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Original Research Article

Mycobacterial profile and antibiotic susceptibility pattern in chronic suppurative otitis media: a cross- sectional study

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a disease of mucoperiosteal lining of the middle ear cleft. The poor compliance of patients to antibiotic treatment, Incomplete treatment, misuse/improper choice of antibiotics have resulted in changes in susceptibility to antibiotics and also development of resistant strains by microbes to commonly used antibiotics. This study aimed to identify the mycobacteriological profile and determine antibiotic susceptibility pattern in CSOM patients.
Methods: This cross sectional study was conducted on 120 clinically diagnosed cases of CSOM attending otolaryngology department. Ear discharges obtained were processed for microbial culture (aerobic, anaerobic and fungi). Antimicrobial susceptibility testing was done by Kirby–Bauer disc diffusion method.
Results: Of total 120 cases, pathogens were isolated from 114 cases. The commonest aerobic organism isolated was *Pseudomonas aeruginosa* (34.5%) followed by *Staphylococcus aureus* (29.4%). *Staphylococcus aureus* showed maximum sensitivity to erythromycin (70%), followed by cotrimoxazole (62.5%) and ampicillin (55%). Maximum resistance was observed for ciprofloxacin (77.5%), followed by amoxiclave (55%). *Pseudomonas aeruginosa* showed maximum sensitivity to piperacillin (89.36%) followed by gentamicin (70.2%), amikacin (70.2%), moderate sensitivity to ceftazidime (51.06%); however resistance to carbpenicillin (60%). Ciprofloxacin which is the most prescribed topical antibiotic showing an increase in resistance to causative organisms of CSOM.
Conclusions: Hence, it becomes essential to study each case of CSOM bacteriologically to formulate local antibiotic policy for appropriate use of antibiotics. This will certainly help in achieving a safe ear and to control the organisms developing resistance to prevalent antibiotics.

Keywords: Antibiotic susceptibility pattern, Mycobacterial profile, Chronic suppurative otitis media, Resistance, Sensitive

INTRODUCTION

Ear is an important sensory organ. It is worthy to note that ear infections are a very common problem worldwide. Otitis media is an inflammation of the middle ear cleft caused by bacteria, fungi and viruses resulting in inflammation of the mucosal lining. It can be acute, subacute and chronic. Chronic suppurative otitis media (CSOM) is a commonly encountered infection of the middle ear.[1,2] CSOM is a chronic inflammation or persistent infection of the middle ear cleft—the eustachian tube, middle ear, and mastoid air cells.[3]

Although the ear infection affects all age-groups, owing to shorter Eustachian tube, more horizontal position and with a more flaccid cartilage and low immunity have made this bacterial infection more severe in children. CSOM, whether it is attico-antral or tubo-tympanic disease, is associated with mixed bacterial flora.[4]

The most common aerobic causative isolates are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Others include *Proteus* species, *E.coli*, *Klebsiella* species, *Streptococcus* species, *Haemophilus influenza*. *Aspergillus* species and *Candida* species are most fungal isolates which are responsible for CSOM.[5] The most common anaerobic causative isolates are *Bacteroides* species, *Peptostreptococcus* species.[6]

The treatment of CSOM depends on the prevalence and antibiogram of these organisms. This has been reported to vary with time and geographical area as well as continent to continent, probably due to indiscriminate use of the antibiotics.[7]

The altering flora of CSOM and emergence of resistant strains of microorganisms to the commonly prescribed antibiotics stimulated us to conduct the study. Hence, the current study was undertaken to identify the mycobacteriological profile in CSOM patients and to determine the antibiotic susceptibility pattern.

