Detection of Macrolide-Lincosamide-Streptogramin B Resistance in Staphylococci Isolated from Ear Discharge of Patients of Chronic Suppurative Otitis Media in a Tertiary Care Hospital

Chetana I. Wahane[1*], Vanita A. Kulkarni[2]

[1]Assistant Professor, Department of Microbiology, Government Medical College, Latur, Maharashtra, INDIA.

[2]Professor and Head, Department of Microbiology, R.C.S.M and CPR hospital, Kolhapur, Maharashtra, INDIA.

[*]Corresponding Address:

wahanec@gmail.com

Research Article

Abstract: Staphylococci act as major aerobic pathogens in the causation of chronic suppurative otitis media (CSOM). Clindamycin is one of the alternative agents used to treat CSOM and accurate identification of clindamycin resistance is important to prevent therapeutic failure. Inducible clindamycin resistance cannot be detected by standard susceptibility tests. This study aimed to detect macrolide-lincosamide-streptogramin B (MLS_B) resistance in staphylococcal isolates causing CSOM in order to assist clinicians in treatment of CSOM by thesegroup of antibiotics. MLS_B resistance in the present study was detected in 59 staphylococcal isolates (41 *S. aureus* and 18 CONS) isolated by standard procedure from ear discharge in CSOM. D-test was performed on these isolates to detect MLS_B resistance. Inducible clindamycin was detected in 5% Methicillin susceptible *staphylococcus aureus* (MSSA), 0% Methicillin resistant *staphylococcus aureus* (MRSA) and 5.5% Coagulase negative Staphylococci (CONS). Constitutive resistance (8.4%) was found more common than inducible clindamycin resistance (5%) in the present study. Detection of inducible clindamycin resistance can help in using clindamycin safely and effectively in patients with true clindamycin susceptible isolates and thus helps to avoid treatment failure while high prevalence of constitutive resistance makes D-test essential when clindamycin is an option for therapy of staphylococci in CSOM.

Key words: Chronic suppurative otitis media, D-test, inducible clindamycin resistance, staphylococci.

Introduction

Chronic suppurative otitis media(CSOM) is probably the most commonest disease seen in ENT outpatient department[1]. CSOM is well known for it's recurrence, bacterial resistance, ototoxicity, fatal complications like meningitis, cerebral abscesses, etc and chronic hearing loss which has negative impact on development of speech, language and social interaction[2,3]. It is a disease of multiple etiology[4]. The major aerobic pathogens responsible for CSOM are S. aureus and P. aeruginosa[5]. Coagulase negative staphylococci (CONS) also assume pathogenic rule when resistance in middle ear is lowered due to invasion by other organisms[4]. Clindamycin is very effective against staphylococci and anaerobes in the treatment of CSOM[6]. However, widespread use of the antimicrobial agents has led to increase in the number of resistant staphylococcal strains[7]. In S. aureus and CONS, an active efflux mechanism encoded by msr A gene confer resistance to macrolides and and streptogramins B antibiotics (so called MS phenotype) and modification of ribosomal target encoded by erm genes cause resistance to macrolides, lincosamides, streptogramins B antibiotics; which called MLS_B resistance. The later mechanism can be constitutive ($cMLS_B$); where the rRNA methylase is always produced, or can be induced ($iMLS_B$); where methylase is produced only in the presence of an inducing agent. Low levels of erythromycin are the most effective inducers of $iMLS_B$ resistance. Previous reports indicated that treatment of patients harbouring $iMLS_B$ resistant-staphylococci with clindamycin might lead to development of $cMLS_B$ resistant strains and subsequent treatment failure. Unfortunately, the $iMLS_B$ phenotype cannot be recognized by using standard susceptibility tests and require specific methods. A test known as disk approximation test or simply D-test detects MLS_B resistance pattern of staphylococci[7]. The purpose of this study was to detect the MLS_B resistance in staphylococcal isolates causing CSOM in order to assist clinicians in treatment of CSOM by these groups of antibiotics.

Material and Methods

The present study was conducted for a period of 1 year from Jan 2010-Dec2010. A tatol of 59 staphylococcal isolates were obtained from ear discharge through a perforated tympanic membrane of patients, using sterile thin cotton swab by no touch technique and with all aseptic precautions from a tertiary care hospital at Miraj

(MS), India [8,9]. The staphylococcal strains were identified by using standard microbiological procedures. Antibiotic susceptibility tests were performed by the Kirby-Bauer disc diffusion method. Methicillin resistance was detected by using 30µg cefoxitin disc[10]. For performing D-test, suspension equivalent to 0.5 McFarland of each freshly cultured isolate in normal saline was prepared and inoculated onto a Mueller-Hinton agar plate as described in the CLSI recommendations. Clindamycin (2µg) and erythromycin (15µg) discs were manually placed 15mm apart (edge to edge) on the Mueller-Hinton agar plate. Plates were read after 18 hours of incubation at 37^0c. Interpretation of diameters of zones of inhibition was done according to CLSI guidelines as follows: For Erythromycin $\geq$ 23mm - S, 14-22mm – I, $\leq$ 13mm – R and Clindamycin $\geq$21mm – S, 15-20mm – I, $\leq$14mm – R. Strains with flattening of clindamycin zone adjacent to erythromycin disc with D shaped zone were reported as $iMLS_B$ phenotype (Fig. 1). Strains resistant to both antibiotics were reported as $cMLS_B$ phenotype, while strains resistant to erythromycin but susceptible to clindamycin with no D shape zone were reported as MS phenotype. Strains susceptible to both antibiotics were reported as Susceptible or S phenotype. Known positive D-test and negative D-test strains were used as control strains.

