Original Research Article

Microbiological profile and antimicrobial susceptibility pattern of chronic suppurative otitis media in a tertiary care centre

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Abstract

Introduction: Chronic suppurative otitis media (CSOM) is a common clinical condition diagnosed in ENT out patients department (OPD). There appears paucity of data on the incidence and pattern of bacterial and fungal flora in patients presenting with ear discharge. **Materials and Methods:** A total of 180 consecutive patients with CSOM attending the ENT OPD of a tertiary care hospital in North India were included in the study. 3 swabs were used to collect the ear discharge and were processed for isolation of bacterial and fungal organisms. The bacterial isolates thus obtained were tested for antibiotic susceptibility testing. Isolates of *Staphylococcus aureus* were also tested for methicillin resistance. Fungal growth was identified and antifungal susceptibility testing of yeast isolates was done using standard recommended procedures. **Results:** Microbiological profile of total 180 cases revealed, bacteria alone in 68.9% cases, bacteria along with fungi in 15.6% cases and fungi alone in 8.3% cases. Amongst these, *Pseudomonas aeruginosa* was the most common bacterium isolated (34.9%) followed by *Staphylococcus aureus* (24.4%). Out of the fungal isolates obtained, *Aspergillus niger*was the commonest isolate (29.4%) followed by *Candida albicans* (19.5%). MRSA strains were 37%. Among the *Pseudomonas aeruginosa* isolates, maximum resistance was found to Ciprofloxacin (74.2%) followed by GentamicinandCeftazidime. All*Candida species* were sensitive to Fluconazole, Itraconazole and Voriconazole. **Conclusion:** Considering the emergence of bacterial resistance and the availability of wide spectrum of newer antimicrobial agents, it is important to know the pattern of infections and the antimicrobial sensitivity of the isolates.

Keywords: Antibiotic resistance, Bacterial isolates, Ear discharge, Mycological profile

Introduction

Discharge from the ear is one of the commonest symptoms of infections of the ear. Infection of the ear is categorized into otitis externa (infection of external ear) and otitis media (infection of middle ear) [1]. Though the most common causes of ear discharge remains the otitis media, other conditions such as otitis externa and otomyocosis may also contribute.

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As external and middle ear are exposed to outer environment and nasopharynx respectively, these sites are likely to be infected when the natural milieu is disturbed. Infection of external ear is called "otitis externa" and middle ear is called "otitis media". Otitis media comprises of the inflammation of the middle ear cleft. It can be acute, subacute or chronic [2].Chronic Suppurative Otitis Media (CSOM) is one of the most common diseases of all age groups especially of childhood. It is prevalent in developing countries and is a disease of poverty [3].

Chronic suppurative otitis media is a stage of ear disease in which there is a chronic infection of the middle ear cleft i.e. eustachian tube, middle ear and mastoid, and in which a non intact tympanic membrane (e.g. perforation or tympanostomy tube) and discharge (otorrhoea) are present. It causes conductive and sensorineural hearing loss and adverse effects on child development [4]. CSOM and its complications are the bugbear of otologists, pediatricians and general practitioners. It is a disease of multiple etiology. Its importance lies in its refractoriness to treatment and chronicity leading to complications [5].

Incidence of CSOM is higher in developing countries like India due to poor socioeconomic standards, poor nutrition and lack of health education. Cases usually present with ear discharge, hearing loss, perforation in tympanic membrane in bacterial or itching, blocked ear in fungal associated CSOM. Over 50% of otitis media are caused by bacteria [6].

In CSOM the most frequently isolated bacteria are *Pseudomonas aeruginosa, Staphylococcus aureus*, *Proteus spp* and *Klebsiellaspp* [3]. Occasionally otitis media may be caused by fungi, viruses, *Mycoplasma pneumoniae* and *Chlamydia trachomatis* [7]. Fungal infections can commonly co-exist with bacterial infections in cases of ear discharge. Predominant fungi in CSOM include *Aspergillus species and Candida species* [8].

There is often a history of recent water exposure and mechanical trauma like scratching or cotton applicators [9]. Mycotic infections of ear are many times overlooked because of accompanying bacterial infections and are often the reason of incorrect treatment [10].