METHODS

Study design

This cross-sectional study was conducted from February 2020 to April 2021 at ear, nose and throat (ENT) department in our tertiary care hospital. A total of 120 clinically diagnosed CSOM cases attending ENT OPD and inpatients were included in the study. Ethical clearance was obtained from institutional ethical committee prior to the study. A written informed consent was also obtained from the patients before their participation in the study. The sample size was calculated using the formula given.

$$n = 4\,pq/d^2$$

Sample collection and processing

Demographic data such as patient's name, age, sex, and clinical features of the patients were recorded in standard proforma. Ear discharge was collected using a cotton swab under aseptic precautions after mopping out excess discharge from external auditory canal by an otolaryngologist. Two out of four samples were transported in thioglycollate medium for anaerobic culture and one swab transported in sterile container for aerobic culture. One swab was immediately inoculated over Sabouraud's dextrose agar media and incubated at 37 degree Celsius. Antimicrobial susceptibility profile of the bacterial isolates to the commonly used antibiotics was determined by Kirby–Bauer disc diffusion method, as per clinical laboratory standard institute (CLSI) guidelines.

Statistical analysis

Data were pooled and analyzed using statistical package for the social sciences (SPSS) v20 software.

RESULTS

Age of the patients ranged from 3 to 85 years with male preponderence (56.7%). Maximum incidence was observed during second decade (28.3%) of life and the incidence decreased as the age advanced. The demographic including clinical features of the study sample are shown in Table 1.

Table 1: Demography and clinical profile of study population.

Variable	N (%)
Sex	
Male	68 (56.7)
Female	52 (43.3)
Age (in years)	
1-10	18 (15)
11-20	34 (28.3)
21-30	21 (17.5)
31-40	14 (11.6)
41-50	17 (14.1)
51-60	11 (9.1)
>60	5 (4.1)
Type of CSOM	
Tubotympanic type	102 (85)
Atticoantral type	18 (15)
Culture results	
Culture positive	114 (95)
Culture negative	06 (5)
Total	120 (100)
Aerobic organisms	
Gram positive	43 (39.1)
Gram negative	67 (60.9)
Polymicrobial growth	
Aerobe and aerobe	5
Aerobe and anaerobe	3
Aerobe and fungi	14

Out of 120 cases, 114 were culture positive and the total number of microbial isolates was 136. The bacteriological profile in this study showed only aerobic growth in 83 cases and only anaerobic growth in 9 cases. Polymicrobial growth of organisms was as follows: aerobe and aerobe in 5, aerobe and anaerobe in 3 and aerobe and fungal growth in 14 cases. The most common aerobic organism isolated was *Pseudomonas aeruginosa*, the most common anaerobic organism isolated was *Prevotella intermedia* and the most common fungal growth seen was *Aspergillus niger*. Also, out of 110 aerobic isolates identified, 60.9% cases were of gram-negative organisms and 39.1% cases were gram-positive organisms. Microbial isolates are shown in Table 2.

The sensitivity and resistance pattern of isolated gram-positive and gram-negative organisms to different antibiotics is reported in Table 3 and 4 respectively. Among the gram-positive organisms, *Staphylococcus*

aureus showed maximum sensitivity to erythromycin (70%), followed by cotrimoxazole (62.5%), and ampicillin (55%). Maximum resistance was observed for ciprofloxacin (77.5%), followed by amoxiclave (55%). Among gram-negative organisms, *Pseudomonas aeruginosa* showed maximum sensitivity to piperacillin (89.36%), followed by gentamicin (70.2%), amikacin (70.2%), moderate sensitivity to ceftazidime (51.06%), and resistant to carbpenicillin (60%).

Table 2: Microbial isolates – aerobes, anaerobes and fungal organisms.

S. no.	Microbe	N (%)
Aerobes		
1	*Pseudomonas aeruginosa*	47 (34.5)
2	*Staphylococcus aureus*	40 (29.4)
3	*Methicillin resistant Staphylococcus aureus*	06 (4.4)
4	*Citrobacter freundii*	05 (3.6)
5	*Proteus mirabilis*	04 (2.9)
6	*Escherichia coli*	03 (2.2)
7	*Klebsiella pneumoniae*	02 (1.5)
8	*Streptococcus pneumoniae*	01 (0.7)
9	*Enterococcus*	01 (0.7)
10	*Coagulase negative staphylococcus*	01 (0.7)
Anaerobes		
1	*Prevotella intermedia*	06 (4.4)
2	*Bacteroides fragilis*	04 (2.9)
3	*Porphyromonas*	02 (1.5)
Fungal organisms		
1	*Aspergillus niger*	06 (4.4)
2	*Aspergillus flavus*	04 (2.9)
3	*Candida albicans*	02 (1.5)
4	*Aspergillus fumigatus*	01 (0.7)
5	*Alternaria*	01 (0.7)

Table 3: Antibiotic susceptibility of gram positive organisms.