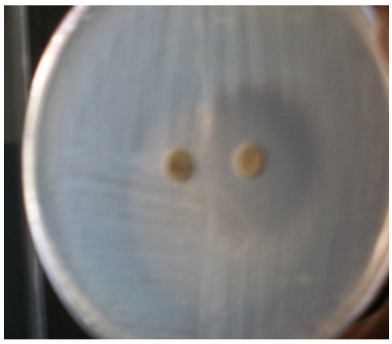

Figure 1: D-test positive isolate showing inducible resistance to Clindamycin

Results

Among the 59 staphylococcal isolates, 41 were *S. aureus* and 18 were CONS. Inducible clindamycin resistance ($iMLS_B$) (D-positive) was found in 5% MSSA, 0% MRSA and 5.5% CONS. On the other hand, 7.5% MSSA, 0% MRSA and 5.5% CONS showed MS phenotype (D-negative). The isolates of 5% MSSA, 100% MRSA and 11.1% CONS were $cMLS_B$ or resistant (R) phenotype, whereas 82.5% MSSA, 0% MRSA and 77.7% CONS were susceptible or S phenotype (Table1). Of the 59 staphylococcal isolates, 5%(3) had inducible resistance phenotype, 8.4%(5) had a constitutive phenotype, 6.7%(4) had a MS phenotype and 79.6%(4) had susceptible or S phenotype (Table 2)

Discussion

Clindamycin is very effective against staphylococcal isolates causing CSOM [6]. It has excellent tissue penetration. It accumulates in abscesses. It is not impeded by high bacterial burden at the infection site and no renal dose adjustments are needed [11]. Good oral absorption makes it an important option in outpatient therapy. Clindamycin is a good alternative antibiotic for penicillin allergic patients and infections due to MRSA [11,12,13]. Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, in vitro susceptibility testing for clindamycin by disc diffusion testing with erythromycin and clindamycin discs in non-adjacent positions may indicate false susceptibility. However, recent reports indicate that treatment failure may occur in the case of $iMLS_B$ resistance, in spite of in vitro susceptibility to clindamycin[14,15]. The inducible clindamycin resistance ($iMLS_B$) was detected in 5%(2) MSSA, 0% MRSA and 5.5% CONS in the present study (Table 1) which correlates with the findings of Chelae S. et al who reported $iMLS_B$ resistance of 4.7% in MSSA and 5.5% in CONS. The test thus can separate strains that have the genetic potential (presence of the erm gene) to become resistant during therapy from strains that are fully susceptible to clindamycin[16]. The $cMLS_B$ resistance in the present study was observed in 5%(2) MSSA, 100%(1) MRSA and 11.1%(2) CONS isolates (Table 1). Chelae S. *et al*[16] reported $cMLS_B$ resistance (R phenotype) in 4.4% MSSA and 61.3% MRSA isolates, while Schmitz FJ *et al*[17] reported $cMLS_B$ resistance in 9.7% MSSA and 89.4% MRSA isolates. Gupta *et al*, however, reported $cMLS_B$ resistance in 10% MSSA and 46% MRSA isolates [18]. Prevalence of $cMLS_B$ among MRSA isolates of CSOM need more studies. In the present study, MS phenotype was found in 7.5% MSSA and 5.5% CONS (Table 1). Gadepalli *et al* [19] reported 12% MS phenotype and Schmitz FJ. *et al* [17] reported 15% MS phenotype of *S. aureus*. This phenotype is caused by efflux mechanism encoded by msr A gene and is increasingly found in MSSA isolates[17]. The susceptible or S phenotype was found in 82.5% MSSA, 0% MRSA and 77.7% CONS, while Chelae S. *et al* [16] observed 90.6% MSSA, 1.6% MRSA and 42.9% CONS isolates of S phenotypes in their study. The $cMLS_B$ resistance (8.4%) in the present study was more common than iMLSB resistance (5%) which is similar to study by Schmitz FJ. *et al* [17] and Delialioglu N. *et al* [20] and in contrast with study by Frank AL. *et al* [21].

The variation in MLS_B (Table 2) resistance pattern depends on the patient population studied, the geographical region, the hospital characteristics and methicillin susceptibility[22].

