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A Study of Aerobic Bacterial Isolates and Their Antibiotic Susceptibility Pattern in Chronic Suppurative Otitis Media

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ABSTRACT

CSOM is a disease of multiple aetiology and is well known for its persistence and recurrence inspite of the treatment. The present study was carried out to determine the aerobic microorganisms involved,their antibiotic sensitivitypattern in patients with Chronic Suppurative Otitis Media (CSOM) and to provide a guideline for empirical antibiotic therapy.The sample were collected from 104 clinically diagnosed case of CSOM E.N.T. department (both OPD & IPD) of J.L.N.hospital and processed using standard microbiological techniques at Microbiology department, J.L.N.Medical College Ajmer (Rajasthan).Out of 104, ear swabs studied, 94.23% were smear positive and culture positive, 1.92% were smear positive and culture negative.The commonest isolates of CSOM are Pseudomonas aeruginosa(30.76%) followed by Staphylococcus aureus(29.80%) followed by Klebsiella pneumoniae 11(10.57%), Proteus mirabilis 7(6.73%), Enterococcus faecalis 4(3.84%), CONS 3(2.88%), E.coli and Citrobacterfreundii isolated in 2(1.92%) and Serratiamarcescens and Streptococcus pyogenes 1(0.96%) of cases each. Follow up of patients after a course of antibiotics will help in cure of some patients andin preparing patients for surgery either tympanoplasty or mastoidectomy. It also helps in preventing development of complications of CSOM.

KEYWORDS Chronic suppurative otitis media, aerobic bacteriology, antibiotic sensitivity.

INTRODUCTION

CSOM is perforation of tympanic membrane with persistent drainage from middle ear for 6-12 weeks. Chronic suppuration can occur with or without Cholesteatoma.[1]

CSOM is a disease of multiple aetiology and is well known for its persistence and recurrence inspite of the treatment. CSOM is a name given to a long standing inflammatory disease affecting mucoperiosteal lining of the middle ear. It is a destructive and persistent disease with irreversible sequelae and can proceed to serious intra and/or extracranial complications.[2]

CSOM is divided into two types according to presence or absence of cholesteatoma. Bacteriologically and etiologically both types are different. Noncholesteatomatous type is usually result of incompletely treated acute suppurative otitis media or recurrent suppurative otitis media. Important feature of this type of disease is the presence of central perforation; however, in cholesteatoma type perforation is either marginal or attic.[3]Otitis media (OM) incidence and prevalence estimates from around the world vary widely, it is clear that OM is a very common childhood disease. It is especially prevalent in children younger than 2 years of age. Furthermore, the earlier the first episode of OM, the greater the risk of subsequent recurrent OM and chronic otitis media with effusion.[4]

Hearing loss associated with CSOM leads on to educational backwardness in children that is well recognized by Otologists, Paediatricians and Educators. Development of speech, language and learning skills are severely hampered in these children making it difficult for them to achieve full academic potentials outdoor activities are also hampered.[5]

The indiscreminate, haphazard and half hearted use of antibiotics and poor follow up of the patients have resulted in persistent low grade infectional changes in the microbiology of the disease, the advent of new antimicrobials, anti-inflammatory and anti-histamine agents make an evaluation of bacterial flora of CSOM important.[6]

Knowledge of local micro-organism pattern and their antibiotic sensitivity is essential for the effective and low cost treatment.[7]

Incidence of CSOM is increasing during the past 10-20 years. The disease prevalence depends on race and socioeconomic factors like poor living conditions, overcrowding, poor hygiene and nutrition.[8]

MATERIALS AND METHODS

Hundred patients with CSOM who presented to the Ear, Nose and Throat (ENT) department, (both OPD & IPD) of J.L.N.hospital and processed at Microbiology department, J.L.N.Medical College Ajmer (Rajasthan).Form 15 September 2012 to 14 September 2013 were prospectively studied. None of them had received topical or systemic antibiotics for earlier 7 days.

Clinical/demographic data were collected using a set format, which included patient age, sex and residential address, duration of illness (ear disease).

