Original Research Article

Bacteriological Profile of CSOM and Antibiotic Susceptibility Pattern of Aerobic Isolates in a Tertiary Care Hospital of Central India

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A B S T R A C T

Chronic Suppurative Otitis Media (CSOM) is one of the most common diseases of the ear of all age groups. It is a destructive and persistent disease with irreversible outcome and a major health problem in developing nations with low socio-economic civilization because of malnutrition, overcrowding, poor hygiene and inadequate health care and laboratory facilities. Aerobic and anaerobic bacteria are the important causative agents of CSOM. Hence this study was conducted to identify the common bacterial isolates from patients diagnosed with CSOM at tertiary center in central India and to determine the antibiotic sensitivity pattern of aerobic bacterial isolates. A total of 157 patients were enrolled and processed for isolation of bacterial isolates using standard microbiological tests with sensitivity pattern. SPSS software 20 and MS Microsoft Excel 2007 was used for statistical analysis. Bacterial cultures were positive in 133 cases out of which 126(94.75%) were aerobes and 7(5.25%) were anaerobes. Antimicrobial susceptibility testing of the aerobic bacterial isolates was performed by disc diffusion method according to clinical laboratory standards institute guidelines. The aerobic bacterial isolates were mostly sensitive to commonly used antimicrobials like 3rd generation cephalosporins, Amoxycillin + Clavulanic acid, Amikacin and Ciprofloxacin. Evidence based judicial treatment and appropriate use of antimicrobials will ensure & reduce the resistance to antimicrobials and the cost of treatment.

Introduction

Chronic Suppurative Otitis Media (CSOM) is a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent otorrhoea through a tympanic perforation. Due to the perforated tympanic membrane, microorganisms can enter into the middle ear via the external ear canal. Infection of the middle ear mucosa subsequently results in ear discharge.[1] Incidence of this disease is higher in developing countries especially among low socio-economic society because of malnutrition, overcrowding, poor cleanliness, insufficient health care, and recurrent upper respiratory tract infection.[2] It is a huge public health problem, and India is one of the countries with highest CSOM prevalence (> 4 %,

WHO 2004). It is a common cause of hearing impairment and can occasionally lead to fatal intracranial infection. Over 50 percent of the cases are caused by bacteria.[3] Most common isolates found in CSOM are Aerobic bacteria (*Staphylococcus aureus*, *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli* etc.), anaerobic bacteria (*Bacteroids, Peptostreptococcus, Peptococcus.* etc.) but distribution of these isolates differ in various geographical areas.[4]

Topical preparations containing antibiotics and steroids, to reduce otorrhea and to provide local anti-inflammatory effect are the bases of medical management of chronic otitis media[5,6]. The knowledge of bacterial etiology of CSOM is very important for the clinicians for appropriate management of the cases, to prevent or minimize the occurrence of complications and prevent emergence of resistant strains [7]

The present study was conducted to isolate aerobic as well as the anaerobic bacteria involved in the CSOM and the antibiotic susceptibility pattern of aerobic bacteria. Study of susceptibility pattern of anaerobic bacteria was not included in this study.

Materials and Methods

This study was performed in the department of Microbiology, People's college of Medical Sciences and Research Centre Bhopal from NOV 2012-APR 2014. Ethical committee approval was obtained before starting the study.

After written informed consent, ear discharge from clinically diagnosed patients was collected and processed in Microbiology department. Three sterile swabs were used for collecting ear discharge. The 1st swab was used to make a smear on a clean glass slide for direct smear examination by Gram's stain and

Ziehl-Neelsen stain. 2nd and 3rd swab was processed for the isolation of aerobic and anaerobic bacteria. 2nd Swab was used to inoculate MacConkey's agar (MA) and Blood agar plates. All plates are incubated aerobically at 37^0C and evaluated at 24 hours, 48 hours and 72 hours and the plates are discarded if there is no growth. The specific identification of bacterial pathogens is done based on microscopic morphology, staining characteristics, cultural and biochemical properties using standard laboratory procedures.[8,9,10]

Under aseptic precaution samples for the anaerobic culture were collected in 3rd swab and inoculated in Robertson's cooked meat (RCM) broth. From RCM inoculation was done on two plates each of blood agar (Columbia agar with 5% sheep blood by Himedia) and two selective blood agar (Vancomycin / Kanamycin (V/K) blood agar, Neomycin/ Vancomycin blood agar) [9]. Primary sensitivity test was done by placing metronidazole disc (5 µg) at the junction of secondary and tertiary streaking area, opposite to primary well of inoculation.