Organism	Antibiotic susceptibility										
	Amp			**Cot**		**Cip**		**Ery**		**Amo**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Staphylococcus aureus	S	22	55	25	62.5	9	22.5	28	70	18	45
	R	18	45	15	37.5	31	77.5	12	30	22	55
MRSA	S	3	50	4	66.67	1	16.7	5	83.3	3	50
	R	3	50	2	33.34	5	83.3	1	16.7	3	50
CONS	S	1	100	0	0	0	0	0	0	1	100
	R	0	0	1	100	1	100	1	100	0	0
Streptococcus pneumoniae	**Gen**			**Ery**		**Amo**		**Lev**		**Cef**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
	S	1	100	0	0	0	0	0	0	1	100
	R	0	0	1	100	1	100	1	100	0	0

S-Sensitive, R-resistance, Amp-ampicillin, Cot-cotrimoxazole, Cip-ciprofloxacin, Ery-erythromycin, Amo-coamoxiclav, Gen-gentamicin, Lev-levofloxacin, Cef-cefotaxime; MRSA-methicillin resistant *Staphylococcus aureus*; CONS-coagulase negative *Staphylococcus*

Table 4: Antibiotic susceptibility of gram negative organisms.

Organism	Antibiotic susceptibility										
	Gen			**Ami**		**Ceft**		**Pip**		**Car**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Pseudomonas aeruginosa	S	33	70.2	33	70.2	24	51.06	42	89.36	19	40.4
	R	14	29.7	14	29.7	23	48.93	5	10.63	28	59.57
	S	2	50	3	75	1	25	4	100	3	75

Continued.

Organism	Antibiotic susceptibility										
	Gen			Ami		Ceft		Pip		Car	
		No.	%	No.	%	No.	%	No.	%	No.	%
Proteus mirabilis	R	2	50	1	25	3	75	0	0	1	25
	Amp			Cot		Gen		Ami		Cefta	
		No.	%	No.	%	No.	%	No.	%	No.	%
Citrobacter freundii	S	1	20	5	100	5	100	4	80	5	100
	R	4	80	0	0	0	0	1	20	0	0
Escherichia coli	S	1	33.4	1	33.4	1	33.4	3	100	0	0
	R	2	66.6	2	66.6	2	66.6	0	0	3	100

S-sensitive, R-resistant, Gen-gentamicin, Ami-amikacin, Ceft-ceftazidime, Pip-piperacillin, Carb-carbpenicillin, Amp-ampicillin, Cefta-ceftriaxone

DISCUSSION

CSOM is a persistent disease with great risk of irreversible complications. Early mycobacteriological diagnosis of all the cases will assume appropriate effective therapy.[8]

In the present study maximum number of patients were in the age group of 11-20 years, i.e. 34 (28.3%) followed by 21-30 years (17.5%). These findings are in correlation with Kumar and co-authors, studies conducted by Poorey and co-authors, and Saraswati and co-authors reported maximum number of patients were below 20 years of age.[5,9,10] Vishwanath and co-authors, and Loy and co-authors have reported maximum number of patients in third decade.[11,12]

Higher incidence of otitis media in paediatric age group may be due to: abundance of lymphoid tissue in pharynx may obstruct the eustachian tube, increased risk of respiratory infection, short and straight eustachian tube in infants and young children allows easy access of microbes to middle ear cleft.

