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DETECTION & CORRELATION OF INDUCIBLE AND CONSTITUTIVE CLINDAMYCIN RESISTANCE WITH METHICILLIN RESISTANCE IN COAGULASE NEGATIVE STAPHYLOCOCCI

Microbiology

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ABSTRACT

Background: CoNS are one of those amongst the commonly isolated organisms in the clinical microbiology laboratory. Interest in CoNS is increasing because of their role as pathogens in hospital-acquired infections (HAI) associated with catheters and implanted medical devices and their marked resistance to antibiotics.

CoNS strains are commonly multi-resistant to various groups of antibiotics.The macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics is commonly used in the treatment of staphylococcal infections.(35) However, one important issue in clindamycin treatment is the risk of clinical failure during therapy.

Materials And Methods:

Methicillin Resistance : In this study methicillin resistance was tested by cefoxitin disk (30µg) diffusion method as per CLSI guidelines, **Inducible Clindamyc in Resistance:** All CoNS isolates were tested for the inducible clindamycin resistance by Double-disk diffusion test (D-zone test) as per CLSI guidelines.

Results: 234 Coagulase Negative Staphylococcial strains from different clinical specimen were processed. Methicillin resistance was shown by 65.38% isolates. Inducible clindamycin resistance was found to be 31.3% & 14.28% respectively in MRCoNS & MSCoNS Similerly constitutive clindamycin resistance was found to behigher in MRCoNS at 57.39% & then in MSCoNS (35.71%).

Conclusion: Clindamycin disk induction test (D-zone test) is simple and must be done routinely in the diagnostic microbiology laboratories. Without the "D-zone test" all the CoNS isolates with inducible clindamycin resistance would be erroneously classified as clindamycin susceptible by routine testing methods. Constitutive & inducible clindamycin resistance was found to be more prevalent in MRCoNS then MSCoNS.

KEYWORDS

Coagulase Negative Staphylococci, Methicillin Resistance, Inducible clindamycin resistance, Constitutive clindamycin resistance, D test.

INTRODUCTION:

Over the past few decades, CoNS have become recognized as important agents of human diseases.[1] CoNS are reported to be the third common causative agent of nosocomial infections and the most common cause of nosocomial blood stream infections.[2,3]

Emergence of methicillin resistance in Coagulase Negative *Staphylococci* has left us with very few therapeutic alternatives available to treat CoNS infections. Antibiotics like Macrolides, lincosamides and type B streptogramin (MLS_B) belong to different class but act through same mechanism of inhibition of protein synthesis, and are routinely used in treatment of such infections [4]. Specially Clindamycin (a lincosamide) in particular, is an attractive choice for clinicians as it is available for both oral and injectable use, tissue penetration is good, and is bacteriostatic against Staphylococci [5]. CoNS strains resistant to Macrolides, lincosamides and type B streptogramin antibiotics have increased in number following there widespread use[6,7]

Mechanism for resistance is different to these antibiotics. Active efflux of drug because of *msr* (A) gene confers resistance to streptogramin and B macrolides (MS phenotype) but not to clindamycin. Another mechanism for resistance is ribosomal target modification, it confers resistance to all above mentioned groups, ie type B streptogramin, macrolide and clindamycin (MLS_B phenotype). MLS_B resistance in *CoNS* is either constitutive ($cMLS_B$) or inducible ($iMLS_B$), in constitutive ($cMLS_B$) rRNA methylase is always present while in inducible ($iMLS_B$) rRNA methylase is produced only in the presence of an inducer[8,9]. Infection with $iMLS_B$ strains of *CoNS* if treated with clindamycin can develop constitutive resistance during therapy and result in treatment failure [10]. So before starting these antibiotics it is important to detect the phenotype (MS, $iMLS_B$, $cMLS_B$).

Macrolids (erythromycin) are an inducer for clindamycin resistance ($iMLS_B$), which induces production of erythromycin ribosomal methylase (erm). D test (Double disc diffusion) is recommended by CLSI for detection of inducible clindamycin resistance [11]. A negative result by D test confirms clindamycin susceptibility and provides a treatment option[12].

