ORIGINAL ARTICLE

Microbial profile with their antimicrobial susceptibility pattern in ear discharge of Chronic suppurative otitis media patients at a tertiary care hospital in Durgapur

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a persistent middle ear disease with high risk of irreversible complications in absence of timely management. It is a massive health problem in India having the highest prevalence rate (>4%) requiring urgent attention. Aims and Objectives: The aim of the study was to find out risk factors, microbiological profile, with their susceptibility pattern, and of ear discharge in CSOM patients to provide guidelines for the empirical treatment. Materials and Methods: Ear discharge samples from 135 clinically diagnosed CSOM patients were collected and processed. Microbial isolates were identified and drug susceptibility testing was conducted using Kirby-Bauer disc diffusion method. Results: Pseudomonas species (59/135, 43.70%) was the predominant isolate followed by Staphylococcus species (37/135, 27.40%) while Aspergillus species (10/12, 83.33%) was the predominant fungus isolated. No anaerobic bacteria were isolated on culture. Gram-negative bacilli were most susceptible to Meropenem (100%) and Amikacin (100%) while Pseudomonas species was to Imipenem (98.35%). Gram-positive cocci showed 100% susceptibility to Vancomycin, Linezolid, and Doxycycline. Conclusion: A continuous and periodic evaluation of microbiological pattern of CSOM and antimicrobial sensitivity of isolates is necessary for forming the basis of empirical treatment which shall aid in decreasing the potential risk of complications. Further, accurate and timely identification, knowledge of the pathogens, and judicious use of antibiotics are the need of the hour.

Key words: CSOM; Methicillin-resistant Staphylococcus aureus; Middle ear disease

INTRODUCTION

Chronic suppurative otitis media (CSOM) is a chronic inflammation of middle ear cleft without reference to etiology or pathogenesis. Sources of infection in otitis media are mainly dependent on the route by which infection reaches the middle ear and the chief route by which this occurs in the Eustachian tube.^{1,2} It is a persistent disease of the middle ear, which is capable of causing severe destruction sequelae with the manifestation of deafness, discharge, and a permanent perforation.¹ This disease is mainly classified into two

types based on the area of tympanic membrane involved – Tubotympanic-pars tensa and Atticoantral-pars flaccid. The causes of infection in such cases are nasopharyngeal disease and in children this usually means the adenoids. The causative infections may be in the nose, paranasal sinuses, or in the oropharynx. All these conditions are ascending infection of the Eustachian tube.³ Tubotympanic is called as a safe type or benign type as there is no serious complication whereas atticoantral is called as the unsafe or dangerous type because of associated complications which may be life threatening at times.² Developing countries like India have higher

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prevalence (>4%) mostly seen in low socioeconomic society. CSOM is mostly caused by bacteria, but fungi and virus can also be associated with CSOM. The aerobic microorganisms most frequently isolated in CSOM are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* spp., *Klebsiella* spp., *Escherichia* spp., *Citrobacter* spp., *Haemophilus influenzae*, and *Moraxella catarrhali*.⁴⁻⁶ Untreated/inadequately treated cases cause intra/ extracranial complications and emergence of resistant strains. Since the introduction of antibiotics, the complications have become less common. However, due to increased and irrational use of wide - spectrum antibiotics, the resistance in the bacterial isolates has become very common. Therefore, the microbial culture and sensitivity will help in appropriate management of otitis media and its complications and thus preventing the emergence of resistant bacterial strains.

Aims and objectives

The aim of the study was to find out risk factors, microbiological profile, with their susceptibility pattern, and of ear discharge in CSOM patients to provide guidelines for the empirical treatment.

MATERIALS AND METHODS

After obtaining approval from the Ethical Committee (GIMSH/MC/IEC/AL/01/19/59), the study was carried out on 135 patients attending the ENT OPD and microbiology department of a tertiary care teaching center having ear discharge with signs and symptoms of CSOM. The ear discharge was collected under aseptic precautions with the aid of an aural speculum, before the instillation of any topical medication. The first swab was used to make a smear on clean grease free glass slide for bacterial differentiation by Gram stain examination and direct microscopy of specimen in KOH for fungal examination. The second swab was used for the bacterial culture on blood agar and MacConkey's agar which is then incubated for 24 h at 37°C and the isolates were identified using colony morphology and standard biochemical tests. After identifying the isolate, their antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines on Muller Hilton Agar. The plates were read out after overnight incubation, by measuring the zone of inhibition around the antibiotic discs (HiMedia Laboratories Private Limited, Mumbai, India) as per Clinical Laboratory Research Institute (CLSI) standards.

