ORIGINAL RESEARCH PAPER

Volume-6 | Issue-10 | October-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CONSTITUTIVE AND INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF COAGULASE NEGATIVE STAPHYLOCOCCI IN A TERTIARY CARE HOSPITAL

Microbiology

DR.B.PADMINI M.D (microbiology) Senior Assistant Professor Department Of Microbiology Coimbatore Medical College Coimbatore

***DR. S.DEEPA** M.D (microbiology) Senior Assistant Professor Department Of Microbiology Coimbatore Medical College Coimbatore *Corresponding Author

ABSTRACT

Background : Coagulase Negative Staphylococci are one of the most common pathogens isolated from nosocomial and community acquired infections. High incidence of antibiotic resistance, in particular methicillin resistance has complicated the treatment of these infections. Clindamycin has been used successfully to treat the methicillin-resistant CoNS (MRCoNS) in adults and children, but this matter is complicated by the possibility of inducible macrolide-lincosamide streptogramin B resistance (MLSBi). The **Aim** of this study is to determine the antimicrobial susceptibility pattern - methicilin resistance, macrolides lincosamides-streptogramin B (MLSB) resistance and multidrug resistance among CoNS isolates.

Materials and Methods: A total of 135 consecutive, non-duplicate strains of CoNS, isolated from various clinical specimens were included in the study. After determining the susceptibility to different antimicrobial agents and methicillin resistance, D-test was performed on all erythromycin-resistant and clindamycin-sensitive isolates to detect constitutive and inducible clindamycin resistance.

Results: Among135 CoNS isolates73 (54%) were found to be MRCoNS. MDR was also prevalent among CoNS isolates 58(43%) and the prevalence was more in the methicillin-resistant isolates (79%). MRCoNS showed higher inducible as well as constitutive resistance to clindamycin as compared to methicillin-sensitive CoNS (MSCoNS). The CoNS isolates exhibited 32% MLSBi phenotype and 43%MLSBc phenotype. All isolates having MLSBi phenotype were sensitive to vancomycin and linezolid.

Conclusions : Clindamycin is a useful drug in the treatment of staphylococcal infections. Hence, routine testing of staphylococcal isolates for inducible clindamycin resistance is strongly recommended.

KEYWORDS

MR CoNs,MDR CoNS, MSCoNS, MLSBi, MLSBc, MS phenotype,D test

INTRODUCTION

Coagulase Negative staphylococci (CoNS) are among the most frequently isolated micro-organisms in clinical microbiology laboratories[1,2] .There is enormous increase in emergence of multi drug resistant CoNS strains over the last decades, particularly methicillin resistant strains[3]. CoNS have historically been more resistant to antimicrobials, including the β - lactum antibiotics, than S. aureus; some hospitals report that the rates of Methicillin resistance in CoNS is approaching 90%[4]..Most of these methicillin-resistant CoNS were also resistant to multiple additional antimicrobial agents. Multi drug resistant CoNS also commonly colonize the skin of hospitalized patients and hospital personnel and these colonizing isolates serve as a reservoir for antibiotic resistance genes that can transfer among CoNS and be acquired by S. aureus[5].

The increasing frequency of the infections with MR Staphylococci and the changing drug susceptibility patterns have led to a renewed interest in the use of macrolide lincosamide streptogramin-B (MLSB) antibiotics to treat such infections, with clindamycin being the preferred agent. Macrolides (erythromycin, roxithromycin, clarithr mycin) and Lincosamides (clindamycin and lincomycin) belong to different classes of antimicrobials but act through the same mechanism that is by inhibition of protein synthesis6. Resistance to MLSB antibiotics can occur due to drug interaction, target site modification or efflux mechanisms[6]