Collection of discharge

The discharge was collected under aseptic precautions. The external ear canal was wiped with sterile cotton and then with 70% alcohol. This was allowed to dry. Then using a sterile auditory speculum, under aseptic conditions, a sterile cotton swab stick was introduced into the middle ear. The stick was rotated and removed with precaution so as, not to touch the external ear canal or any other part of the skin. The cotton swab stick was immediately put into its container. Two specimens from a single ear were collected in such manner. Labeled and taken to the laboratory immediately for processing.

Processing of Sample: 1st swab stick was used for gram staining and IInd swab stick was used for culture. Direct smear with gram stain were screened for the presence of inflammatory cells and type of microbial flora. IInd swab was inoculated on MacConkey agar, Blood agar and Brain Heart Infusion Broth. It was incubated at 37°C for 24-48 hrs. Observe the

growth, if there was no growth on MA & BA but BHI was turbid, and then subculture was done on MA & BA. The colonial morphology and identification was done by standard procedures (Collee etal. 1996). Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, indole production, motility, carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Mathur et al. (2006) and Senthilkumar et al. (2012).

Antibiogram Testing

The antibiotiogram testing was done as per CLSI guidelines using modified Kirby-Bauer method. Few colonies from the culture plate were inoculated into 2 ml of peptone water. Incubated at 37^{0}C for 2 hours. Turbidity was compared to that of 0.5 Mc Farlandstandard. A cotton swab was immersed & rotated in this inoculum, the swab was then pressed to the sides of the tube so as to remove excess inoculum. It was then used for carpet streaking on Muller Hinton Agar plate. The required antibiotic discs were then placed aseptically on this using sterile forceps. The plates were then incubated 24 hours at 37^{0}C. Next day, the zone size was recorded and reported as sensitive or resistant by comparing the zone size to the Kirby-Bauer chart

Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. Depending on the isolate, antibiotic discs were selected from among the following: Co-trimaxazol (25µg), Erythromycin (15µg) , Gentamicin (10µg),Ciprofloxacin (5µg), Oxacillin(1µg),Amoxyclav (30 µg), Linazolid (30 µg),Vancomycin (30*µg), Tetracycline* (30µg),Cefotaxime(30µg),Amikacin(30µg),Amoxyclav(30 µg),Ceftazidime (30 µg),Imipenem (10 µg), Pipercilline (100 µg).

RESULT AND DISCUSSION

In the study it was observed that,out of hundred and four ear swabs studied, 94.23% were smear positive and culture positive, 1.92% were smear positive and culture negative, 3.84% were smear negative and culture negative.

Maximum number of cases falls in the age group 11-20 years (29%) and 21-30 years (22%) and 11-20 years 21%.(Table 1) Male to female ratio 1.08: 1.0. Males (52%) predominately affected than female (48%), which is similar to the studies done by Vijay D et al[9], Sinhna et al[10]. & Gupta vineet et al[3].

Incidence of CSOM was higher in males (52) compared to females (48).Out of 52 males studied 51(54.26%) were positive for culture and out of 48 females 43(45.74%).Majority of patients were from rural areas (67%) compared to urban areas (33%).Unilateral infection (96%) was more common than bilateral infection (4%). The side of involvement showed left ear was predominant (50%) compared to right ear (46%). Maximum no of cases were seen during November-February (25%) i.e winter season and July-October i.e. early rainy season (11%)

Gram negative organisms were more common 57 (54.80%) than gram positive 37 (35.57%) organisms, which can be correlated to the studies done by Sirvastava V.K et al[11] , Nandy A et al[6]., Malkappa S K et al[12].Negative cultures can be attributed to-Non-bacterial growth,Anaerobic growth and Prior-antibiotic therapy.Among positive cultures monomicrobial isolates were seen in 94 (90.38%) and only polymicrobial isolates were seen in 4(3.84%) no growth in 6 (5.76%) cases. Rama Rao M.V. et al. (1980)[9] found equal incidence of mixed and pure culture and Baruah P.C. et al. (1972)[14] found predominance of mixed culture.Availability and use of topical and systemic broad spectrum antibiotics in the period before consultation was probably responsible for the lower incidence of mixed infection.[15]