Table 1: D-test phenotype of *S. aureus* and CONS

Isolates	Phenotype				
	D-Positive ($iMLS_B$) No(%)	D-Negative (MS) No(%)	Resistant (R) ($cMLS_B$) No(%)	Susceptible(S) No(%)	Total
MSSA	2(5%)	3(7.5%)	2(5%)	33(82.5%)	40
MRSA	0	0	1(100%)	0	01
CONS	1(5.5%)	1(5.5%)	2(11.1%)	14(77.7%)	18

Table 2: MLS_B resistance among all staphylococcal isolates

Phenotype	No. of isolates	Percentage(%)
$iMLS_B$ (D-Posirive)	3	5%
MS (D-Negative)	4	6.7%
$cMLS_B$	5	8.4%
Susceptible (S)	47	79.6%
Total	59	100%

Conclusion

Failure to identify inducible clindamycin resistance may lead to clinical failure when clindamycin is used therapeutically. On the other hand, if inducible clindamycin resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in those patients with true clindamycin susceptible isolates. Along with this, high prevalence of $cMLS_B$ in the present study showed that antimicrobial susceptibility testing is essential when clindamycin is an option for therapy of staphylococci in chronic suppurative otitis media.

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References

1. Srivastava VK, Agarwal SK, Malik GK. Chronic suppurative otitis media in children. Indian J paediatrics;46:363-67,1979.
2. Nikakhlagh S, Khosravi AD, Fazlipour A, Safarzadeh M, Rashidi N. Microbiological findings in patients with CSOM. J Med Sci;8(5):503-06,2008.
3. Maji PK, Chatterjee TK, Chatterjee S, Chakrabarty J, Mukhopadhyay BB. The investigation of chronic suppurative otitis media in patients attending a tertiary care hospital with special emphasis on seasonal variation. IJO & HNS;59:128-31,2007.
4. Rama Rao MV, Jayakar PA. Bacteriological study of Chronic suppurative otitis media. J Indian M A ,75(2):30-34,1980.
5. Klein J. Strategies for decreasing multidrug antibiotic resistance: role of ototopical agents for treatment of middle ear infections. The American J managed care ,8(14):345-52,2002.
6. Jha A.K.. Bacteriology and treatment of chronic otitis media. Journal of Nepal medical association,41:518-521,2002.
7. Saderi H, Owlia P, Eslami M. Prevalence of Macrolide-Lincosamide-Streptogramin B (MLS_B) resistance in S. aureus isolated from patients in Tehran, Iran. Iranian journal of pathology,4(4);161-166,2009.
8. Mackie & McCartney. Laboratory strategy in the diagnosis of infective syndrome. In. Collee JC, Fraser AG, Marmion BP, Simmons A. Practical medical microbiology. 14th edn. New Delhi: Elsevier;. pp 53-94,2006.
9. Forbes BA, Sahm DF, Weissfeld AS. Specimen management. Bailey & Scott's diagnostic microbiology. 12th edn. Philadelphia: Elsevier;.pp 65,2007.
10. Clinical laboratory standard institute guidelines 2010.
11. Kasten MJ. Clindamycin, metronidazole and Chloramphenicol. Mayo Clin Proc,74:825-33,1999.
12. Martinez-Angular G, Hammerman WA, Mason EO,Jr, Kaplon SL. Clindamycin treatment of invasive infections caused by community-aquired, methicillin resistant and methicillin susceptible S. aureus in children. Paediatr Infect Dis J,22:593-8,2003.
13. Roberts S, Chambers S. Diagnosis and management of staphylococcus aureus infections of the skin and soft tissue. Intern Med J,35(2):S97-105,2005.
14. Lewis JS, Jorgensen TH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? Clin Infect Dis,40:280-5,2005.
15. Rao GG. Should clindamycin be used in treatment of patients with infections caused by Erythromycin-resistant staphylococci? J Antimicrob Chemother,45:715,2000.
16. Chelae S, Laohaprertthisarn V, Phengmak M, Kongmuang U, Kalnauwakul S. Detection of inducible Clindamycin resistance in staphylococci by disk diffusion induction test. J Med Assoc Thai,92;7:947-951,2009.
17. Schmitz FJ, Verhoef J, Fluit AC. Prevalence of resistance to MLS antibiotics in 20 European university hospitals participating in the European SENTRY surveillance programme. Sentry participants group. J Antimicrob Chemother,43(6):783-92,1999.
18. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in staphylococcus aureus: A study from north India. J Postgrad Med,55:176-9,2009,
19. Gadepalli R, Dhawan B, Mohanty, Kapil A, Das BK, Chaudhary R. Inducible clindamycin resistance in clinical isolates of S. aureus. Indian J Med Res,123:571-3,2006.
20. Delialiaglu N, Aslan G, Ozturk L, Baki V, Sen S, Emekdas G. Inducible inducible resistance in staphylococcal isolates from clinical samples. Jpn J Infect Dis.,58:104-6,2005.
21. Frank AL, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC, et al. Clindamycin treatment of methicillin resistant S. aureus infections in children. Paediatr Infect Dis J,21(6):530-4,2002.
22. Shantala GB, Shetty AS, Rao RK, Vasudeva, Nagarathnamma T. Detection of inducible clindamycin resistance in clinical of S. aureus by disk diffusion induction test. Journal of Clinical and Diagnostic Research,5(1):35-37,2011.