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DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCAL ISOLATES FROM CHRONIC SUPPURATIVE OTITIS MEDIA

Microbiology

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ABSTRACT

Chronic Suppurative Otitis Media (CSOM) is a major health problem in developing countries. Most common organisms associated with CSOM are *Staphylococcus aureus* and *Pseudomonas*

Clindamycin is very effective against staphylococcal isolates causing CSOM

Resistance (Constitutive or Inducible) to Clindamycin may result in treatment failure. Inducible Clindamycin resistance cannot be determined using standard susceptibility test methods but by Double disk diffusion test (D-test).

Present study was undertaken to provide baseline data on inducible Clindamycin resistance among S*taphylococcal* isolates from CSOM patients in our area.

Total 157 patients having CSOM were included in the study.

Aerobic bacteriological culture yielded 67 staphylococcal isolates. Inducible Clindamycin resistance was detected in 9.8% Methicillin susceptible *Staphylococcus aureus* (MSSA), 28.57% Methicillin resistant *Staphylococcus aureus* (MRSA) and 12.5% Methicillin susceptible coagulase negative Staphylococci (MSCONS).

Detection of inducible Clindamycin resistance can help in using the drug safely and effectively.

KEYWORDS

Chronic Suppurative Otitis Media, Staphylococci, Inducible Clindamycin resistance

INTRODUCTION

Otitis media is one of the most common conditions diagnosed in primary health care settings.[1] Chronic suppurative otitis media (CSOM) is a result of an initial episode of acute otitis media (AOM) and is characterized by a persistent discharge from the middle ear through a tympanic perforation along with pain, irritation, rashes and fever. It is an important cause of preventable hearing loss particularly in developing world.[2,3,4]

The most common organisms associated with CSOM are *Staphylococcus aureus* and *Pseudomonas.*[5]

Treatment of CSOM should be guided by culture and sensitivity testing to suggest choices for prescribing narrowest spectrum antibiotics assuring a most effective and cost–effective protocol of treatment as antibiotic resistance pattern of microorganisms has changed.[6,7] The resistance to antimicrobial agents among staphylococci is an increasing problem.[8]

Clindamycin, a lincosamide antibiotic active against gram-positive microorganisms including staphylococci and Streptococci, inhibits bacterial protein synthesis.[9] It is preferred by clinicians because it is highly bacteriostatic against *Staphylococcus aureus* (both MRSA and MSSA), distributes well in tissues and is available in both parenteral and oral forms.[10,11]

However, resistance to Clindamycin may be seen in clinical isolates of Staphylococci which may be either constitutive or inducible.[9] Inducible clindamycin-resistant staphylococci show susceptible results in conventional susceptibility tests, but can be converted to a constitutively resistant phenotype during clindamycin treatment. As this may result in treatment failure, detection of inducible clindamycin resistance is necessary.[9]

Inducible Clindamycin resistance cannot be determined using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests.[8]

Clinical and Laboratory Standards Institute (CLSI)[12] recommends double disk diffusion test (D-test) to detect the presence of inducible Clindamycin resistance.

Cross-resistance exists for 3 antibiotic families that share a common binding site—Macrolides (e.g., erythromycin, clarithromycin, azithromycin), Lincosamides (e.g., clindamycin), and group B Streptogrammins (e.g., quinupristin) which is called as the MLS_B phenotype.[13]

Resistance to macrolides mediated by an active efflux mechanism encoded by msr A gene confer resistance to macrolides and streptogramins B antibiotics (so called MS phenotype)[14] The clindamycin resistance mechanism is primarily due to ribosomal modificationby methylases encoded by *erm* genes. Methylation of 23S rRNA decreases the affinity for clindamycin, all macrolides and type B streptogramins (the MLSB phenotype).MLSB resistance can be either constitutive ($cMLS_B$) resistance to all three antibiotic classes or inducible ($iMLS_B$).[9,15]

In vitro, *Staphylococcus aureus* isolates with constitutive resistance are resistant to Erythromycin and Clindamycin, while isolates with inducible Clindamycin resistance are resistant to Erythromycin but appear susceptible to Clindamycin.[11] Incidence of resistance varies with geographic area and therefore local statistics are crucial to guide antibiotic therapy.[10]

This is one of the first studies of this type from this semi-rural area of Maharashtra.