The present study was undertaken with an aim to detect methicillin resistance and their property of constitutive or inducible clindamycin resistance in Clinical Isolated of CoNS.

MATERIALS AND METHODS:

The present study was conducted in the Department of Microbiology, Govt Medical College, Nagpur, Maharastra, India. The study included 234 non-duplicate isolates of *CoNS* from various clinical samples of IPD patients received over the period of study. Various clinical samples included pus, blood, urine, tracheal aspirates and cerebrospinal fluid (CSF). The organism was identified by conventional laboratory methods such as colony morphology, gram stain, catalase test, slide and tube coagulase test.

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method and methicillin resistance was identified by using cefoxitin (30 µg) disc and interpreted as per CLSI guidelines [11]

All isolates were subjected to inducible clindamycin resistance testing by CLSI recommended D test on Mueller Hinton agar by keeping erythromycin (15µg) disc and clindamycin (2 µg) disc at 15 mm apart (edge to edge) [11]

Blunting of the circular zone of inhibition around clindamycin disc towards erythromycin disc indicated the presence of $iMLS_B$ resistance and was reported as resistant to clindamycin. Quality control (QC) for erythromycin and clindamycin discs was done by using *S. aureus* ATCCC 25923 according to standard disc diffusion QC procedure. We interpreted the results as follows:

1. The isolate sensitive to erythromycin and clindamycin was considered susceptible phenotype,
2. Erythromycin resistant and clindamycin sensitive isolate (no D zone), MS phenotype,
3. Erythromycin resistant and clindamycin sensitive isolate (D zone present), $iMLS_B$ phenotype and
4. Erythromycin and clindamycin resistant isolate, cMLSB phenotype.

RESULT:

The study was conducted during the period from November 2012 to October 2014 in Department of Microbiology Govt.Medical College Nagpur. Isolates of Coagulase Negative Staphylococci from the various clinical specimens received in the diagnostic bacteriology laboratory from inpatient departments were included in this study.

We processed a total of 12358 clinical samples in our laboratory and were able to isolate 234 Coagulase Negative Staphylococcial strains from these specimens

Table no-1: Methicillin resistance pattern of CoNS isolates

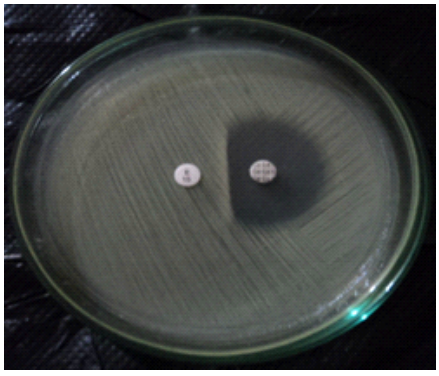
Isolates	NUMBER	PERCENTAGE
MRCoNS	153	65.38%
MSCoNS	81	34.62%
TOTAL	234	100%

MRCoNS DETECTION

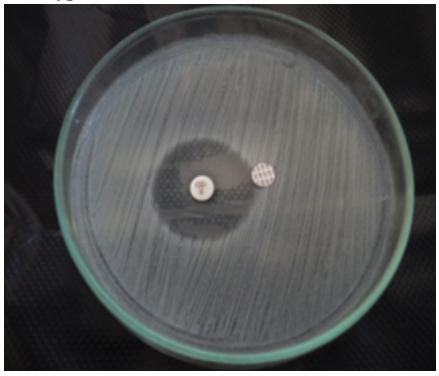
Cefoxitin disk (30 µg) diffusion test

In this study, methicillin resistance among CoNS isolates was identified by cefoxitin disk diffusion method. Table No 1 shows that out of 234 strains of CoNS, 153 strains (65.38%) were detected as Methicillin resistant(MRCoNS) and 81 strains (34.62%) as Methicillin sensitive(MSCoNS) by cefoxitin disk diffusion method.