The most common organism isolated was Pseudomonas aeruginosa. Pseudomonas aeruginosa was isolated in 32 cases accounting for 30.76% of the total single isolated organisms, study is correlated with the above workers like Ballal M. et al. (1992),[16] Saurabh V. et al. (1999),[15]Hiremath S.L. et al. (2001)[17] and Loy A..C. (2002)[7] have found Pseudomonas spp. as the predominant organism causing CSOM. The second common organism was Staphylococcus aureus 31 (29.80%) This finding is correlated with other workers shown in the table like Arya S.C. et al. (1966),[18]Nandy A. et al. (1991),[6]Grevel R.S.et al. (1995),[19]Urmil Mohan et al. (1998),[20]Hiremath S.L. et al. (2001),[17] Loy A.H.C.et al. (2002)[7] have found Staphylococcus aureus as the second most common organism causing CSOM.

Other organisms isolated were Klebsiella pneumoniae 11(10.57%) and Proteus mirabilis 7(6.73%), Enterococcus faecalis 4(3.84%), CONS 3(2.88%), E.coli and Citrobacterfreundii isolated in 2(1.92%) and Serratiamarcescens and Streptococcus pyogenes 1(0.96%) of cases each. (Table 2)

Among mixed isolates S.aureus was associated with Ps.aeruginosa in 1 (25%) cases. Ps.aeruginosa associated and Enterobacter aerogenes in 1 (25%) and Klebsiella pneumoniae associated with Serratiamarcescens 1 (25%) and Citrobacterfreundii with Klebsiella pneumoniae in 1 (25%) cases.

The organisms like Pseudomonas spp. and Proteus spp. are considered mostly as secondary invaders from external auditory canal gaining access to the middle ear via a defect in tympanic membrane resulting from an acute episode of otitis media.The frequency of Staphylococcus aureus in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.Organisms like E. coli and Klebsiella spp. become opportunistic pathogens in the middle ear when resistance is low.Although CONS are generally considered as non-pathogenic, their association in some cases can be attributed to the extreme lowering of resistance in middle ear due to invasion by other organisms. Under these circumstances they assume pathogenic role either singly or more often in combination with other organisms.[13]

Out of 31 single isolated S. aureus, MRSA were isolated in 12 (38.70%) isolates.

Out of 55 gram negative single isolates 19(34.54%) are ESBL producers and 36 (65.45%) are non- ESBL producers. Among single isolated ESBL producers, P.aeruginosa was common 12(63.15%) and followed by Klebsiella pneumoniae 4(21.05%) and Citrobacterfreundii, Proteus mirabilis and E.coli 1(5.26%) each.Among mixed isolates MRSA+NON ESBL seen in one case. NON ESBL+NON ESBL in one case, ESBL +NON ESBL in two cases.

S.aureus showed sensitivity of 100% to Vancomycin, 93.54% to Linezolid, 70.96% to Gentamicin, (64.51%) to Amoxyclav, 61.29% to Oxacillin, 51.61% to Ciprofloxacin, 32.25% to Erythromycin and Cotrimoxazole. S.aureus is 100% resistant to Ampicillin.

Enterococcus faecalis were 100% sensitive to Linezolid, Amoxyclav Gentamycin and oxacillin, 75% sensitive to Vancomycin and Erythromycin but 50 % resistant to Ciprofloxacin, Ampicillin and 100 % resistant for Cotrimoxazole.

Coagulase negative Staphylococcus were 100% sensitive to Amoxyclav, Gentamycin, Erythromycin, Linezolid, Oxacillin and Vancomycin but 33.33% resistance to Ciprofloxacin, Ampcillin and Cotrimoxazole seen.

Maji PK et al[21], Study by Vijay D et al.[22] and Gulati and others[23] shows the similar results.