However in Staphylococci, Macrolide (erythromycin) resistance is usually caused either by an active efflux mechanism encoded by msrA gene (conferring resistance to macrolides and group B streptogramins only) or by ribosomal modification mediated by 23S rRNA methylases encoded by the erm genes. In target-site modification,7the modifica tion of the ribosomal target is encoded by a multiallele plasmid borne erythromycin ribosome methylase (erm) gene that causes the production of the methylase enzymes. Methylases lead to MLSB resistance which can either be inducible (MLSBi resistance) or constitutive (MLSBc resistance)[7]. If the erm genes are consistently expressed, the organisms may show in vitro resistance to erythromycin (ER), clindamycin (CL) and to other members of the MLSB group and they are said to be of the MLSBc phenotype. However, if the erm genes require an inducing agent to express the resistance to CL, then the organisms are said to be of the MLSBi phenotype. The organisms which belong to the MLSBi phenotype are resistant to ER and sensitive to CL in vitro. The CL therapy in such patients can lead to clinical failures8. Low levels of ER is an inducer of the MLSBi phenotype and this forms the basis of the D-test9. Thus standard methods for antimicrobial susceptibility tests do not recognize MLSBi resistance and this may lead to therapeutic failure when clindamycin is used. Disc diffusion induction methods (D-test) should be used routinely in microbiological laboratories to detect MLSBi resistance Staphylo cocci can also develop macrolide resistance, based on the presence of the efflux pump which is encoded by the macrolide streptogramin resistance (msrA) gene, leading to resistance to the macrolides and the type B streptogramins, but not to the lincosamides. These isolates are known to be of the MS phenotype and they show in vitro resistance to ER and susceptibility to CL. But the CL therapy can safely be given in infections which are caused by the organisms of this phenotype and there is no risk of clinical failures 10. Therefore, it is important to differentiate these two mechanisms of resistance.

MATERIALS AND METHODS

A total of 135 strains of CoNS, isolated from various clinical samples over a period of one year were used in this study. The isolates were identified using conventional methods and they were tested for antibiotic susceptibility by Kirby Bauer disc diffusion method on Muller Hinton agar[11].Commercially available antibiotic discs (Himedia) were used. Resistance to cefoxitin, erythromycin, and clindamycin was assessed based on the guidelines from the Clinical Laboratory Standards Institute (CLSI) along with routine antibiotic susceptibility testing[10,12],.The isolates that were found to be erythromycin resistant were further studied for inducible clindamycin resistance.

Isolates that were CL-S and ER-R were tested for inducible resistance by the D-test[6,15] The ER disc was placed 15mm apart, edge to edge from the CL disc on the inoculated MHA plate and was incubated at 37°C for [18-24] hrs. If an isolate was ER-R and CL-S, with a D-shaped inhibition zone around the clindamycin disc, it was considered to be positive for inducible resistance (D-test positive). If the isolate was ER-R and CL-S, but with both zones of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D-test negative), but to have an active efflux pump. If the isolate was ER-R and CL-R, the isolate was considered to have an MLSBc phenotype [13,14]

RESULTS

Antimicrobial susceptibility testing revealed wide-spread (54%) methicillin resistance among the CoNS isolates. MDR was also prevalent among the CoNS isolates, with a higher incidence among the methicillin-resistant isolates than the methicillin-sensitive isolates. Among the (54%) methicillin resistant CoNS isolates,79% exhibited MDR; however, overall MDR among CONS was 43% only..

Clindamycin susceptibility rates were 68% and 85% among methicillin-resistant CoNS and methicillin-susceptible CoNS, respectively. Erythromycin susceptibility rates were10.6% and 59.1%, respectively. Among MRCoNS isolates, 47% had the constitutive and 27% had the inducible clindamycin resistance. In MSCoNS isolates, 18% and 20% isolates exhibited the constitutive and inducible resistance phenotypes respectively. Thus, both the constitutive and inducible resistance phenotypes were found to be significantly higher in MRCoNS isolates compared to MSCoNS. Isolates with MS phenotype and sensitive to both erythromycin and clindamycin were predominant among MSCoNS.

Table 1: Resistance Phenotypes of Isolates

ISOLATES	Phenotype			
	ER-R, CL-R MLSBc Phenotype (%)	ER-R, CL-S D-test positive MLSBi Phenotype (%)	ER-R, CL-S Dtest Negative MS phenotype (%)	ER-S, CL-S (Both Sensitive) (%)
MRCONS (n=66)	31(47%)	18(27%)	8(12%)	9(14%)
MSCONS (n=69)	13(18%)	14(20%)	12(17%)	31(45%)

Chart 1 MLSB resistance phenotypes among MRCoNS and MSCoNS isolates

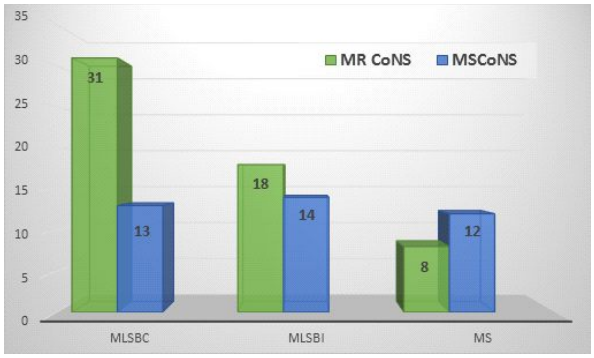
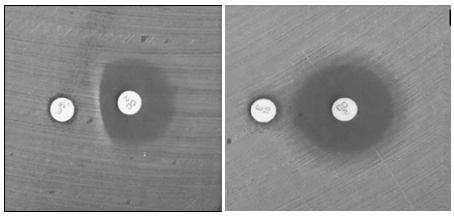