There is a dearth of knowledge of CSOM and its bacteriological and mycological etiology. So the present study was aimed to isolate, characterize and identify the bacteriological and mycological etiological agents of CSOM in this tertiary care centre.

Considering the emergence of bacterial resistance and the availability of wide spectrum of newer antimicrobial agents, it appears logical to study the pattern of infection and antimicrobial susceptibility of isolates from the cases, so that a database may be established that can serve as a basis of empirical treatment in patients with this very common infection.

Materials and Methods

The prospective study was carried out in the department of Microbiology in conjunction with department of ENT, Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar.A total of 180 consecutive patients with CSOM attending the ENT outdoor patients department were included in the study.

After taking informed consent from the patients, age, sex, nature of discharge, duration of ear discharge and any antibiotic treatment taken were noted in the structured proforma. Three ear swabs were collected from each patient.

Inclusion criteria: Any patient presenting with CSOM.

Exclusion criteria: Patient on antibiotics or antifungal drugs for more than seven days before presenting to the ENT outpatients department (OPD).

Method: Discharge samples from the ear were collected with three sterile swabs taking care not to touch the external acoustic canal. These swabs were transported to the department of Microbiology and were processed without any delay.

Laboratory processing of samples were done as follows

a) Direct microscopic examination (First swab): From the first swab Gram stained smear was made and examined under oil immersion lens to see the presence of pus cells, bacteria and fungal elements.

A KOH mount was also prepared and examined under 40X magnification for fungal elements.

(b) Culture for bacterial isolation (Second swab): The second swab was inoculated onto the plates of blood agar and Mac Conkey agar and also into a tube of BHI broth. The inoculated medium were incubated under aerobic conditions at 37°C for 24 hours. If no growth was observed on blood agar and Mac Conkey medium and BHI broth showed turbidity, subcultures were made onto the blood agar and MacConkey medium.

(c) Culture for fungal isolation (Third swab): The third swab was plated on two sets of Sabouraud's Dextrose Agar medium (SDA).

(1) One set containing SDA with Chloramphenicol (16µg/ml).

(2) The other set with Chloramphenicol (16µg/ml) plus Cycloheximide (0.5µg/ml).

Media used were prepared as per the standard procedures. One set of inoculated slants was incubated a22^{0}Cand the other at 37^0 C and examined every other day for fungal growth up to 4-6 weeks before discarding as negative.

Various aerobic bacterial isolates obtained were identified by their colony morphology, motility by hanging drop technique, gram staining and a battery of biochemical tests.

Antimicrobial susceptibility testing- All the aerobic bacterial isolates obtained were subjected to antimicrobial susceptibility testing for a wide range of antimicrobial agents, by Kirby Bauer Disc diffusion method against following antimicrobial agents.

Screening for Methicillin resistance in *Staphylococcus aureus*- All the strains of *Staphylococcus aureus* isolated were also tested for methicillin resistance by Cefoxitin disc method as recommended by the CLSI[11].

Identification of fungal isolates

(A) Fungal isolates

Fungal growth was identified by:
1. Colony morphology
2. Gram's stain
3. Lactophenol cotton blue preparation
4. Slide culture as per standard recommended procedures [12, 13, 14].

(B) Yeast Isolates: Identification and speciation of yeast isolates were done on the basis of the following tests:

1. Production of germ tube
2. Morphology on Corn Meal agar
3. Carbohydrate fermentation test
4. Carbohydrate assimilation test
5.CHROM agar medium as per standard recommended procedures [12,13,14].

Antifungal susceptibility testing was done using Disk Diffusion Susceptibility Testing of Yeast (M44-A, NCCLS, USA). Antifungal discs used were–Fluconazole (25mcg), Itraconazole (10mcg), Voriconazole (1 mcg). To control the precision and accuracy of the results two quality control strains were used.