Strptococcus pyogenes was sensitive to AmpcillinAmoxyclav, Ciprofloxacin, Gentamycin, Erythromycin, Linezolid, Oxacillin and Vancomycin but resistant to Cotrimoxazole.

Pseudomonas aeruginosa were 100% sensitive to Imipenem and Amikacin followed Pipercillin (68.75%), Ceftazime (62.5%), Amoxyclav, Ciprofloxacin and Cefotaxime (56.25%) and Tetracyclin (50%).

Other Gram negative bacilii were more sensitive to Imipenem (100%), Cefotaxime, Amikacin, Ceftazidime, Tetracycline and Amoxyclav.It was observed from above study that gram positive organisms were sensitive to Gentamicin, Amoxyclav, Linezolid and Vancomycin but resistant to Ampicillin, Cotrimoxazole. For Ciprofloxacin and Erythromycin sensitivity was moderate.Gram negative organisms were sensitive to Amikacin, Cefotaxime and Imipenem. For Tetrecyclin, Ciprofloxacin and Amoxyclav, all are moderately sensitive.

In mixed isolates

When both the S.aureus as well as Ps.aeruginosa were isolated from the culture then it was observed that S.aureus was sensitive to Gentamicin, Amoxyclav, Linezolid and Vancomycin but resistant to Ampicillin, Ciprofloxacin, Cotrimoxazole, Erythromycin, Oxacillin, whereasPs.aeruginosa was sensitive to Amikacin, Amoxyclav, Ciprofloxacin, Cefotaxime, Ceftazidime, Imipenem, Piperacillin and Tetracycline

Similarly when Ps.aeruginosa and E.aerogenes were found then Ps.aeruginosa was sensitive to Amikacin, Ciprofloxacin, Imipenem, and Piperacillin but resistant to Amoxyclav, Cefotaxime, Ceftazidime, and Tetracycline and E.aerogenes was sensitive to Amikacin, Amoxyclav, Cefotaxime, and Ceftazidime Imipenem and resistant to Ciprofloxacin and Tetracycline.

K.pneumoniae and S.marcescens were isolated then K.pneumoniae was sensitive to Amikacin, Ciprofloxacin, and Ceftazidime Imipenem and resistant to Amoxyclav, Cefotaxime, and Tetracycline whereas S.marcescens was sensitive to Amikacin, Ciprofloxacin, Cefotaxime, Ceftazidime Imipenem, and Tetracycline and resistant to Amoxyclav.

When both C.freundii as well as K.pneumoniaewere found in the culture then it was found C.freundii was sensitive to Amikacin, Ciprofloxacin, Imipenem, and tetracycline and resistant to Amoxyclav Cefotaxime, Ceftazidime. K.pneumoniae was sensitive to Amikacin, Ciprofloxacin, Cefotaxime, Ceftazidime, Imipenem, Tetracycline and resistant to Amoxyclav.

Vijay D and others[22] showed mixed culture of S.aureus+P. aeruginosa was predominant.Asiri SA and others[24]also showed P. aeruginosa was common with mixed cultures. Sinha A and others[10] also isolated P.aeruginosa in mixed cultures.

MRSA was sensitive to Amoxyclav 78.12%, Gentamicin 75%, Linezolid 90.62%, Oxacillin 59.37% and Vancomycin 100% but resistant to Ampicillin. The resistance For Ciprofloxacine is 71.87 % and for Erythromycin is 40.62%.ESBL producers were sensitive to Amikacine (91.93%) and Amoxyclav (67.74%), Cefotaxime (66.12%), Ceftazidime (66.12%), Imipenem (100%), and Tetracycline (66.12%) but resistant to Ciprofloxacin (62.90%).This was compared with Choi and others[25] which showed MRSA of 28% in CSOM. Park DC and others showed MRSA 45.9% in CSOM.[26] Park MK and others showed MRSA in 4.9% of CSOM.[27]Varsha G and other[28] showed 24% ESBL producers in urine, pus and sputum.