Detection Of Inducible Clindamycin Resistance: D-zone Test



MLSBiPhenotype



MS Phenotype

Table no- 2: Inducible And Constitutive Clindamycin Resistance in CoNS isolates

Resistance	Number	Percentage
MLSBi	38	16.24%
MLSBc	71	30.34%
MS	20	8.55%

129 (55.13%) of CoNS isolates were erythromycin resistant. 38 isolates (16.24%) showed positive D-zone test indicating inducible clindamycin resistance (MLSBi phenotype). 71 (30.34%) isolates were resistant to both erythromycin and clindamycin indicating constitutive clindamycin resistance (MLSBc phenotype). 20 (8.55%) CoNS isolates were erythromycin resistant and susceptible to clindamycin with no induction on D-zone test indicating MS phenotype. Table No 2

Table No- 3: Inducible And Constitutive Clindamyc in Resistance in MRCoNS

Resistance	Number	Percentage
MLSBi	36	31.30%
MLSBc	66	57.39%
MS	13	11.30%

Of 153 MRCoNS 115 which were resistant to erythromycin were tested for D-zone test, of which 36 (31.3%) showed inducible clindamycin resistance and 66 (57.39%) MRCoNS showed constitutive resistance to clindamycin. 13 (11.3%) MRCoNS belonged to MS phenotype as shown in Table no. 3.

Table No-4 : Inducible And Constitutive Clindamycin Resistance In Mscons

Resistance	Number	Percentage
MLSBi	2	14.28%
MLSBc	5	35.71%
MS	7	50.00%

Of the 81 methicillin sensitive CoNS 14 were erythromycin resistant and were tested for D-test. 2 (14.28%) showed inducible and 5 (35.71%) showed constitutive clindamycin resistance respectively. 7 (50.0%) showed clindamycin sensitive i.e. MS phenotype as shown in **Table 4.**

DISCUSSION:

Coagulase negative staphylococci (CoNS) were generally regarded to be the contaminants, having little clinical significance in the past.[1] Formerly regarded as harmless inhabitants of the skin and mucous linings, CoNS are now recognized as a major cause of nosocomial infections in critically ill patients especially in intensive care units, which leads to morbidity and even mortality.[13]

CoNS are an important cause of nosocomial infections.[14] The increasing importance of CONS also may be due in part to the growing appreciation of this group of organisms as opportunistic pathogens and to the increase in the use of transient or permanent medical devices, such as intravascular catheters and Prosthetic devices, in seriously ill and immunocompromised patients (i.e intensive care patients, premature newborns, and cancer and transplant patients).

CoNS tend to be more resistant to antimicrobial agents than S. aureus, especially to methicillin.[15] Currently, more than 70% CoNS isolates worldwide are resistant to methicillin. In the present study, the total numbers of methicillin sensitive CoNS (MSCoNS) were 81 (34.62%) and methicillin resistant CoNS (MRCoNS) were 153 (65.38%). Singhal et al[14] also determined 37.3% MSCoNS and 62.7% MRCoNS in total 83 CoNS. Mohan et al[16] found 71.7% MSCoNS and 28.3% MRCoNS isolates.

The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics is commonly used in the treatment of staphylococcal infections. However, one important issue in clindamycin treatment is the risk of clinical failure during therapy. Therapeutic failures caused by MLS_B inducible resistance are being more commonly reported.[17]

In the present study, out of total 234 CoNS strains, 105 were sensitive to erythromycin and remaining 129 were resistant to erythromycin. Of these 129 erythromycin resistant strains, 71 showed resistance and 58 showed susceptibility to clindamycin on disc diffusion testing. These 58 erythromycin resistant and clindamycin sensitive (on disc diffusion testing) strains were when subjected to D-test, 38 (16.24%) strains showed inducible clindamycin resistance. The remaining 20 strains (erythromycin resistant and clindamycin sensitive) which were D- test negative were referred as MS phenotype. The total 71 (30.34%) strains which were resistant to both the clindamycin and erythromycin were the constitutive clindamycin resistant CoNS.