1. Candida parapsilosisATCC 22019
2. Candida albicansATCC 90028

Recommended quality control zone diameter (mm) ranges

Antifungal agent	Disk content	C.albicans ATCC 90028	C.parapsilosis ATCC 22019
Fluconazole[15]	25µg	28-39mm	22-33mm
Itraconazole[16]	10µg	-	28-35mm
Voriconazole[17]	1µg	31-42mm	28-37mm

Interpretative guidelines for Fluconazole, Itraconazole and Voriconazole:

Antifungal drugs	Susceptible(S)	Susceptible-dose dependent(S-DD)	Resistant(R)
Fluconazole25µg	≥19mm	15-18mm	≤14mm
Itraconazole10µg	≥23mm	14-22mm	≤13mm
Voriconazole 1µg	≥17mm	14-16mm	≤13mm

Data so obtained was statistically analyzed.

Results

The present study consisted of 180 CSOM cases. All the cases under study were investigated and observed as described under material and methods. Maximum number of patients (36.7%) belonged to the age group of 11-20 years and minimum number of patients (0.6%) belonged to the age group of 71-80 years (Table 1).

Table-1: Age Distribution (n=180)

Age	Number	Percentage
0-10	22	12.2
11-20	66	36.7
21-30	31	17.2
31-40	25	13.9
41-50	13	7.2
51-60	14	7.8
61-70	8	4.4
71-80	1	0.6
Total	**180**	**100**

In this study, M: F ratio was 1.53: 1 with males accounting for 60.6% of the patients. Most of the patients (57.8%) in this study belonged to the rural group, the study being conducted in a tertiary care hospital in rural area. The common complaints in the patients presenting with ear discharge were loss of hearing (53.3%) and pain in ear (17.2%). Other complaints were itching, fever, tinnitus etc. Out of 180 cases, two-thirds were of the safe variety. Bacteria alone were isolated in 68.9% cases, bacteria along with fungi in 15.6% cases and fungi alone in 8.3% cases. Sterile culture was obtained in 7.2% of cases(Table 2).

Table-2: Distribution of Culture Isolates (n=180)

Types of isolates	No. of cases	Percentage
Bacteria alone	124	68.9
Bacteria + fungi	28	15.6
Fungi alone	15	8.3
Sterile	13	7.2
Total	**180**	**100**

Table-3: Bacterial Isolates (n=189)

Bacteria	Number	Percentage
Gram Negative Bacteria	**107**	**56.61**
Pseudomonas aeruginosa	66	34.9
Proteus mirabilis	12	6.3
Escherichia coli	09	4.8
Klebsiella pneumoniae sub spp. pneumoniae	06	3.2
Providencia stuartii	05	2.6
Enterobacter aerogenes	04	2.1
Acinetobacter baumanii	03	1.6
Alcaligenes faecalis	02	1.1
Gram Positive Bacteria	**82**	**43.33**
Staphylococcus aureus	46	24.4
Streptococcus pyogenes	08	4.2
Streptococcus pneumoniae	04	2.1
Staphylococcus auricularis	04	2.1
Staphylococcus epidermidis	04	2.1
Enterococcus faecalis	02	1.1
Diphtheroids	14	7.4
Total	**189**	**100**

There were 189 bacterial isolates. Amongst these, *Pseudomonas aeruginosa* was the most common bacterium isolated (34.9%) followed by *Staphylococcus aureus* (24.4%) and *Proteus mirabilis* (6.3%). Nine isolates of *Escherichia coli,* followedbyeight isolates of *Streptococcus pyogenes,*six isolates of*Klebsiella pneumoniae sub spp. pneumoniae,* five isolates of *Providencia stuartii*and four isolates each of *Enterobacter aerogenes, Streptococcus pneumoniae, Staphylococcus auricularis, Staphylococcus epidermidis* were also grown (Table 3).

Out of the 41 fungal isolates obtained, *Aspergillus niger* was the commonest isolate (29.4%) followed by *Candida albicans* (19.5%) and *Aspergillus fumigatus* (12.2%) (Table 4).