MATERIAL AND METHODS

The study was conducted in Department of Microbiology, Govt. Medical College, Latur, Maharashtra, India during January 2012 to June 2013. A total 157 patients of CSOM were examined. Clearance of the institutional ethical committee was obtained before initiation of the study. It was a Descriptive Cross Sectional study.

INCLUSION CRITERIA

Patients of all age groups and both sexes attending outpatient department (OPD) and those admitted in our tertiary care center and clinically diagnosed as suffering from CSOM by ENT Surgeon.

Exclusion criteria

Patients who have taken topical or systemic antibiotics in last 7 days.

Sample collection [16]

Written consent of patient / parent of a minor patient was taken before collection of sample. The external auditory canal of discharging ear was cleaned with sterile cotton. Under all aseptic precautions discharge was collected with two cotton swabs with help of sterile ear speculum, taking care not to touch external ear canal. Swabs were transported in sterile containers to the Microbiology laboratory

Laboratory procedure [16,17,18,19]

Gross appearance of the discharge including colour, odour and consistency were noted. Primary smear was made from one of the swabs. Gram staining was done.

Second swab was cultured on Blood Agar (BA), MacConkey agar (MA) and incubated aerobically at 37^{0}C for 24 hours. Blood and MacConkey agar plates were observed for growth of microorganisms. If no growth was present plates were further incubated for next 24 hours, if still there was no growth then it was reported as sterile and plates were discarded. In case of growth, colony/colonies were identified by standard procedures.

Antibiotic susceptibility test [12]

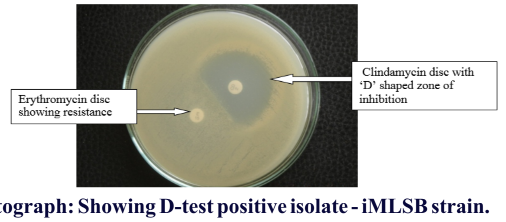
It was done for all bacterial isolates by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs procured commercially (Hi-media Laboratories ltd. India) were used for staphylococci.[8,9,12]

Interpretation of diameters of zones of inhibition was done according to CLSI guidelines. Methicillin resistance among staphylococci was detected by using 30µg Cefoxitin(CX) disc. Inducible resistance was detected by D-test. For performing D-test, Clindamycin (CD) 2µg and Erythromycin (E) 15 µg 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^{0}c.Interpretation of the diameters of zones of inhibition was as follows:

(E) Sensitive ≥ 23 mm,	(CD) Sensitive ≥ 21 mm,
(E) Intermediate= 14 to 22 mm,	(CD) Intermediate=15 to 20 mm,
(E) Resistant ≤13 mm,	(CD) Resistant ≤ 14 mm

Different phenotypes observed were appreciated and interpreted as follows:

1. S phenotype — both Erythromycin and Clindamycin sensitive.
2. MS phenotype — Erythromycin resistant and Clindamycin sensitive (no D zone).
3. iMLSB phenotype — Erythromycin resistant with D zone around Clindamycin.
4. cMLSB phenotype — both Erythromycin and Clindamycin resistant.



Photograph: Showing D-test positive isolate - iMLSB strain.

RESULTS

A total of 157 patients having CSOM were included in study. There were 12 patients having bilateral infection. Hence total 169 swabs were examined.

On aerobic bacteriological culture they yielded 165 bacterial isolates, *Staphylococcus aureus* 58 (35.1%) being most common. Other causative agents included *Pseudomonas aeruginosa* (21.2%), *Proteus* Spp. (19.4%), *Klebsiella pneumoniae* (10.3%), Coagulase negative *Staphylococci* (5.5%), *E coli* (4.2%), C*itrobacter freundii* (3%), *Acinetobacter baumannii* (1.2%).