Various studies gave variable results. Yilmaz et al[17] processed a total 803 CoNS and found 195 (24.3%) to be inducible and 253 (31.5%) as the constitutive clindamycin resistant. As many as 113 (14.1%) CoNS isolate showed MS phenotype. Delialioglu et al[18] reported 14.7% inducible, 40.2% constitutive and 18.2% isolates as MS phenotype amongst the total 291 CoNS. Hammilton Miller et al[19] demonstrated

31% of inducible, 11% constitutive and 13% MS phenotype CoNS. Jenssen at al[20] reported 16.6% inducible and 35.9% of constitutive clindamycin resistance in CoNS. Angel et al[21] reported 5 (10%) inducible clindamycin resistance and 10 (19%) MS phenotype. No constitutive resistance was reported.

Percentage of inducible(MLS_Bi) and constitutive(MLS_Bc) clindamycin resistance

Study	MLSBi	MLSBc	MS
Jenssen et al 1987	16.6%	35.9%	-
Delialioglu et al 1995	14.7%	40.2%	18.2%
Hammilton Miller et al 2000	31%	11%	13%
Yilmaz et al 2007	24.3%	31.5%	14.1%
Present study 2014	16.24%	30.24%	8.55%

(MLSBi- induciable clindamycin resistant phenotype, MLSBc- constitutive clindamycin resistant phenotype, MS- clindamycin sensitive phenotype)

In the present study, it was seen that both the inducible and constitutive clindamycin resistance were seen in higher proportion in MRCoNS as compared to MSCoNS. Inducible clindamycin resistance was present in 31.30% isolates of MRCoNS and 14.28% isolates of MSCoNS. Similarly constitutive clindamycin resistance was present in 57.39% isolates of MRCoNS as compared to only 35.71% MSCoNS. 11.30% isolates of MSCoNS and 50.0% MRCoNS were of MS phenotype.

Shreckenberger et al[22] reported that inducible clindamycin resistance was more prevalent in methicillin resistant S. aureus (MRSA). But Lim et al[23] found that inducible clindamycin resistant strains were more prevalent in CoNS (91%) than MRSA (32%). The D test is acceptable for all the staphylococcus species including methicillin-susceptible or methicillin resistant S. aureus or CoNS. Many of the recently recognized MRSA, which cause community-associated infections, have the msrA gene, and oral clindamycin may be a treatment option for these patients. Although clindamycin can be effective in some Patients, it is not recommended to use it before conducting the D-test.[24]

CONCLUSION :

The study documents the importance of *CoNS* as important Gram-positive pathogen special in hospital settings, resistant to commonly used antibiotics

Clindamycin disk induction test (D-zone test) is simple and must be done routinely in the diagnostic microbiology laboratories. Without the "D-zone test" all the *CoNS* isolates with inducible clindamycin resistance would be erroneously classified as clindamycin susceptible by routine testing methods.

MRCoNS showed higher degree of inducible and constitutive clindamycin resistance as compared to MSCoNS.

REFERENCES:

1. Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods GL. Gram positive cocci: Staphylococci and related gram Positive Cocci. In: Koneman's colour atlas and textbook of diagnostic microbiology. 6th ed. Lippincott Williams and Wilkins; 2006. p 624-73.
2. Pittet D, Tarara D, Wenzel RP, Nosocomial Bloodstream Infection in Critically ill patients JAMA 1994; 271: 1599-601.
3. Spencer RC. Predominant pathogens found in the European prevalence of infection in intensive Care study. Eur J Clin Microbiol Infect Dis 1996; 15: 281-85.
4. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J Clin Microbiol. 2003;41:4740–44.
5. Forrest GN, Oldach DW. Infectious diseases. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2004. Macrolides and clindamycin. In: Gorbach SL, Bartlett JG, Blacklow NR, editors; p. 223. [Google Scholar]
6. Gadepalli R, Dhawan B, Mohantay S, Kapil A, Das BK, Chaudhary R. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res. 2006;123:571–73. [PubMed] [Google Scholar]
7. Yilmaz G, Aydin K, Iskender S, Calyan R, Koksal I. Detection and prevalence of inducible clindamycin resitance in staphylococci. J med Microbiol. 2007;56:342–45. [PubMed] [Google Scholar].
8. Chelae S, Laohaprertthisarn V, Phengmak M, Kongmuang U, Kalnauwakul S. Detection of inducible clindamycin resistance in staphylococci by disc diffusion induction test. J Med Assoc Thai. 2009;92(7):947–51. [PubMed] [Google Scholar]
9. Leclercq R. Mechanism of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis. 2002;34:482–92. [PubMed] [Google Scholar]
10. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment in methicillin resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. C Lin Infect Dis. 2003;37:1257–60. [PubMed] [Google Scholar]
11. Clinical Laboratory Standards Institute (CLSI) guidelines. Performance standards for antimicrobial susceptibility testing: twenty second informational supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute. Pennsylvania; Wayne; 2012.
12. Rodrigues Perez LR, Caierao J, Souza Antunes AL, Alves d'Azevedo P. Use of D test method to detect inducible clindamycin resistance in coagulase negative staphylococci (CoNS) Braz J Infect Dis. 2007;11:186–88. [PubMed] [Google Scholar]
13. Huang SY, Tang RB, Chen SJ, Chung RL. Coagulase negative staphylocoaccal bactaeremia in critically III children: risk factors and antimicrobial susceptibility. J Microbiol Immunol Infect 2003; 36:51-5.
14. Singhal R, Dhawal S, Mohanty S, Sood S, Das B, Kapil A. Species distribution and antimicrobial susceptibility of CoNS in tertiary care hospital. Indian J Med Res 2006; 123: 569-70.
15. Hussain Z, Stoakes L, Lannigan R. Evaluation of screening and commercial methods for Detection of methicillin resistance in coagulase-negative staphylococci. J Clin Microbiol 1998; 36: 273-4.
16. Mohan U, Jindal N and Aggarwal P, Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococci isolates from various clinical specimens. Indian Journal of Med Microbiol 2002; vol 20, no 1: 45-46.
17. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. Journal of Medical Microbiology 2007; 56: 342-5.
18. Delialioglu N,Aslan G,Oztuk C,Baki V,Sen S, Emekdas G, Inducible clindamycin resistance in staphylococci isolated from clinical samples ,Jpn J Infect Dis 1995;58;104-6.
19. Hamilton Miller JMT,shah S. patterns of phenotypic resistace to macrolides-lincosarnides-ketolide-streptogramin group of antibotics in staphylococci , J Antimicrobiol chemother 2000;46;941-9.
20. Jenssen WD Thakkervaria S,Dublin DT, Weinstin MP,prevalence of macrolides-sreptograriIn-B resistance and erm gene classes among clinical strains of staphylococci and streptococci.Antimicrob Agents Chemother 1987;31;883-8 .
21. Angel MR, Balaji V, Prakash JAJ, Brahmadathan KN, Mathews MS. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care center. Indian j Med Microbial 2008; 26: 262-4.
22. Schreckenberger PC, Ilendo E, Ristow incidence of const'kuNti 20 ' and inducible clinciarnycin resistance in Staphylococcus aurous al\ci coagulase-negative staphylococci in a community and a tertiary Care Hospital. J Clin Microbiol 2004; 42: 2777-9.
23. Lim HS, Lee H; Roh KH,Yum JH,Yong D,Lee K,chong Y. prevalence of inducible clindarnycin resistance in staphylococcal isolates at a Korean tertiary care hospital. Yonsei Med J 2006;47;480-4.
24. Perez LRR, Caierao J, Antunes ALS, d'Azevedo PA. Use of the D test method to detect inducible clindamycin resistance in coagulase negative staphylococci (CoNS). The Brazilian journal of Infectious Diseases 2007; 11: 186-8