Mathur et al found 68% and Tankhiwal et al found in 48%. Most of the studies showed E.coli was commonest ESBL producers followed by K.pneunomiae, Citrobacter spp., P.aeruginosa and Proteus spp. in pus, urine and sputum. Sensitivity of ESBL producer in Varsha G and other showed as resistance to Amikacin(24%), Gentamicin (75%), Ciprofloxacin (65%), Cefotaxime (90%) and Amoxyclav (69%) which is comparable to present study.[29]

CONCLUSION

Chronic otitis media is major health problem in many populations around the world and a significant cause of morbidity and mortality.It is a major global cause of hearing impairment and the effect is major concern particularly in children because it may have long-term effects on early communication language development, auditory processing, psychosocial, cognitive development and educational progress.It is necessary to know the causative agent and drug sensitivity pattern for better treatment where antibiotics are commonly abused. This will enhance better treatment and reduce the burden of the infection on the patients and in long term, it may reduce the cost of treatment. Proper selection of antibiotics also helps in preventing drug resistance and also clearing of infection. Hence isolation of bacteria and sensitivity study is important for all CSOM cases.

Age group	Male		Female		Total	
	No. of Cases	%	No. of Cases	%	No. of Cases	%
0-10	14	26.92	7	14.58	21	21
11-20	17	32.69	12	25	29	29
21-30	8	15.38	14	29.16	22	22
31-40	3	5.76	8	16.66	11	11
41-50	1	1.92	3	6.25	4	4
51-60	5	9.61	1	2.08	6	6
>60	4	7.69	3	6.25	7	7
Total	**52**	**100**	**48**	**100**	**100**	**100**

Table 1: Agewise distribution

S. No.	Organisms	No. of organisms (n=104)	Percentage (%) (n=104)
1	Pseudomonas aeruginosa	32	30.76%
2	Staphylococcus aureus	31	29.80%
3	Klebsiella pneumonia	11	10.57%
4	Proteus mirabilis	7	6.73%
5	Enterococcus faecalis	4	3.84%
6	CONS	3	2.88%
7	E.coli	2	1.92%
8	Citrobacter freundii	2	1.92%
9	Serratia marcescens	1	0.96
10	Streptococcus pyogenes	1	0.96
11	Mixed infections	4	3.84%
12	No growth	6	5.76%
	Total	**104**	**100%**

Table- 2: The Single isolate in the present study and their percentage:

BIBLIOGRAPHY

- Michael A Rubin,RalphGonzales,Merle A Sande ,Harrison principles of internal medicine Kasper, Braunwald, Fauci, Mc Graw Hill Publication,16th edition, volume 1, 185-193].
- Poorey VK, Iyer A. Study of Bacterial flora in Chronic Suppurative Otitis Media and its clinical significance. Indian Journal of Otolaryngology and Head and Neck Surgery 2002; 54: 91-95.
- Gupta V, Gupta A, Sivarajan K. Chronic Suppurative Otitis Media: An Aerobic Microbiological study. Indian Journal of Otology 1998; 4: 79-82.
- Kathleen A Daly. Epidemiology of otitis media. Otollaryngolclin North Am 1991;24:775-83
- Mustafa E, Tabsin A, Erdogan S, Erciban G. Bacteriology of chronic suppurative otitis media. Ann OtolRhinolLaryngol 1994;103;771-4.
- Nandy A, Mully PS, Sivarajan K. Chronic suppurative otitis media – A bacteriological study. Indian Journal of Otolaryngology 1991; 43(3): 136-138.
- Loy AHC, Tan AL, Lu PKS. Microbiology of chronic suppurative otitis media in Singapore. Singapore Med J 2002; 43(6):296-299.
- Goyal N, Kakkar V, Goyal P, Yadav SPS. Myringoplasty for chronic otitis media,Ind J of pediatrics 2002;69:223-4.
- Vijay D, Nagarathanmma T. Microbiologial study of chronic suppurative otitis media. Ind J Otol 1998 Dec; 4(4); 172- 4.
- Sinha A , Kapil A, Gupta V , Aerobic Bacteriological study of chronic suppurative otitis media:Ind J Otol 1999 December; 5(4):203-06.
- Srivastava VK, Agarwal SK, Malik G. Chronic suppurative otitis media in children. Indian Journal of Paediatrics 1979; 46(3&1):363-367.
- Malkappa SK, Kondapaneni S, SurpamRB, Chakraverti TK. Study of aerobic bacterial isolates and theirantibiotic susceptibility pattern in chronic suppurative otitis media.Indian J Otol 2012;18:136-9.

