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Original Research Paper

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PHENOTYPIC DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF COAGULASE NEGATIVE STAPHYLOCOCCI- STUDY FROM TERTIARY CARE CENTRE

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ABSTRACT

Background : Coagulase negative staphylococci(CoNS) are colonisers of skin and mucous membrane. When isolated from clinical samples they were considered as contaminants but recently have gained importance as agents of nosocomial infections. and their antimicrobial resistance patterns to commonly used agents that limits therapeutic options. The study was done to detect phenotypic expression of inducible clindamycin resistance in clinically significant isolates of coagulase negative staphylococci.

Materials And Methods: The study was a cross sectional study done in the Department of Microbiology RIMS, Ranchi, for a period of 1 year from July2017 to June2018. A total of 111 clinically significant isolates of Coagulase negative staphylococci were identified from various clinical samples by using standard microbiological methods. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and results interpreted using the CLSI guidelines. Methicillin resistance was detected by using cefoxitin discs (30 µg). The isolates showing clindamycin sensitive and erythromycin resistance were selected for D –test to test for inducible clindamycin resistance.The strains showing flattening of or blunting of zone of inhibition(D shaped) around CL were designated as D-test positive.

Results: Methicillin resistant coagulase negative Staphylococi (MRCoNS) accounted for 39(35.2%). Three types of phenotypes were found, constitutive resistance(cMLSB), inducible resistance(iMLSB) and MS phenotype or D -test negative. The three types were determined in Methicillin sensitive and Methicillin resistant coagulase negative staphylococci(MSCoNS, MRCoNS).D-test was found to be positive in 32.1% of MRCoNS and 11.3% of MSCoNS. Constitutive resistance (cMLSB) phenotype was found in 10.2% of MRCoNS, iMLSB(inducible resistance) phenotype was found in 32.1% of MRCoNS, MS phenotype was found in 67.8% of MRCoNS. Constitutive resistance(cMLSB) phenotype was found in 13.8% of MSCoNS, iMLSB(inducible resistance) phenotype was found in 11.3% of MSCoNS, MS phenotype was found in 88.6% of MSCoNS. MS phenotype or D test negative was found in 67.8% of MRCoNS and 88.6% of MSCoNS.

Conclusion: D-test is a simple and easy method for determining phenotypic expression of inducible clindamycin resistance, therefore it may be helpful to guide empirical therapy in patients with skin and soft tissue infection and avoid clinical therapeutic failures.

KEYWORDS : MRCoNS, Clindamycin, Erythromycin, D-Test

INTRODUCTION

Coagulase negative staphylococci(CoNS) are colonisers of skin and mucous membrane, when isolated from clinical samples were considered as contaminants but recently have gained importance as agents of nosocomial infections. (1) They have become an important cause of morbidity in immunocompromised patients due to their multidrug resistance pattern. Increasing antimicrobial drug resistance pattern limits therapeutic options. Human and animal origin CoNS harbour large reservoir of mobile genetic elements leading to resistance to beta lactams, aminoglycosides, quinolones, macrolides and tetracyclines. An increase in incidence of nosomial infections caused by CoNS resistant to methicillin have been reported. Resistance to methicillin is determined by the presence of *mec*A gene carried on mobile genetic element staphylococcal chromosomal cassette *mec (SCCmec)*.(2) The increasing frequency of Methicillin resistant coagulase negative staphylococcus(MRCoNS) and changing pattern in antimicrobial resistance have led to renewed interest in the use of macrolide lincosamide streptogramin B(MLSB) antibiotics to treat such infections.

MLSB antibiotics are structurally unrelated but have similar mode of action.(3) Resistance to MLSB can occur by two different mechanisms: an active efflux mechanism encoded by *msr* A gene (macrolides, lincosamides and streptogramin resistance) and ribosomal target modification affecting macrolides, lincosamide and type B streptogramins coded by the *erm* gene(MLSB resistance).The *erm* genes encode enzymes that confer inducible and constitutive resistance to MLS agents. The presence of *erm* gene causes methylation of 23S rRNA leading to reduced binding of these agents to ribosomes.(4) The msrA gene confirs MS phenotype (resistance to erythromycin, inducible resistance to streptogramins and susceptibility to clindamycin) by efflux.(5) MLSB resistance can either be constitutive (cMLSB) or inducible(iMLSB). In vitro MRCoNS isolates with constitutive resistance are resistant to erythromycin and clindamycin, while isolates with inducible resistance are resistant to ER but appear to be susceptible to CL(6,7,8)

The iMLSB strains show in vitro resistance to erythromycin while appearing susceptible to clindamycin which may cause inability to identify resistance and clinical failure to clindamycin therapy. As erythromycin is an effective inducer of iMLSB resistance, D-test helps to detect this kind of resistance in CoNS.(9) The iMLSB resistance can be detected by a disc induction test, a distorted D shaped zone of inhibition is observed around CL if an ER disc is placed nearby(15-20mm).(10)

There are few studies done in our area demonstrating the detection of inducible clindamycin resistance among clinical isolates of CoNS using the D-test by disc diffusion method. Therefore, the study was taken up to identify isolates showing inducible clindamycin resistance that lead to clinical failure of clindamycin therapy in skin and soft tissue infection.