The present study shows male preponderance with CSOM 68 (56.7%) than the females 52 (43.3%). This finding is correlated with the studies done by Deb and co-authors.[13]

Even today CSOM is a disease seen among the lower (48.3%) and middle (33.3%) class of the individuals in the present study. This finding is correlated with studies of Gulati and co-authors (1997). It is presumed that multiple factors such as poor sanitation, unhygienic living conditions, overcrowding, malnutrition, illiteracy and lack of health consciousness in low socio-economic status may contribute to the increased development of otitis media.[14]

In the present study mucosal (tubotympanic) type of CSOM seen in 102 (85%) cases, whereas squamosal (atticoantral) type of CSOM was seen in 18 (15%) cases. This finding is correlated with study done by Vishwanath and co-authors.[11]

In the present study 114 (95%) specimens were positive and 06 (5%) were negative for the culture. The culture results correlates with Vishwanath and co-authors with 88.3% positive culture reports.[11]

The total number of organisms isolated is 136 microbial isolates. The mycobacteriological profile in this study shows aerobic growth in 110 (80.8%) cases, anaerobic growth in 12 (8.8%) cases and fungal growth in 14 (10.3%) isolates. This correlates with Vishwanath and co-authors shows 74.8% of aerobic growth, 15.6% of anaerobic growth 9.6% of fungal growth.[11] In the present study, gram negative organisms (60.9%) outnumber the gram positive organisms (39.1%).

In the present study, 10 aerobic bacterial organisms were grown in 114 positive culture growths. The most common organism isolated was *Pseudomonas aeruginosa* 47 (34.5%) followed by *Staphylococcus aureus* 40 (29.4%). *Methicillin resistant staphylococcus aureus* was isolated in 06 (5.17%) cases, *Citrobacter freundii* in 05 (3.6%) cases, *Proteus mirabilis* in 04 (2.8%) cases, *Escherichia coli* in 03 (2.2%) cases, *Klebsiella pneumoniae* in 02 (1.5%) cases and each (0.7%) case of *Enterococci*, *Streptococcus pneumoniae* and *Coagulase negative staphylococcus* were isolated.

The occurrence of *Pseudomonas aeruginosa* as the predominant offending organism could be linked to several factors. Pollock stated that *Pseudomonas aeruginosa* survives competition with other pathogens which could be due to minimally required nutrition, its relative resistance to antibiotics and its antibacterial byproducts i.e. pyocyanin and bacteriocin. Apart from the above said reasons, it uses the pili to attach to the necrotic or diseased epithelium of middle ear mucosa. Once attached, the organism produces enzymes like proteases, and lipopolysaccharides to elude from normal defense mechanism of the body required for fighting infections. In addition, the organism acts as an opportunistic pathogen, flourishes in external ear and causes suppurative disease.[15]

Pseudomonas aeruginosa was the predominant organism isolated from patients with CSOM in studies reported by Vishwanath and co-authors (32.2%), Kumar and co-authors (46%) which correlates with our study.[11,16]

In the present study the second most common organism isolated was *Staphylococcus aureus* (29.4%) which correlates with studies conducted by Kumar and co-authors 2013 (33%), some studies showed *Staphylococcus aureus* as the predominant isolate conducted by Parween and co-authors (40.2%) which differ from our study.[16,17]

The high frequency of *Staphylococcus aureus* in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in ear and upper respiratory tract.

In the present study, the anaerobic organisms (8.8%) isolated were *Prevotella intermedia, Bacteroides fragilis, Porphyromonas*. This correlates with study conducted by Loy and co-authors isolated 6.6% of anaerobes.[12]

Antibiotic susceptibility pattern for *Pseudomonas aeruginosa* was determined for gentamicin, amikacin, ceftazidime, piperacillin, and carbpenicillin. *Pseudomonas aeruginosa* showed maximum sensitivity to piperacillin (89.36%), followed by gentamicin (70.2%), amikacin (70.2%), moderate sensitivity to ceftazidime (51.06%), and resistance to carbpenicillin (59.5%). According to study by Vishwanath and co-authors, *Pseudomonas aeruginosa* showed maximum sensitivity to piperacillin (97.3%) followed by gentamicin (73%), amikacin (78.4%), ceftazidime (91.2%), and resistance to netilmycin (2.7%) which correlates with our study.[11]

According to Sharma and co-authors, *Pseudomonas aeruginosa* showed sensitive to piperacillin (25%), gentamicin (30%) which differ from our study.[18]

Gentamicin and amikacin were found to be the most effective aminoglycoside; however, the risk of ototoxicity caused by aminoglycoside preparations remains a subject of discussion and prevents their routine use.