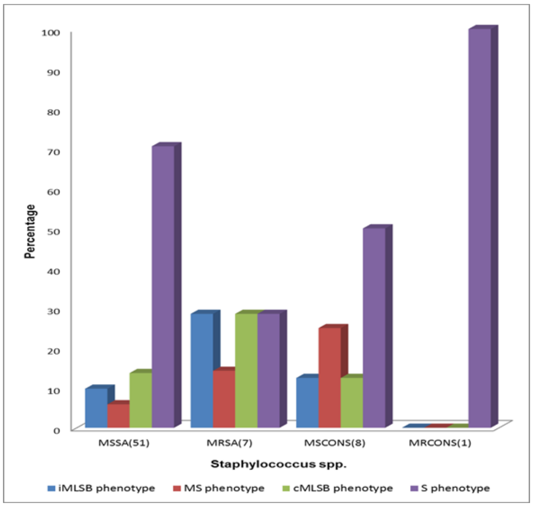
Fungal culture yielded19 (11.2%) isolates, mostly along with one or the other bacterial growth.

The study of staphylococcal isolates for detection of inducible Clindamycin resistance revealed following findings (Table 1 and Figure 1).

Table 1: Showing susceptibility of *Staphylococcus* spp. to Erythromycin and Clindamycin

Phenotype	MSSA (51)	MRSA (7)	MSCONS (8)	MRCONS (1)	Total (67)
iMLSB E-Resistant, **CD**-Sensitive D zone positive	5 (9.8%)	2(28.6%)	1(12.5%)	0	8 (11.9%)
MS **E**-Resistant, **CD**-Sensitive D zone negative	3(5.9%)	1(14.3%)	2(25%)	0	6 (9%)
cMLSB **E**-Resistant, **CD**-Resistant	7 (13.7%)	2(28.6%)	1(12.5%)	0	10 (14.9%)
S **E**-Sensitive, **CD**-Sensitive-	36 (70.6%)	2(28.6%)	4(50%)	1(100%)	43 (64.2%)
Total	51 (100%)	7(100%)	8(100%)	1(100%)	67 (100%)

Figure 1: Susceptibility of Staphylococcus spp. to Erythromycin and Clindamycin



DISCUSSION

In our study majority of Staphylococci (64.2%) were sensitive to both, Erythromycin and Clindamycin (S phenotype). Mokta et al (2015)20 found 60.9% sensitivity among Staphylococcus Aureus, Debdas & Joshi (2011) 10 found 62% sensitivity while Wahane & Kulkarni (2013)14 found it to be 79.6% among all Staphylococcal isolates.

Table 2: Various studies across the world showing Clindamycin resistance in Staphylococcus Aureus

Place	Study	MRSA			MSSA		
		iMLSB %	cMLSB %	MS %	iMLSB %	cMLSB %	MS %
India	Gadepalli et al (2006)	**30**	**38**	**12**	**10**	**15**	**12**
	Debdas & Joshi (2011)	18	23	48	2	3	6
	Sasirekha et al (2012)	1	5	6	9	8	13
	Wahane & Kulkarni (2013)	0	1	0	5	5	8
	Mokta et al (2015)	**28**	**29**	**13**	**9**	**13**	**7**
	Shetty & Afroz (2017)	27	52	17	11	22	15
World	Paul et al (2004)	7	84	1	20	3	1
	Yilmaz et al (2007)	**24**	**44**	**5**	**15**	**5**	**4**
	Moosavian & Shoja et al (2014)	29	13	0	3	0	0
	Nwokah & Abbey (2016)	75	24	-	59	4	-
India	Present study	**28.6**	**28.6**	**14.3**	**9.8**	**13.7**	**5.9**

Inducible Clindamycin resistance (iMLS$_B$ Phenotype) was seen in 9.8% of MSSA isolates in our study which correlates with findings of some of the Indian studies like Shetty & Afroz (2017)[21] who found it to be 11%, Mokta et al (2015)[20] reported it to be 9% while Gadepalli et al (2006)[11] reported it as 10%. Yilmaz *et al* (2007)[8] in their study in Turkey found 14.8% incidence of Inducible Clindamycin resistance among all Staphylococcal isolates.

While Wahane and Kulkarni (2013)[14] found 5% incidence, Debdas and Joshi (2011)[10] found 2% incidence of inducible Clindamycin resistance in Staphylococci isolated from ear discharge of patients of CSOM. Nwokah and Abbey (2016)[22] in their study in Nigeria found 59% incidence while Moosavian et al (2014)[23] in Iran reported it to be 3%.