- Rama Rao MV, Jayakar PA. Bacteriological study of chronic suppurative otitis media. Indian Journal of Medical Association 1980; 75:30-33.
- Baruah PC, Agarwal SC, Arora MML, Mehra YN. Clinical and microbiological studies in suppurative otitis media. Indian Journal of Otology 1972; 24(4):157-159. 48.
- Saurabh Varshney, Pratima Gupta. Bacteriological study of chronic suppurative otitis media. Indian Journal of Otology 1999; 5(2):87-91.
- Ballal M, Jyothirlatha, Kishor J, Rajan R, Shivananda PG. Chronic suppurative otitis media – A bacteriological and mycological study. Indian Journal ofOtolaryngology and Head and Neck 1992; 1(1):10-13.
- Hiremath SL, Kanta RC, Yeshwanathrao M, Vasantha Kumar CM. Aerobic bacterial isolates of CSOM and their antibiotic sensitivity pattern. TheIndian Practitioner 2001; 54(7):486-489.
- Arya SC, Mohapatra LN. Bacteriological and mycotic flora in cases of chronic suppurative otitis media. Journal of Indian Medical Association 1966; 47(8):369-372.
- Greval RS, Ram Shobha. Bacteriological patterns of chronic suppurative otitis media in Ludhiana. Indian Journal of Medical Sciences 1996; 50(6):191-95.
- Mohan Urmil, Jindal Neerja. Fungal and bacterial flora of chronic suppurative otitis media in Amritsar. IJO & HNS 1998; 50(2):175-177.
- Maji PK, Chatterjee TK, Chatterjee S, Chakrabarty J etal. The investigation of bacteriology of chronic suppurative otitis media in patients attending a tertiary care hospital with special emphasis on seasonal variation. Ind J Otolaryngol and HNS 2007;59:128-31.
- Vijaya D, Aerobes, Anaerobes and Fungi in chronic suppurativeotits media. Ind J Otol 2000 Sep;6(3);55-8.
- Gulati, Sudesh Kumar. Investigative profile in patients of chronic suppurative otitis media. Indian Journal of Otology 1997; 3(2):59-62.
- Asiri SA, Banjar AA. Microbiological evaluation and the management of chronic suppurative otitis media among Saudi children. Ind J Otol 1999 Mar; 5(1) : 33-6
- Choi HG, Park KH, Park SN, Jun BC, Lee HM, The appropriate medical management of Methicillin resistant Staphalococcus aureus in chronic suppurative otitis media, Actaotolarynglogica 2010:130(1):42-6.
- Park DC, Lee SK, Chaci, Lee SO, Lee MS et al Antimicrobiological Resistance of Staphylococcus from otorrhoea in chronic suppurative otitis media and comparision with results of all isolated Staphylococci. Eur J ClinMicrobiol and Inf Dis 2008 Feb:27(7):571-7.
- Park MK, Jung MH, Kang HS, Woo JS, Lee HM et al. The ch; 1317-1342. nges of MRSA infections in chronic suppurative otitis media. Journal Otoloayngol Head Neck surg 2008 Sep:139(3):395-8.
- Varsha G, Priya D. Extended spectrum Betalactamases (ESBL) in community isolates from North India: frequency and predisposing factors. International J Inf Dis 2007:11(1):88-9.
- Khan MKR, Thukral SS, Gand R. Evaluation of a modified double disk synergy test for detection of Extended spectrum Beta-lactamases in AmpC-Beta-lactamase producing P.mirabilis. Ind J MedlMicrobiol 2008:26(1):58-61