One agar plate was incubate aerobically, and rest of the plates were incubated anaerobically by using anaerobic jar (3.5 litre from Himedia) with anaerogas Pack (3.5L from Himedia). After 24 hours one blood agar plate and one selective blood agar plate were checked, remaining plates were left for 48 hours. Plates were examined for typical colonies. Re-incubation of plates for 48 hours was done for slow growers. After incubation, zone of inhibition around the metronidazole disk was examined. An aero-tolerance test on Chocolate agar (CA) was set up to rule out facultative anaerobes.

Identification of anaerobes was done by using rapid ID 32 A (biomerieux India) and ATB system. As per manufacturer's instructions

one well isolated colony was sub-cultured on a Columbia agar with 5% sheep blood. Incubation was done for 24 hours at 37^0C in anaerobic conditions. Inoculum was prepared by mixing a growth obtained from blood agar with sterile distilled water with turbidity equivalent to 4McFarland measure with the DENSIMAT. With the help of pipette 55 µl of the suspension, dispensed into each cupule of the strip. Then strip was covered with lid and incubate at 37^0C for 4 hours in aerobic conditions. Strip was placed in the ATB system for identification of anaerobes.

Antimicrobial susceptibility of the aerobic isolates was performed on Mueller Hinton Agar plates by Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. [11]Data was compiles and statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) software 20 and MS Microsoft Excel 2007.

Results and Discussion

Among 157 studied cases 92(58.60%) cases were males and 65(41.40%) cases were females. Age of the subjects in the study group varies from 1 year to 73 years. Maximum patients of CSOM belong to age group 11-20 years which was 37 (23.57%) and least cases of CSOM which was 13 (8.28%) found in age group 41-50 years (Table 1).

Out of 157 samples, 133 were culture positive and 24 were culture negative. The results of bacteriological studies on the 133 cases showed that positive aerobic bacterial cultures were obtained in 126(94.75%) cases while positive anaerobic cultures were obtained in only 7(5.25%) cases (Table 2).

Out of 126 aerobes *Staphylococcus aureus* was most commonly isolated organism 62(49.20%) followed by *Staphylococcus*

aureus 33(26.20%). Among 7 anaerobic isolates *Fusobacterium spp* was most common 3(42.86%) followed by *bacteroides* 2(28.57%) and *Peptostreptococcus* 2(28.57%) (Table 3).

Table 4 and 5 shows the antibiotic sensitivity pattern of various aerobic bacteria isolated from CSOM cases. Out of 126 (100.00%) isolates 109 (86.50%) were sensitive to Cefotaxime, 108 (85.71%) were sensitive to Amoxyclav, 94 (74.60%) were sensitive to Amikacin, 60 (47.62%) were sensitive to Ciprofloxacin, 55 (43.65%) were sensitive to Gentamicin and 51 (40.47%) were sensitive to Cotrimoxazole. that Cefotaxime had highest sensitivity 53 (48.62%) for *Staphylococcus aureus* followed by Amoxyclav 52 (48.14%) and Amikacin 42 (44.68%). Antibiotic susceptibility pattern of anaerobes was not included in the study

For prescribing appropriate treatment of CSOM Identification of causative organisms is important as a wide range of Organisms are isolated in CSOM. Local damage to the middle ear is often seen in CSOM patients as the organisms may spread to adjacent structures near the ear or middle ear itself. Intracranial and extra-cranial complications may develop in untreated cases of CSOM. Early bacteriological diagnoses of all cases will ensure accurate and appropriate therapy. This study was conducted for the assessment of aerobic and anaerobic bacteriological profile of CSOM, with antimicrobial susceptibility testing of the aerobic isolates. The results are compared with the other studies and discussed as follows.

In our study out of total 133 positive case, males 78(58.65%) were more affected than females 55 (41.35%). This finding is in consonance with the study conducted by Ahmed *et al*., [12] who showed (57.29%) male and (42.70%) female cases out of 192 (100.00%) cases in their study.

Table.1 Age wise distribution of study population

Age	No. of patients	Percentage (%)
0-10 years	36	22.93
11-20 years	37	23.57
21-30 years	27	17.19
31-40 years	23	14.65
41-50 years	13	8.28
>50 years	21	13.38
Total	**157**	**100.00**

Table.2 Distribution of isolates among study population

Types of isolates	Frequency	Percentage (%)
Aerobes	126	80.25
Anaerobes	7	4.45
No organism	24	15.30
Total	**157**	**100**

Table.3 Distribution of various bacterial isolates in study population

	Organism	Frequency	Percentage (%)
	Staphylococcus aureus	62	49.20
	Staphylococcus aureus	33	26.20
	Klebsiella species	10	7.94
Aerobic Isolates	*Escherichia coli*	09	7.14
	Streptococcus Pyogenes	06	4.76
	Citrobacter freundii	04	3.17
	Acinetobacter species	02	1.59
	Total	**126**	**100.00**
	Bacteroids species	03	42.86
Anaerobic Isolates	*Fusobacterium species*	02	28.57
	Peptostreptococcus	02	28.57
	Total	**07**	**100.00**