Among MRSA 28.57% of isolates showed inducible Clindamycin resistance. These findings are similar to the findings of following Indian studies like Shetty and Afroz (2017)[21] who found it to be 27%, Mokta et al (2015)[20] who reported it to be 28% while Gadepalli et al (2006)[11] reported it as 30%.

Moosavian et al (2014)[23] in Iran reported it to be 29% while Yilmaz *et al* (2007)[8] in their study in Turkey found 24% incidence of Inducible Clindamycin resistance among all Staphylococcal isolates.

These findings differ from Wahane and Kulkarni (2013)[14] did not find any MRSA isolate showing inducible Clindamycin resistance, Sasirekha et al (2012)[24] found 1% incidence in MRSA.

Nwokah and Abbey (2016)[22] in their study in Nigeria found 75% incidence while Paul et al (2007)[15] in Chicago reported it to be 7%.

Among CONS one (12.5%) isolate was found with inducible Clindamycin resistance among MSCONS while there was no isolate from MRCONS with inducible Clindamycin resistance. Yilmaz *et al* (2007)[8] found 19.2% incidence among MSCONS while 25.7% incidence was found amongst MRCONS, Wahane and Kulkarni (2013)[14] found 5.5% incidence of inducible Clindamycin resistance among CONS from CSOM. Hawan et al(2009)[9] reported 30% resistance among MRCONS and 21% among MSCONS. Paul et al (2004)[15] reported 14% resistance among all CONS.

In our study, constitutive resistance (cMLS$_B$ Phenotype) was found in 28.6% of MRSA, 13.7% of MSSA, 12.5% of MSCONS isolates which correlates with the findings of Mokta et al (2015)[20] reported it as 29% among MRSA and 13 % among MSSA while Wahane and Kulkarni (2013)[14] reported 1% incidence among MRSA, 5% among MSSA and 11.1% among CONS.

In our study MS Phenotype (E-Resistant, CD-Sensitive D zone negative) was found in 14.3% of MRSA, 5.9% of MSSA and 25% of MSCONS isolates while Mokta et al (2015)[20] reported it as 13% among MRSA and 7 % among MSSA isolates and Debdas et al (2011)[10] reported 48% incidence in MRSA and 6% in MSSA isolates whereas Moosavian et al (2014)[23] in Iran did not report any. Wahane and Kulkarni (2013)[14] reported 5.5% incidence among CONS.

Various researchers across the world reported the incidence of inducible Clindamycin resistance in MRSA ranging from 0% to 75% and 2% to 59% in MSSA. This wide variation in resistance pattern may be due to differences in patient papulations studied, geographical area, hospital characteristics, types of clinical specimen and the pattern of Methicillin resistance.

Thus incidence of iMLS$_B$ was found to be higher in MRSA than in MSSA strains in our study. As MRSA strains are also resistant to other antibiotics, the choice of antibiotics gets restricted. In such situation correctly identifying iMLSB would be of great help in deciding whether should be used or not.

CONCLUSION

Without the double-disc test (D test), all the Staphylococcal isolates with inducible Clindamycin resistance would have been misclassified as susceptible resulting in treatment failure. In our view this is a feasible option as it is easy to perform and gives clear cut results. Hence it should be performed routinely wherever Clindamycin is indicated for the treatment of Staphylococcal infections. In depth studies should be done at regional levels as there is vast difference in inducible Clindamycin resistance in different regions across the world.

