin may show in vitro $iMLS_B$ phenotype due to *erm* gene expression. These strains however would then be mutated to form $cMLS_B$ during clindamycin therapy (Siberry et al., 2003). A study of 1976 was first to report clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin in a case of *Staphylococcus aureus* endocarditis (Drinkovic et al., 2001). Therefore caution must be taken to test for $iMLS_B$ phenotype in erythromycin resistant *Staphyloccocus species* before switching over to clindamycin therapy.

In this present study 43.36% (63/142) *S.aureus* were detected as MRSA and maximum number was found in sputum (80%) followed by tip (65.62%) and blood (27.77%). Various Indian studies published, show different patterns of drug resistance (Table: 3) at different geographic locations. 42.8% (27/63) strains of MRSA were $iMLS_B$ (D+) phenotype, whereas only16.4% (13/79) MSSA expressed $iMLS_B$ phenotype.

Constitutive resistance in our study was seen as 31.7% (20/63) of MRSA isolates as compared to 29% reported from other part of Uttarakhand (Juyal et al., 2013). The prevalence varies according to geographical location as found in various studies from 7.3% to 46.9% in MRSA (Table: 3) (Deotale et al., 2010; Pal et al., 2010).

Table: 2. Inducible clindamycin resistance in different studies.

Study	Year	MRSA	$iMLS_B$	$cMLS_B$
This study	2012	37.5%	42.8%	31.7%
Gupta *et al*.,	2009	25%,	20%	23%
Pal *et al*.,	2010	31.60%,	23.48%	46.97%
Deotale *et al*.,	2010	49.79%	27.6%	7.3%
Bansal *et al*.,	2011	44.8%,	33.9%	44.7%
Shantala *et al*.,	2011	54.78%	32.53%	25.39%

Reporting *S.aureus* as sensitive to clindamycin without checking for inducible resistance may result in failure in clindamycin therapy. However negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a good therapeutic option (Prabhu et al., 2011). In this study 44.36% (63/142) of isolates of *S. aureus* (both MRSA and MSSA) were susceptible to clindamycin suggesting a potential role in treatment. It was also found that 37.97% (30/79) MSSA isolates were MS phenotypes and this finding is consistent with previous studies (Jethwani et al., 2011; Manjunath et al., 2013).

Pal et al. (2010) reported 22.42% $iMLS_B$ in CoNS, where as in this study only 3.84% (1/26) iMLSB methicillin resistant among CoNS was detected. Therefore clinical microbiology laboratories should include detection of indicible clindamycin resistance for both *S. aureus* and CoNS along with susceptibility test report.

Conclusion

Macrolides are used worldwide as first line of treatment against skin and soft tissue infections due to their wide spectrum of action and comparatively low toxicity. Owing to the complex mechanisms of drug resistances for macrolides and indiscriminate use of antibiotics, it is losing its beneficial role as a crucial member of primary line of treatment, especially for staphylococcal infections. It is thus advisable to perform and report simple tests like D-test along with standard antibiotic susceptibility testing to rule out the possibility of treatment failure with macrolides.

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