In the present study, antibiotic susceptibility pattern for *Staphylococcus aureus* was determined for ampicillin, cotrimoxazole, ciprofloxacin, erythromycin, amoxiclave. *Staphylococcus aureus* showed maximum sensitivity to erythromycin (70%), followed by cotrimoxazole (62.5%), moderate sensitivity to ampicillin (55%). Maximum resistance was seen for ciprofloxacin (77.5%), followed by amoxiclave (55%).

In our study, methicillin resistant *Staphylococcus aureus*, coagulase negative *Staphylococcus* and *Streptococcus pneumoniae* also showed resistance to antibiotic ciprofloxacin.

According to study by Vishwanath and co-authors, *Staphylococcus aureus* showed maximum sensitivity to erythromycin (75%), cotrimoxazole (95%), moderate sensitivity to ampicillin (55%). ciprofloxacin (45%) which correlates with our study.[11] Deb and co-authors, showed 50% resistance to ciprofloxacin.[13]

Ciprofloxacin, an important drug in the management of otitis media as it is less ototoxic, affordable and widely available as topical preparations makes it the most prescribed topical agent. Various studies have shown sensitivite to the organisms previously but recent studies are reporting an increase in resistance of antibiotic ciprofloxacin to commonly causing organisms of CSOM.

Methicillin resistant *Staphylococcus aureus*, a strain of staphylococcus aureus showed maximum sensitivity to erythromycin (83.3%), followed by cotrimoxazole (66.67%) and ampicillin (50%), and amoxiclave (50%). Maximum resistance was seen for ciprofloxacin (83.3%).

In the present study, *Citrobacter freundii* showed 100% sensitive to cotrimoxazole, gentamicin, ceftazidime, followed by amikacin (80%), resistant to ampicillin (80%). According to Sharma and co-authors, *Citrobacter freundii* showed 100% sensitive to ciprofloxacin, gentamicin and amikacin which correlate with our study.[18]

Proteus mirabilis showed maximum sensitivity to piperacillin (100%) followed by amikacin (75%), carbpenicillin (75%), gentamicin (50%), resistant to ceftazidime (75%). According to Sharma and co-authors, *Proteus mirabilis* showed sensitive to gentamicin (100%), amikacin (83.3%), and piperacillin (66.6%) which mildly differ from our study.[19]

The *Prevotella intermedia* showed maximum sensitivity to colistin (100%), followed by kanamycin (80%), and showed resistance to vancomycin (80%). *Bacteroides fragilis* showed sensitivity to colistin (50%), kanamycin (50%), and resistance to vancomycin (75%). *Porphyromonas* showed sensitivity to kanamycin (100%), and resistance (100%) to vancomycin, and colistin. Even though anaerobes are thought to play a pathogenic role in CSOM, the large variability in their isolation rates among different studies may be due to differences in prior use of antibiotics and differences in the timing of sampling during the course of the disease.

In our study, five different fungal organisms were isolated as polymicobial growth, those are *Aspergillus niger* 6 cases, *Aspergillus flavus* 4 cases, *Candida albicans* 2 cases, *Aspergillus fumigatus* 2 cases, *Alternaria* species 1 case. This could be correlated to study conducted by Vishwanath and co-authors.[11] Fungi isolated as polymicrobial growth could be because, they can become pathogenic in already inflamed discharging ear.

CONCLUSION

In the present era, where we encounter drug resistance to commonly prescribed antibiotics, the regular periodic monitoring of mycobacteriological profile and antibiotic susceptibility pattern has become essential. In our study, Ciprofloxacin, the most prescribed topical agent, showed an increased resistance to common organisms of CSOM. This emergence of antibiotic resistance is becoming more

common due to human negligence which is seen as stopping antibiotics before completion of course due to symptoms subside and this allows partially resistant microbes to flourish. Such practice should be discouraged strongly and patients should be educated to avoid the same. Thus, studies should be undertaken to determine bacteriological profile to formulate local antibiotic policy for appropriate use of antibiotics. This will enhance better treatment to achieve safe ear, reduce the incidence of drug resistance and reduce the burden of infection on the patients and in the long term, it may also reduce the cost of treatment.

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