RESULTS

A total of 135 samples, including 80 ear swabs and 55 aspirates, were collected from clinically diagnosed CSOM patients. Age-wise distribution of these patients is shown in

Table 1. Among the patients, 57.03% (77/135) were males and 42.96% (58/135) were females, with male to female ratio being 1.5:1. The growth positivity was higher in male patients (75/77, 97.4%) as compared to females (47/58, 81.03%) and the difference was statistically significant (P=0.04). In this study, 72/135 (53.33%) patients were from rural area and 63/135 (46.66%) from urban area. The growth positivity was higher in patients living in rural areas (98.57%, 69/70)as compared to patients from urban area (81.53%,53/65) and the difference was statistically significant (P=0.02). Of the 135 samples processed, 122 (90.37%) samples showed growth positivity in which 120 (88.8%) showed bacterial growth while 12 (8.8%) had fungal growth. Pure growth was seen in 112 (93.33%) samples and mixed growth in 10 (8.3%) samples. All the 122 bacterial isolates were aerobic. No anaerobic bacteria were isolated on culture. Various bacteria and fungi isolated from the clinical samples in our study are shown in Table 2. Antimicrobial susceptibility pattern of the various microorganisms isolated in our study is shown in Tables 3-5.

DISCUSSION

The present study was undertaken on 135 clinically diagnosed CSOM patients. Out of 135 cases, the majority of the CSOM patients 46 (37.70%) were in 11–20 years of age group which is in concordance with the findings of various other studies^{7,8} while Gulati et al., Baruah PC

Table 1: Age-wise distribution of patients				
Age	Number of samples	Growth Positivity		
0–10	24 (17.77%)	22 (18.03%)		
11–20	49 (36.29%)	46 (37.70%)		
21–30	32 (23.70%)	29 (23.77%)		
31–40	15 (11.11%)	12 (9.83%)		
41–50	10 (7.40%)	08 (6.55%)		
51–60	05 (3.70%)	05 (6.55%)		
Total	135	122		

Table 2: Various microorganisms isolated fromclinical samples

Isolated Organisms	Number	Percentage
Pseudomonas species	59	48.36
Staphylococcus aureus	37	30.32
Coagulase Negative Staphylococcus	12	9.83
Escherichia coli	6	4.91
Proteus mirabilis	2	1.63
Enterococcus species	2	1.63
Enterobacter aerogenes	1	0.81
Proteus vulgaris	1	0.81
Acinetobacter species	1	0.81
Citrobacter species	1	0.81
(Fungal isolates) Aspergillus niger	4	33.33
Aspergillus flavus	2	16.66
Aspergillus fumigatus	2	16.66
Candida species	2	16.66

et al., and Nandy et al., reported higher incidence Goyal A et al., have lower incidence of CSOM in the first decade of life. ⁹ In the present study, males were affected more than female which is in concordance with the other studies^{7,8,10-12} but in contrast with the studies done by Prakash et al., Mansoor T et al., Prakash R et et al., and Loy AHC et al., who reported a higher percentage of females being affected in their study. ^{2,13,14} The cause of predominance of male patients over female is unknown.