Table.4 Antibiotic sensitivity pattern for Aerobic bacterial isolates

Antibiotic	Sensitive N(%)	Total N (%)
Amikacin	94(74.60)	126(100)
Ciprofloxacin	60(47.62)	126(100)
Amoxyclav	108(85.71)	126(100)
Gentamicin	55(43.65)	126(100)
Cefotaxime	109(86.50)	126(100)
Cotrimoxazole	51(40.47)	126(100)

Table.5 Sensitivity pattern of aerobic bacterial isolates in CSOM

Isolates	No. of isolates N (%)	Type of Antibiotics N (%)					
		AK	CIP	CTX	GEN	AMC	COT
Pseudomonas aeruginosa	62 (49.21)	42 (44.68)	31 (51.66)	53 (48.62)	22 (40.00)	52 (48.14)	19 (37.25)
Staphylococcus aureus	33 (26.19)	30 (31.91)	20 (33.33)	32 (29.35)	19 (34.55)	31 (28.70)	17 (33.33)
Klebsiella species	10 (7.93)	8 (8.51)	4 (6.67)	7 (6.42)	7 (12.72)	9 (8.33)	6 (11.76)
Escherichia coli	09 (7.14)	5 (5.31)	2 (3.33)	9 (8.26)	5 (9.09)	8 (7.40)	5 (9.80)
Streptococcus Pyogenes	06 (4.76)	5 (5.31)	2 (3.33)	3 (2.75)	0 (0.00)	4 (3.70)	0 (0.00)
Citrobacter freundii	04 (3.17)	4 (4.25)	1 (1.67)	3 (2.75)	2 (3.64)	2 (1.85)	4 (7.84)
Acinetobacter species	02 (1.59)	0 (0.00)	0 (0.00)	2 (1.83)	0 (0.00)	2 (1.85)	0 (0.00)
Total	126 (100.00)	94 (74.60)	60 (47.62)	109 (86.50)	55 (43.65)	108 (85.71)	51 (40.47)

The male predominance may be because of males are more actively involved in outdoor activities, hence more likely to be exposed to contaminated environment.[13]

In the present study the age ranged from less than 1 year to more than 50 years. In this study the maximum number of cases were found in the age group of 11-20 years 37 (23.57%) followed by 0-10 years 36 (22.93%). These findings were correlated with a study conducted by Akinpelu and Amusa in their study of 160 (100.00%) patients the maximum number of patients was in the age group of 0-10 years 69 (43.00%).[14]

The most common organism isolated in the present study was *Staphylococcus aureus* 62 (42.17%) followed by *Staphylococcus aureus* 44(29.93%). These findings were in consistency with a study conducted by workers like Hiremath *et al.,* (2001)[15] and Loy (2002).[16] The most common anaerobe

isolated in present study was Bacteroids spp. 3(42.86%) followed by *Fusobacterium* spp. 2(28.57%) and *Peptostreptococcus* 2(28.57%). Geeta found 45.61% *Peptostreptococcus* followed by bacteroids 17.54% and *Fusobacterium* 7% in their study.[17] Prakash *et al.,* isolated 8 (11.59%) Bacteroids species out of 69 (100.00%) anaerobes in their study[18]. Kusuma Bai *et al.,* found 13 (28.26%) *Peptostreptococcus* among 46(100.00%) anaerobes in their study.[19]

Antibiotic sensitivity was carried out for 126 aerobic isolates by Kirby-Bauer disc diffusion method by using antibiotic discs. In the present study 109 (86.50%) of organisms were sensitive to Cefotaxime, followed by Amoxyclav 108(85.71%), Amikacin 94 (74.60%), Ciprofloxacin 60 (47.62%), Gentamicin 55 (43.65%), Cotrimaxazole 51(40.47%). The most effective drugs in the present study are Cefotaxime, Amoxyclav and

Amikacin. Similar sensitivity pattern was reported by Shyamala and Reddy[20]. However Singh *et al.,* have found Ciprofloxacin as the most effective drug.[21]

This study was conducted with the aim of identification of causative agents associated with ear infections especially in an environment where antibiotics are commonly abused. Based on the findings from this study, it is therefore recommended that treatment of ear infection is better done when the causative agents as well as the drug sensitivity patterns are known and properly administered. Also the role of anaerobes should be studied. This will enhance the chances of better treatment and reduce the burden of the infection on the patients and reduce the cost of treatment.

It can be concluded that a variety of bacteria are responsible for CSOM with predominance of *Staphylococcus aureus* followed by *Staphylococcus aureus, Klebsiella* species, *Escherichia coli* and *Streptococcus pyogenes.* The antibiotic susceptibility testing showed Cefotaxime as the most effective drug followed by Amoxyclav, Amikacin, Ciprofloxacin, Gentamicin and cotrimoxazole.

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