

The Prevalence of Inducible and Constitutive Clindamycin Resistance Among the Nasal Isolates of *Staphylococci*

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ABSTRACT

Context: One of the important sources of *Staphylococci* which causes nosocomial infections, is the nasal carriage of *Staphylococci* among Health Care Workers (HCWs). The commonest antibiotic which is preferred for the treatment of the methicillin and multi drug resistant *Staphylococcal* infections is clindamycin. The inducible clindamycin resistance in *Staphylococci* is not detected by the routine antibiotic susceptibility testing and it results in treatment failures.

Aim: The present study was undertaken to know the prevalence of constitutive and inducible clindamycin resistance and its correlation with the methicillin resistance among the nasal isolates of *Staphylococci* which were obtained from different HCWs.

Material and Methods: Nasal swabs were collected from 206 HCWs and they were processed. The *Staphylococci* which were isolated were tested for methicillin resistance by using cefoxitin

(30 µg) discs. The inducible clindamycin resistance was tested by using erythromycin (15 µg) and clindamycin (2µg) discs and the D test according to the CLSI guidelines.

Results: Inducible clindamycin resistance was seen in 21(16.40%) of the *S.aureus* and 14 (7.56%) of the coagulase negative *Staphylococcal* isolates. Constitutive clindamycin resistance was seen in 23(17.96%) of the *S.aureus* and 43(23.24%) of the coagulase negative *Staphylococcal* isolates. The inducible and constitutive clindamycin resistance was more common among the methicillin resistant *Staphylococcal* isolates.

Conclusion: The prevalence of inducible and constitutive clindamycin resistance in the nasal *Staphylococcal* isolates which were obtained from the HCWs was high, especially among the methicillin resistant *Staphylococci*. The D test which is recommended by the CLSI should be routinely done to detect inducible clindamycin resistance, to prevent treatment failures.

Key words: Inducible clindamycin resistance, D test, Methicillin resistance, *Staphylococci*, MLS_B

INTRODUCTION

Staphylococcus aureus and coagulase Negative *Staphylococci* (CoNS) are recognized as pathogens which cause nosocomial and community acquired infections in every region of the world. The resistance to antimicrobial agents among *Staphylococci* is an increasing problem [1]. The methicillin resistance in *Staphylococci* is an increasing problem in the clinical practice, because the Methicillin Resistant *Staphylococcus Aureus* (MRSA) strains are resistant to other antimicrobial agents and isolates with a reduced susceptibility and resistance to vancomycin have also emerged [2]. Once such a strain is recognized to be the causative agent of an infection, it is of interest, for determining as to which of the alternatives to vancomycin is suitable for the therapy. The commonest antibiotic which is preferred for the treatment of these *Staphylococcal* infections is clindamycin (CL) [3]. Its low cost, fewer severe side effects, the availability of oral and parenteral forms, the lack of a need for a renal adjustment and good tissue penetration and ability to directly inhibit toxin production are its advantages. Moreover, CL is a useful choice in cases of penicillin allergy [4].

Clindamycin is an antimicrobial which belongs to the Macrolide–Lincosamide–Streptogramin B (MLS_B) family. The wide spread use of the MLS_B family of antimicrobials has led to the emergence of resistance [5].

The macrolide antibiotic resistance in *Staphylococci* can be mediated by the *msr A* gene which codes for an efflux mechanism which confers resistance to the macrolides and the type B streptogramin only or via the *erm* gene which encodes for the enzymes that cause a ribosomal target modification by causing methylation of the 23S rRNA, thereby reducing the binding of the

MLS_B agents to the ribosomes, thus conferring resistance to the macrolides, lincosamides and the type B streptogramins (MLS_B resistance). The MLS_B resistance may be of the constitutive (cMLS_B) or the inducible (iMLS_B) type. The isolates with cMLS_B are resistant to both erythromycin (ER) and CL and they are readily detected by in vitro testing. The isolates with iMLS_B are resistant to ER, but they appear to be susceptible to CL in the routine susceptibility testing and are easily missed [6]. This results in an inappropriate clinical use of clindamycin and a treatment failure. These isolates can be detected easily by the D test [7]. One of the important sources of *Staphylococci* which cause nosocomial infections is the nasal carriage among Health Care Workers (HCWs) [8].

Hence, the present study was undertaken to know the prevalence of the constitutive and inducible clindamycin resistance and its correlation with the methicillin resistance among the nasal isolates of *Staphylococci* which were obtained from different HCWs by the CLSI 2011 recommended D test at our tertiary health care centre.