Table 3: Antimicrobial susceptibility pattern ofGram-positive cocci					
Antibiotics	COPS (3)	CONS (12)	Enterococcus (2)		
Ampicillin	20 (54.05%)	9 (75%)	1 (50%)		
Amoxyclav	25 (67.56%)	10 (83.33%)	1 (50%)		
Ceftriaxone	32 (86.48%)	9 (75%)	0 (0%)		
Cephalexin	35 (94.59%)	11 (91.66%)	0 (0%)		
Clindamycin	27 (72.97%)	12 (100%)	0 (0%)		
Erythromycin	28 (75.67%)	12 (100%)	0 (0%)		
Gentamycin	32 (86.48%)	12 (100%)	1 (50%)		
Piperacillin	22 (59.45%)	8 (66.66%)	1 (50%)		
Vancomycin	37 (100%)	12 (100%)	2 (100%)		
Cephoxitin	32 (86.48%)	7 (58.33%)	1 (50%)		
Doxycycline	37 (100%)	10 (83.33%)	2 (100%)		
Linezolid	37 (100%)	11 (91.66%)	2 (100%)		

COPS: Coagulase Positive Staphylococcus, CONS: Coagulase Negative Staphylococcus

Table 4: Antimicrobial susceptibility pattern of Pseudomonas species

Antibiotics	Percentage Susceptibility			
Aztreonam	78.27			
Carbencillin	36.68			
Cefoperazone	79.27			
Ceftazidime	73.24			
Cefoperazone Sulbactum	85.05			
Gentamycin	40.98			
Imipenem	98.35			
Piperacilin Tazobactum	88.44			
Tobramycin	53.56			
Meropenam	94.22			
Polymyxin-B	95.75			
Colistin	93.22			
Levofloxacin	39.28			

Among the patients, 53.33% (72/135) were from rural area and 46.92% (63/135) were of low socioeconomic status. Our study correlates well with Lasisi AO et al., Agrawal A et al., Gulati et al., and Waqar-Uddin et al. ^{9,14,15} Increased incidence in lower socioeconomic status and rural population might be due to unhygienic surroundings, overcrowding, and lack of nourishment.

In the present study, 90.37% (122/135) samples were culture positive which is in accordance with other studies.^{2,16} However; Mansoor T et al.,¹³ have reported lower culture positivity, that is, 78% and 77%, respectively. High percentage of negative cultures could be probably due to presence of lysozyme in samples, initiation of antimicrobials before sample collection or poor patient selection criteria, or sample collection technique.⁴

Percentage of pure bacterial isolates of 93.33%, pure fungal isolates 8.88%, mixed growth, that is, fungus plus bacteria 8.31% is in agreement with many previous researchers.^{3,5} This study revealed that Gram-negative organisms 58.1% (71/122) outnumber the Gram-positive organisms 41.8% (51/122) in CSOM which is reported similarly in other studies.^{6,17-19} Pseudomonas species (59/122, 48.36%) was the most common isolate followed by Coagulase Positive Staphylococcus (COPS) (37/122, 30.32%) in this study which is in accordance with the other studies.^{13,20,21} This could be attributed to the fact that Pseudomonas is known to be resistant to antibiotics and can survive in nutrient deficient environment. Its ability to produce bacteriocins, presence of pili and enzymes like proteases gives it added advantage of survival over the other bacteria in middle ear infections.8 However, certain authors have reported Staphylococcus species as the most common isolate.^{2,13,22} This signifies the need for studying the microbiological profile for each geographical area. Of the 10 fungal isolates, 08 (80%) were Aspergillus species and 2 (20%) were Candida species which is in accordance with the studies of Saraswati Jayanthi R et al., and Loy AHC et al.23,

Table 5: Antimicrobial susceptibility pattern of Gram-negative bacilli						
Antibiotics	Escherichia coli (6)	<i>Proteus</i> mirabilis (2)	vulgaris vulgaris (1)	Acinetobacter spp. (1)	Citrobacter spp. (1)	Enterobacter aerogenes (1)
Amikacin	6 (100%)	2 (100%)	1 (100%)	0	1 (100%)	1 (100%)
Ampicillin	4 (60%)	2 (100%)	1 (100%)	0	1 (100%)	0
Cefepime	6 (100%)	2 (100%)	0	0	1 (100%)	0
Cefixime	6 (100%)	2 (100%)	0	0	1 (100%)	0
Ceftriaxone	6 (100%)	2 (100%)	0	0	1 (100%)	1 (100%)
Cefoperazone+	6 (100%)	2 (100%)	0	0	1 (100%)	1 (100%)
Sulbactum						
Cefuroxime	5 (80%)	2 (100%)	0	0	0	0
Cephalexin	4 (60%)	1 (50%)	0	0	0	0
Ciprofloxacin	3 (40%)	2 (100%)	1 (100%)	0	1 (100%)	0
Gentamycin	6 (100%)	2 (100%)	1 (100%)	0	1 (100%)	0
Doxycycline	6 (100%)	1 (50%)	0	1 (100%)	0	0
Meropenem	6 (100%)	2 (100%)	1 (100%)	0	1 (100%)	1 (100%)