FIGURE 1

D TEST POSITIVE **D TEST NEGATIVE**



The increasing frequency of coagulase-negative staphylococcal infections and ever increasing problem of methicillin resistance among staphylococci have led to renewed interest in the use of clindamycin therapy to treat such infections[15,16]. Clindamycin is a good alternative for the treatment of both methicillin-resistant and -susceptible staphylococcal infections, but therapeutic failures caused by MLSBi resistance are being reported morecommonly.[10] Therapeutic failures caused by MLSB inducible resistance phenotypes are being more commonly reported due to selection of constitutive mutants both in vitro and in vivo during clindamycin therapy[17,18]Conversely, labeling all erythromycin-resistant staphylococci as clindamycin resistant prevents the use of clindamycin in infections caused by truly clindamycin-susceptible staphylococcal isolates. A therapeutic decision is not possible without the relevant antibiotic susceptibility data. This is where the D-test becomes significant.

Antimicrobial susceptibility testing revealed wide spread methicillin resistance (54.4%) among the CoNS Isolates .Comparable levels have previously been reported by Chaudury et al (2007)[65](68.4% oxacillin resistant CoNS) and Marry et al (1996)[63] (49% methicillin resistant CoNS). In a study by Shubhra Singh et al (2006)64, 38% of the CoNS exhibited oxacillin resistance.

MDR was also prevalent among the CoNS isolates; the prevalence was more in the methicillin-resistant isolates than in the methicillin-sensitive isolates. These findings are very similar to those reported by Archer et al (1994)[70] and Diekema et al[90] (2001).

In our study we found that among 66 MRCoNS isolates,47%, 27% and 12% isolates had the constitutive MLSB resistance, inducible clindamycin resistance and the MS phenotype respectively. But in our MSCoNS isolates, MS phenotype was 17% with 18% and20% isolates having MLSBc and MLSBi resistance respectively. Both constitutive and inducible resistances were significantly higher in MRCoNS isolates in comparison to MSCoNS

Similarly Azap et al and other researchers reported a higher percentage of constitutive and inducible clindamycin resistance in MRCoNS compared to MSCoNS isolates from a hospital in Turkey, 30.8% of MRCNS isolates and 15.3% of MSCNS isolates were determined to have the inducible CL-R phenotype20. Schmitz et al,[21] documented a fairly high incidence of both constitutive (69%) and inducible (30%) resisatnce in CoNS. Pal et al, [22] from western India reported higher inducible resistance rates in MRCoNS than MSCoNS which is in accordance with our study.On the contrary Hamilton-Miller et al,[23] determined higher rates of inducible resistance compared to constitutive in CoNS. From India, Ciraj et al,[24] reported an incidence of 6.3% of inducible clindamycin resistance in CoNS which is significantly lower than our results. Similarly, Angel et al[25] reported 19% MS phenotype and 10% MLSBi resisatnce but no MLSBc phenotype in their CoNS isolates.. Possible variations in the prevalence of constitutive, inducible clindamycin resistance and MS phenotype could be explained due to differences in bacterial susceptibility in different geographical areas and also due to varying antimicrobial subscribing patterns of physicians. These differences highlight the significance of inducible clindamycin resistance in our geographical setting.

CONCLUSION

Recent reports on surveillance data have indicated that CoNS are among the five most commonly reported pathogens (in fifth place at 9 to 9.7%, compared with 10 to 11.2% for *S.aureus*) in hospitals conducting hospital-wide surveillance[15]. Most noticeably, the shifts have been toward the more antibiotic resistant pathogens, of which the CoNS are a major group and clindamycin is an attractive therapeutic alternative to vancomycin in staphylococcal infections[16]. Hence, to conclude it is important for laboratories to be aware of the local prevalence of MLSBi resistance as the incidence is highly variable with regard to geographical area. The D-test is an easy, sensitive, and reliable means for detection of MLSBi strains in a clinical laboratory setting and should be performed routinely.