MATERIAL AND METHODS

The present study was conducted in the Microbiology Department from January 2013 to April 2013 and it included the nasal swab which were collected from a total of 206 different HCWs of our tertiary care hospital. The standards of the ethical committee on human experimentation were followed during the study.

Nasal swabs were collected from all the participants by using sterile cotton swabs which was soaked in sterile saline, by rotating the swabs in both the anterior nares consecutively. The swabs were processed immediately by inoculating the samples from them onto sheep blood agar plates. The plates were incubated aerobically

at 37°C for 24-48 hours. The *Staphylococcal* species which were isolated were identified on the basis of their colony morphologies and the catalase, coagulase, mannitol fermentation and the DNAase tests by following the standard microbiological techniques. Methicillin resistance was detected by the Kirby–Bauer disc diffusion method by using cefoxitin 30µg discs according to the CLSI-2011 guidelines [7].

The detection of the inducible clindamycin resistance was performed by using the D test according to the CLSI-2011 [7] guidelines. An erythromycin disc (15µg) was placed about 15-26mm apart from a clindamycin disc (2µg) in the standard disc diffusion method of Kirby- Bauer. The plates were incubated at 35 ± 2°C for 18-24 hours. The different phenotypes were appreciated after testing and they were interpreted as follows [7,9,10].

The MS phenotype: The *Staphylococcal* isolates which exhibited resistance to erythromycin (zone size ≤ 13mm) and sensitivity to clindamycin (zone size ≥ 21mm) and which showed circular zones of inhibition around clindamycin were labeled as having the MS phenotype.

The Inducible MLS_B (iMLS_B) phenotype: The *Staphylococcal* isolates which showed resistance to erythromycin (zone size ≤ 13 mm) and sensitivity to clindamycin (zone size ≥ 21mm) and which showed a 'D' shaped zone of inhibition around clindamycin, with flattening towards the erythromycin disc or a hazy growth within the zone of inhibition around clindamycin (even if no D-zone was apparent), were labeled as having the iMLS_B phenotype.

The constitutive MLS_B (cMLS_B) phenotype: The *Staphylococcal* isolates which showed resistance to both erythromycin (zone size ≤ 13mm) and clindamycin (zone size ≤ 14mm), with circular zones of inhibition, if any, around clindamycin, were labeled as having the cMLS_B phenotype.

The source of the antibiotic discs was Hi-Media Ltd, Mumbai, India.

RESULTS

A total of 313 *Staphylococcal* species were isolated from the nasal swabs of the 206 HCWs. Among them, 128 (40.89%) were *S.aureus* and 185(59.10%) were coagulase negative *Staphylococci*. Among the *Staphylococcal* isolates, 45(35.15%) were MRSA, 83(64.84%) were MSSA (methicillin sensitive *S.aureus*), 61(32.97%) were MRCoNS (methicillin resistant CoNS) and 124(67.02%) were MSCoNS (methicillin sensitive CoNS).

Erythromycin resistance was seen in 46(35.93%) of the *S.aureus* isolates. Among these isolates, iMLS_B was seen in 21(16.40%) isolates. 23(17.96%) isolates showed cMLS_B and the MS phenotype was seen in 5(3.90%) isolates. In [Table/Fig-1], it can be observed that the iMLS_B and the cMLS_B isolates were more among the MRSA than the MSSA isolates. Similarly, erythromycin resistance was seen in 72(38.91%) of the CoNS isolates. Among these isolates, iMLS_B was seen in 14(7.56%) isolates. 43 (23.24%) isolates showed cMLS_B and the MS phenotype was seen in 15(8.11%) isolates. In [Table/Fig-1], it can be observed that the iMLS_B and the cMLS_B isolates were more among the MRCoNS isolates than among the MSCoNS isolates.

DISCUSSION

The prime step before the initiation of the antimicrobial therapy in infected individuals, is performing the antimicrobial susceptibility testing for the clinical isolates, to avoid an indiscriminate usage of antibiotics on a trial and error basis. The empirical treatment for the *Staphylococcal* infection is more endangered, due to the emergence of multi drug resistant strains, especially MRSA.

The increasing frequency of the *Staphylococcal* infections among the patients and the changing patterns in antimicrobial resistance have led to a renewed interest in the use of the clindamycin therapy in treating such infections [11].