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Even though anaerobes play a role in the pathogenesis of CSOM, this study did not find any isolate, which is in concordance with the results of Suman Yeli et al., and Swayamsidha Andhale et et al.^{24,25} All the pathogenic strains isolated in the present study were tested against various antibiotics as per standard protocol. Various studies from different parts of India in the past couple of years report 100% sensitivity of Pseudomonas to Imipenem as compared to 98.35% in the present study.^{21,26} Sensitivity with amikacin (87.8%) was in concordance with study by Madana,²⁷ but presence of resistance to Imipenem in Pseudomonas is an alarming scenario of emerging resistant strains which could be due to indiscriminate use of Imipenem without using first line empirical antibiotics for therapy or horizontal spread of resistant strains in the hospital setup. This calls for strict monitoring to prevent the spread of resistance to other susceptible strains. At the same time, high rate of resistance in *Pseudomonas* to Gentamicin in the present study (40%) as compared to that reported by similar other studies (12% to 24%) indicates that there is a need to review empirical treatment of CSOM from time to time.9,13,28

On the other hand, if we see the susceptibility pattern of Gram-positive cocci and Gram-negative bacilli (other than *Pseudomonas*), resistance to Gentamicin is very less (14% and 5%, respectively). This indicates that susceptibility pattern of different micro-organisms to same drug is different, so there is a need for preliminary identification of pathogens in ear discharge of CSOM patients before starting the empirical treatment which is possible in <24 h, so as to prevent the emergence of resistant strains.

COPS showed 100% sensitivity to Vancomycin, Linezolid, and Doxcycline in our study, which is a heartening situation but calls for their judicious use so that resistance to them can be prevented and regular monitoring for emergent resistance. In case of *Escherichia coli, Proteus species,* and *Citrobacter species,* Amikacin was found to be the most effective drug to which the sensitivity was 100% while Arya C et al.,²² reported 100% sensitivity to Ceftazidime and Yang et al.,¹¹ Sharma et al.,⁸ and Rao and Reddy¹⁶ to Ciprofloxacin. When the results of various studies were compared, it became obvious that the bacteriology and antibiotic sensitivity pattern of CSOM has been changing from region to region and over time period too.

Coagulase negative Staphylococcus is a normal skin flora but can sometimes become an opportunistic pathogen. Majority of them were sensitive to almost all the antibiotics tested. In our study, the highest sensitivity was toward Vancomycin and Gentamycin 100%, followed by Linezolid 91.6% and Doxycyline 83.3%. Antifungal susceptibility pattern of *Candida species* showed 100% sensitivity to Voriconazole while sensitivity to Amphoteracin B, Flucytosine, and Fluconazole was 50%. We cannot draw any inference from this pattern due to very small number of *Candida* isolates (2/12) in our study.

Limitations of the study

The present study has some limitations. The sample size was small. Nutrition status of iodine and thyroid autoimmunity in the study patients was not assessed.

CONCLUSION

The increasing rate of micro-organisms' resistance to traditional antimicrobials has had an important effect on empirical selection of antimicrobials. Thus, we recommend that constant evaluation of the antimicrobial susceptibility pattern of locally prevalent etiological agents of CSOM against commonly used antimicrobials is necessary to formulate a protocol for empirical antibiotic therapy and second line treatment and also to prevent the emergence and spread of resistant pathogens. Further, we also recommend a basic preliminary identification of all CSOM ear discharge for *Pseudomonas* (the most common isolate in our region), as its resistance to Gentamicin, a first line topical antibiotic is much higher than other Gram-negative bacilli and Gram-positive cocci.

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