REFERENCES

1. Kong K, Coates HLC. Natural history, definition, risk factors and burden of otitis media. MJA 2009;91(9):S39-43.
2. Motayo BO, Ojiogwa IJ, Adeniji FO, Nwanze JC, Onoh CC, Okerentugba PO et al. Bacteria isolates and antibiotic susceptibility of ear infecton in Abeokuta, Nigeria. Report and Opinion 2012;4(4):23-6.
3. Prakash R, Juyal D, Negi V, Pal S, Adekhandi S, Sharma M, et al. Microbiology of chronic suppurative otitis media in a teritiary care setup of Uttarakhand State, India. North American Journal of Medical Sciences 2013;5(4):282-7.
4. Acuin J. Chronic suppurative otitis media: burden of illness and management options. Switzerland: WHO library cataloguing-in-publication; 2004.
5. Shrivastava A, Singh RK, Varshney S, Gupta P, Biat SS, Bhagat S,et al. Microbiological evaluation of an active tubotympanic type of chronic suppurative otitis media. Nepalese Journal of ENT Head & Neck surgery 2010;4(2):14-7.
6. Ayson PN, Lopez JEG, Llanes EG. Chronic suppurative otitis media: bacteriology and drug sensitivity apttern at the quirino memorial medical centre (2004-2005): A preliminary study. Phillipine journal of otolaryngology head and neck surgery.2006;21(1&2):20-3.
7. Nikakhlagh S, Khosravi AD, Fazlipour A, Sarafzadeh M, Rashidi N. Microbiologica findings in patients with chronic suppurative otitis media. J. Med. Sci 2008;8(5):503-6.
8. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible resistance in staphylococci. Journal of Medical Microbilogy 2007;56:342-5
9. Lim H, Lee H, Roh K, Yum J, Yong D, Lee K, Chong Y. Prevalence of Inducible Clindamycin Resistance in Staphylococcal Isolates at a Korean Tertiary Care Hospital. Yonsei Med J 2006;47(4)480-4
10. Debdas D, Joshi S. Incidence of resistance in clinical isolates in Staphylococcus aureus.J Infect Dev Ctries 2011;5(4):316-7
11. Gadepalli R, Dhawan B, et al. Inducible resistance in clinical isolates of Staphylococcus aureus. Indian Journal of Medical Research 2006; P 571-573.
12. CLSI. Performance standards for antimicrobial susceptibility testing; 22nd International supplement. CLSI document Wayne PA:Clinical Laboratory standards Institute;2012.
13. Woods C. Macrolide-Inducible Resistance to Clindamycin and the D-Test. Pediatr Infect Dis J 2009;(28)1115-8
14. Wahane CI, Kulkarni VA. 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. International Journal of recent trends in science and technology. 2013;9(1): 5-7
15. Schreckenberger P, Ilendo E, Ristow K. Incidence of Constitutive and Inducible Clindamycin Resistance in Staphylococcus aureus and Coagulase-Negative Staphylococci in a Community and a Tertiary Care Hospital. JCM2004;42(6)2777-9
16. Collee JG, Fraser AG, Marmion BP, Simmons A, Mackie and McCartney Practical Medical Microbiology. 14th ed. New Delhi: Elsevier, a division of Reed Elsevier India Private Limited;2012. p. 113-178
17. Cheesbrough M, Laboratory practice in tropical countries. 2nd ed. Cambrige: Cambrige universitypress;2009. P. 35-45, 80-85.
18. Winn WC, Koneman EW, Allen SD, Procop GW, Schreckenberger PC, Janda WM, Woods GL. Koneman s Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. United States of America, Lippincott Williams and Wilkins; 2006.p67-110, 635
19. Forbes BA, Sahm DF, Weissfeld AS. Baily and Scott s Diagnostic Microbiology, 12th ed. Missouri: Mosby Elsevier Missouri; 2007. p.187-216, 251-3.
20. Mokta K, Verma S, Chavanuhan D, Ganju S, Singh D, Kanga A, Kumari A, Mehta V. Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus aureus from Sub Himalayan Region of India. JCDR2015;9(8)DG 20-3
21. Shetty J, Afroz Z Prevalence of constitutive and inducible clindamycin resistance among clinical isolates of Staphylococcus aureus in a tertiary care institute in North India. Int J Res Med Sci 2017;5(7)3120-5
22. Nwokah E,Abbey S. Inducible-Clindamycin Resistance in Staphylococcus aureus Isolates in Rivers State, Nigeria. Ajcm 2016; 4(3)50-5
23. Moosayian M, Shojas, Rostami S, Torabipour M, Farshadzadeh Z Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus due to erm genes, Iran. IJM 2014; 6(6) 421-7
24. Sasirekha B, Usha M, Amruta J, Ankit S, Brinda N, Divya R. Incidence of constitutive and inducible clindamycin resistance among hospital-associated Staphylococcus aures