MATERIALS AND METHODS

The study was a cross sectional study, conducted in the

Department of Microbiology from July 2017 to June 2018. The study was approved by Institutional Ethical Committee. A total of 111 clinical isolates of CoNS was isolated from various clinical samples. The isolates were identified by colony morphology, gram staining, catalase test, coagulase test, both slide and tube coagulase, hemolysis using blood agar, and growth on Mannitol salt agar. The tests done for speciation included susceptibility to Novobiocin, Voges Proskauer, nitrate reduction, urease test, ornithine and arginine decarboxylase, resistance to polymyxin B, acid production from maltose, xylose, lactose, sucrose, fructose, mannitol and mannose. The isolates were considered clinically significant when isolated in pure culture from infected sites.

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and results interpreted using the CLSI guidelines.(11) Methicillin resistance was detected by using cefoxitin discs 30 µg, zone diameter >=25mm was considered as methicillin sensitive(MSCoNS) and zone diameter<=24mm was considered as methicillin resistant(MRCoNS). The isolates showing clindamycin sensitive and erythromycin resistant were selected and subjected to D –test to test for inducible clindamycin resistance.(10) using erythromycin disc 15mcg and clindamycin disc 2 mcg procured from Hi media Laboratories Pvt. India Ltd. The discs were placed at a distance of 15mm from centre to centre on Muller Hinton agar plates inoculated with test organisms. After incubation the plates were read using transmitted light to detect any flattening of or blunting of the shape of clindamycin zone. The strains showing flattening of or blunting of zone of inhibition(D shaped) around CL were designated as D-test positive.

Three Types Of Phenotypes Were Identified-

1. cMLSB phenotype- growth upto clindamycin(CL) and erythromycin(ER) discs indicate resistance to both(resistant to both ER-zone of inhibition<=13mm &CL<=14mm).
2. iMLSB phenotype- inducible clindamycin resistance, showing flattened clindamycin zone between erythromycin and clindamycin disc(D-test +ve)(resistant to ER<13mm, sensitive to CL>=21mm).
3. MS phenotype- resistance to erythromycin but susceptible to clindamycin or negative for inducible clindamycin resistance, with no flattening CL zone.(D- test-ve).

RESULTS

Methicillin resistant coagulase negative Staphylococi (MRCoNS) accounted for 39(35.2%). Among the MRCoNS maximum strains belonged to *S. haemolyticus* accounting for 59.2%, followed by *S. epidermidis* 54.5%(Table 1). D-test was performed to study inducible clindamycin resistance(iMLSB) for the isolated organisms and 32.1% of Methicillin resistant coagulase negative staphylococci were found to be D- test positive. (Table 4, Fig3). Constitutive resistance (cMLSB) phenotype was found in 10.2% of MRCoNS, iMLSB(inducible resistance) phenotype was found in 32.1% of MRCoNS, MS phenotype was found in 67.8% of MRCoNS (Table 3, Fig2).

Constitutive resistance(cMLSB) phenotype was found in 13.8% of MSCoNS, iMLSB(inducible resistance) phenotype was found in 11.3% of MSCoNS, MS phenotype was found in 88.6% of MSCoNS(Table 3, Fig2).

D-test was found to be positive in 32.1% of MRCoNS and 11.3% of MSCoNS.

MS phenotype or D test negative was found in 67.8% of MRCoNS and 88.6% of MSCoNS(Table 4, Fig3)

Table1: Methicillin Resistance Among Spp Of CoNS

CoNS spp.	No. of strains tested	% of MRCoNS
Staphylococcus epidermidis(55)	19	34.5%
Staphylococcus haemolyticus(27)	16	59.25%
Staphylococcus saprophyticus(11)	2	18.8%
Staphylococcus cohnii(9)	1	11.1%
Staphylococcus hominis(3)	1	33.3%
Staphylococcus warneri	-	0%
Staphylococcus lugdunensis	-	0%

Table 2: Pattern Of Sensitivity Of Isolates To ERY And CL Based On Disc- Diffusion Method

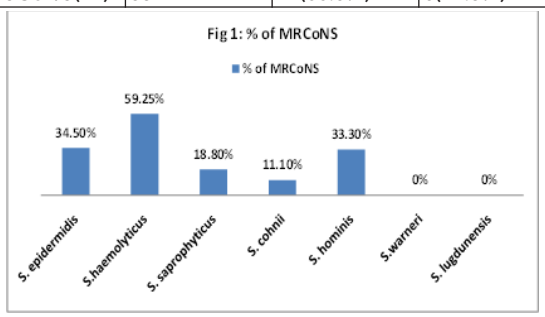
Organism	Total no. of isolates	Erythromycin -S & Clindamycin-S	Erythromycin -R& Clindamycin -S	Erythromycin R & Clindamycin-R
MRCoNS	39	7(17.9%)	28(71.7%)	4(10.2%)
MSCoNS	72	9(12.5%)	53(73.6%)	10(13.8%)