REFERENCES

1. Cervera C., Almela M., Martínez-Martínez J. A., Moreno A., Miró J. M.). Risk factors and management of Gram-positive bacteraemia. Int J Antimicrob Agents (2009) 34 (Suppl. 4), S26–S30
2. Hung K. H., Yan J. J., Lu Y. C., Chen H. M., Wu J. J. Evaluation of discrepancies between oxacillin and cefoxitin susceptibility in coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis(2011). 30, 785–788.
3. R Goyal, NP Singh, A Kumar, I Kaur, M Singh, N Sunita, M Mathur. Simple and economical method for speciation and resistotyping of clinically significant Coagulase Negative Staphylococci. Indian Journal of Medical Microbiology, July (2006)24(3):201-4.
4. Joseph F John, Alexander M Harvin. History and evolution of antibiotic resistance in coagulase-negative staphylococci: Susceptibility profiles of new anti staphylococcal agents. Therapeutics and Clinical Risk Management 2007:3(6) 1143-1152
5. Gordon L. Archer and Michael W. Climo. Antimicrobial Susceptibility of Coagulase-Negative Staphylococci. Antimicrobial Agents and Chemotherapy, Oct. 1994, p. 2231-2237
6. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al. Testing for induction of clindamycin resistance in erythromycin-resistant isolates of Staphylococcal aureus. J Clin Microbial 2005; 43:1716-21. 4
7. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. Clin Infect Dis 2002;34:482-92.
8. Lewis JS 2nd, Jorgensen JH. Inducible clindamycin resistance in Staphylococci: should the clinicians and the microbiologists be concerned? Clin Infect Dis. 2005;40:280–85
9. Weisblum B, Demohn V. Erythromycin-inducible resistance in Staphylococcus aureus: a survey on the antibiotic classes which were involved. J. Bacteriol. 1969;98:447–52

10. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disc diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J Clin Microbiol 2003;41:4740-44.
11. Kloos WE, Banerman TL. Staphylococcus and Micrococcus. In: Chapter 22, Manual of clinical Microbiology 7th ed. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Washington DC: ASM Press; 1999. p. 264-82.
12. The Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing: eighteenth informational Supplement. Wayne, Pa: CLSI; (2008). pp. M100–S18.
13. B PC Schreckenberger, E Ilendo, KL RistowIncidence of constitutive and inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci in a community and a tertiary care hospital,J Clin Microbiol, 42 (2004), pp. 2777-2779
14. C. JH Jorgensen, SA Crawford, ML McElmeel, KR FiebelkornDetection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing J Clin Microbiol, 42 (2004), pp. 1800-1802
15. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev 1994;7:117-40.
16. Novotna G, Adamkova V, Janata J, Melter O, Spizek J. Prevalence of resistance mechanisms against macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic and occurrence of an undefined mechanism of resistance to lincosamides. Antimicrob Agents Ch 2005;49:3586-9.
17. Panagea S, Perry JD, Gould FK. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? J Antimicrob Chemother 1999;44:581-2.
18. Rao GG. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? J Antimicrob Chemother 2000;45:715.
19. Frank AL, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC, et al. Clindamycin treatment of methicillin resistant Staphylococcus aureus infections in children. Pediatr Infect Dis 2002;21:530-4.
20. Azap, O. K., Arslan, H., Timurkaynak, F., Yapar,G., Oruc, E. & Gagir, U. (2005). Incidence of inducible clindamycin resistance in staphylococci: first results from Turkey. Clin Microbiol Infect 11, 582–584.
21. Schmitz FJ, Verhoef J, Fluit AC; The Sentry Participants Group. Prevalence of resistance to MLS antibiotics in 20 European university hospitals participating in the European SENTRY surveillance programme. J Antimicrob Chemother 1999;43:783-92.
22. Pal N, Sharma B, Sharma R, Vyas L. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. J Postgrad Med 2010;56:182-5.
23. Hamilton-Miller JMT, Shah S. Patterns of phenotypic resistance to macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci. J Antimicrob Chemother 2000;46:941-9.
24. Ciraj AM, Vinod P, Sreejith G, Rajani K. Inducible clindamycin resistance among clinical isolates of staphylococci. Indian J Pathol Microbiol 2009;52:49-51
25. Angel MR, Balaji V, Prakash JAJ, Brahmadathan KN, Mathews MS. Prevalence of inducible clindamycin resistance in gram-positive organisms in a tertiary care centre. Indian J Med Microbiol 2008;26:262-4.