Resistance phenotype	<i>S. aureus</i> N=128 (40.89%)			Coagulase negative <i>staphylococci</i> N=185 (59.10%)		
	MRSA	MSSA	Total (%)	MRCoNS	MSCoNS	Total (%)
	N=45 (35.15%)	N=83 (64.84%)		N=61 (32.97%)	N=124 (67.02%)	
Erythromycin resistant	26 (64.45)	20 (24.09)	46 (35.93)	45 (73.77)	27 (21.77)	72 (38.91)
iMLS _B	11 (24.44)	10 (12.04)	21 (16.40)	10 (16.39)	4 (3.22)	14 (7.56)
cMLS _B	15 (33.33)	8 (9.63)	23 (17.96)	25 (40.98)	18 (14.51)	43 (23.24)
MS	3 (6.66)	2 (2.40)	5 (3.90)	10 (16.39)	5 (4.03)	15 (8.11)

[Table/Fig-1]: Susceptibility of staphylococcal species to erythromycin and clindamycin. MRSA- methicillin resistant *S.aureus*, MSSA-methicillin sensitive *S.aureus*, MRCoNS- methicillin resistant CoNS, MSCoNS- methicillin sensitive CoNS

CL resistance can develop in the *Staphylococcal* isolates with the inducible phenotype and spontaneous constitutively resistant mutants have been selected from such isolates, both in vitro and in vivo during the CL therapy [12-14].

The health care workers are at the interface between the hospitals, the long term care facilities, and the nursing homes on one hand and the community on the other and they may serve as the reservoirs, vectors, or the victims of the multi drug resistant *Staphylococci*. The health care workers who carry such *Staphylococci* can transmit the pathogen to the patients who are under their care, thereby leading to various complications which are associated with the *Staphylococcal* infections [15].

The constitutive CL resistance is easily detected by in vitro susceptibility testing, whereas the inducible CL resistance is easily missed in the in vitro susceptibility testing. In the present study, inducible CL resistance was seen in 24.44%, 12.04%, 16.39%, and 3.22% of the MRSA, MSSA, MRCoNS and the MSCoNS nasal isolates respectively, which were obtained from the HCWs. [Table/Fig-2] shows the iMLS_B which was reported in various studies. The findings of the present study are in agreement with those of most

Study	MRSA (%)	MSSA (%)	MRCoNS (%)	MSCoNS (%)
Present study (2013)	24.44	12.04	16.39	3.22
Rao VR et al., [16]	45.71	0	0	0
Reddy PS et al., [17]	46.2	22.2	-	-
N Pal et al., [18]	43.56	6.93	43.56	6.0
Fasih N et al., [19]	70	73	-	-
Yilmaz G et al., [1]	24.4	14.8	25.7	19.9

[Table/Fig-2]: Inducible clindamycin resistance (iMLS_B) in various studies

of the studies, in that iMLS_B is more common in the methicillin resistant *Staphylococcal* isolates. The data also suggest that the occurrence of the iMLS_B and the cMLS_B phenotypes varies widely by hospital, the geographic area, and the methicillin susceptibility of the isolates. Hence, the local data regarding the CL resistance is helpful in guiding the anti *Staphylococcal* therapy. A macrolide induced clindamycin resistance was observed among the clinical isolates of *Staphylococcus* since 1968, which could not be detected by the routine disc diffusion method [20]. From such isolates, constitutively resistant mutants emerge in vivo and they result in a treatment failure. Conversely labeling all the erythromycin resistant *Staphylococci* as CL resistant or not reporting the CL resistance, will prevent the use of CL in treating the infections that are likely to respond to the CL therapy [11,21]. An accurate susceptibility data is an important factor in the making of appropriate therapy decisions. The sensitivity of the D test which was performed at

15–20 mm of disc spacing was 100%, when it was correlated with the detection of the *erm* and the *msr* genes by PCR. The true sensitivity to CL can easily be judged by performing the D test on the erythromycin resistant isolates [22,23]. During the application of the susceptibility test to the *Staphylococcal* isolates, the clinical microbiology laboratories should place the ER disc 15 mm apart from the CL disc. Consequently, the treatment with the use of CL can be omitted in the patients with infections which are caused by the inducibly resistant strains, and therapeutic failures may thus be avoided.

To conclude, the prevalence of inducible and constitutive clindamycin resistance in the nasal *Staphylococcal* isolates of the HCWs was high, especially among the methicillin resistant isolates. The D test can be used as a simple, auxiliary and a reliable method for delineating the inducible and the constitutive CL resistance in the routine clinical laboratories. Misclassification of the isolates with iMLS_B resistance without doing the D test would lead to treatment failures.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 01, 2013**
Date of Peer Review: **May 13, 2013**
Date of Acceptance: **May 21, 2013**
Date of Publishing: **Aug 01, 2013**