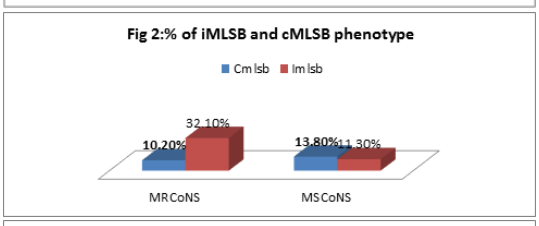
Table3: Phenotypes Of CoNS Isolates

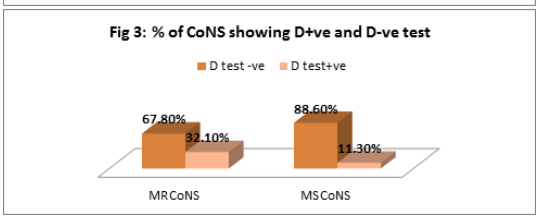
Organism	cMLSB phenotype	iMLSB phenotype	MS phenotype
MRCoNS	4(10.2%)	9(32.1%)	19(67.8%)
MSCoNS	10(13.8%)	6(11.3%)	47(88.6%)

Table 4: Inducible Clindamycin Resistance Among Isolates Based On D- Test

Organism	Total No. of isolates	D test-ve	D test+ve
MRCoNS(39)	28	19(67.8%)	9(32.1%)
MSCoNS(72)	53	47(88.6%)	6(11.3%)







DISCUSSION

Methicillin resistant coagulase negative Staphylococi (MRCoNS) accounted for 39(35.2%). Among the MRCoNS maximum strains belonged to *S. haemolyticus* accounting for 59.2%, followed by *S. epidermidis* 54.5%. In the present study Methicillin resistance was seen in 39 (35.2%) isolates which is different from study done by Amita V Jain et al. (66%) , Saroj Golia (60%) and Asangi et al. (67.7%) (12,13). Studies done by

KL Shobha et al. reported 14% methicillin resistance. Yasar F. Koksal and U. Farooq determined methicillin resistance to be 67.5% and 52.83% respectively.(14,15,16)

The present study showed 10.2% of the Methicillin Resistant Coagulase Negative Staphylococci with constitutive macrolide lincosamide streptogramin B (cMLSB) phenotype and 32.1% of these isolates with inducible macrolide lincosamide streptogramin B (iMLSB) phenotype which is different from study done by D Juyal et al with 29% of MRCoNS with cMLSB phenotype and 15.7% with iMLSB phenotype.(17)

Mohanasoundaram (18) from Tamil Nadu demonstrated iMLSB resistance to be 17% in CoNS whereas the present study showed it to be 32.1% in MRCoNS & 11.3% in MSCoNS. The inducible clindamycin resistance phenotype varies on geographical basis, hospital environment , patient age and species involved. To report clindamycin accurately, the staphylococci must first be subjected to D- test to exclude the isolates with an induced clindamycin resistance (iMLSB), as such isolates when treated with Clindamycin can undergo in vitro conversion to a constitutive resistance (cMLSB) and this may result in Clindamycin treatment failure. There is high burden of staphylococcal infections require some alternative to vancomycin and Clindamycin is a good option, so the prevalence of inducible resistance against it must be known.

Clindamycin is a good option in the treatment of bone, skin and soft tissue infection because of its low cost, good proven efficacy, availability of oral and parenteral forms, tolerance, good tissue penetration, good accumulation in abscess and no dose adjustments required in renal insufficiency. It directly inhibits staphylococcal toxin production and is a good alternative in patients allergic to penicillin. It is active against methicillin sensitive as well as methicillin resistant staphylococci. Appropriate clindamycin susceptibility requires D-test to exclude isolates with iMLSB resistance as such isolates may undergo rapid in vitro conversion to constitutive MLSB(cMLSB) resistance causing therapeutic failures.

CONCLUSION

CoNS are important colonizers of skin and mucous membrane of the patients and are easily transmitted by the hands of health care professionals. Their multidrug resistant nature has helped them in causing various nosocomial infections. As CoNS harbour large reservoir of mobile genetic element they are resistant to most antibiotics. The inducible resistance can be easily missed by routine in vitro susceptibility tests when erythromycin and clindamycin discs are not placed in adjacent position. D- test is an easy method for phenotypic detection of inducible clindamycin resistance and it may guide the clinician in judicious use of it in patients with skin and soft tissue infections for its maximum clinical utility. The method may be used routinely to guide empirical therapy and avoid any clinical failures, thus reducing morbidity and mortality of patients.

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