Table-4: Fungal Isolates (n=41)

Fungi	Number	Percentage
Moulds (n=26)		
Aspergillus niger	12	29.4
Aspergillus fumigatus	05	12.2
Aspergillus flavus	04	9.7
Alternaria species	02	4.9
Fusarium species	02	4.9
Penicillium species	01	2.4
Yeast (n=15)		
Candida albicans	08	19.5
Candida parapsilosis	04	9.7
Candida tropicalis	01	2.4
Rhodotorula species	02	4.9
Total	**41**	**100**

On antimicrobial susceptibility testing of bacterial isolates by disc diffusion method, maximum resistance was seen to Pencillin G and Ampicillin (100%) followed by Flouroquinolones i.e. Ciprofloxacin (67.4%) & Ofloxacin (47.8%),

whereas 32.6% resistance was seen to Erythromycin and Clindamycin, followed by Cephalexin (30%) and Gentamicin (23.9). No resistance was found to Amikacin, Netilmicin, Chloramphenicol, Fusidic acid, Vancomycin and Telcoplanin. All isolates of Coagulase negative Staphylococcal species showed maximum resistance to Pencillin G and Ampicillin (100%) followed by Ciprofloxacin (75%) and 37.5 % resistance to each of Ofloxacin and Gentamicin. 12.5% resistance was seen to both Erythromycin and Clindamycin.

On statistical analysis, the difference in the resistance pattern shown by CONS and *Staphlyococcus aureus* against Ciprofloxacin, Gentamicin and Erythromycin is not statistically significant (p value > 0.05) (Table 5).

Table-5: Resistance Pattern in Staphylococcal Isolates.

Name of antibiotics	*Staphylococcus aureus* (n=46)		Coagulase negative Staphylococcal species (CONS) (n=8)		p value
	No.	% Resistance	No.	% Resistance	
Penicillin G	46	100	8	100	NA
Ampicillin	46	100	8	100	NA
Ciprofloxacin	31	67.4	6	75	0.669
Ofloxacin	22	47.8	3	37.5	0.589
Cephalexin	14	30	0	0	0.110
Amikacin	0	0	0	0	NA
Gentamicin	11	23.9	3	37.5	0.418
Erythromycin	15	32.6	1	12.5	0.250
Clindamycin	15	32.6	1	12.5	0.250
Fusidic acid	0	0	0	0	NA
Chloramphenicol	0	0	0	0	NA

All 8 isolates of *Streptococcus pyogenes* were sensitive to Penicillin G, Ampicillin, Cephalexin, Erythromycin, Clindamycin, Chloramphenicol, Ciprofloxacin, Ofloxacin, Gentamicin, Amikacin, Vancomycin and Teicoplanin. Similarly the four isolates of *Streptococcus pneumoniae* were also sensitive to Penicillin G, Cephalexin, Gentamicin, Amikacin, Erythromycin, Clindamycin, Chloramphenicol, Ciprofloxacin and Ofloxacin. Both isolates of *Enterococcus faecalis* were found resistant to Penicillin G, Ampicillin, Ciprofloxacin, Erythromycin and Clindamycin, while being sensitive to Chloramphenicol, netilmicin. Vancomycin and Teicoplanin. However, one out of the two isolates was found to be resistant to Ofloxacin, Gentamicin and Amikacin (Table 5).

37% of total *Staphylococcus aureus* were found to be Methicillin resistant. None of the coagulase negative Staphylococcal species were found to be Methicillin resistant in this study. On statistical analysis, the difference in resistance pattern between *Staphylococcus aureus* and CONS against Methicillin showed p value of 0.038 which is statistically significant (p value < 0.05) (Table 6).

Table-6: Methicillin Resistance amongst Staphylococcal Isolates.

Staphylococcus aureus (n=46)	No.	%	Coagulase negative staphylococcal species (n=8)	No.	%
Methicillin Resistant *Staphylococcus aureus* (MRSA)	17	37	Methicillin Resistant	0	0
Methicillin Sensitive *Staphylococcus aureus* (MSSA)	29	63	Methicillin Sensitive	8	100
Total	**46**	**100**	**Total**	**8**	**100**

Among MRSA, maximum resistance was observed for Ofloxacin and clindamycin. No resistance was encountered for Amikacin, Netilmicin, Chloramphenicol, Fusidic acid, Vancomycin and Teicoplanin. The difference in resistance pattern between Ciprofloxacin and Gentamicin among MRSA strains is not statistically significant (p-value> 0.05). Among the *Pseudomonas aeruginosa* isolates maximum resistance was found for Ciprofloxacin (74.2%) followed by Gentamicin and Netilmicin (63.6% each), Ofloxacin (54.5%), Cefotaxime and Ceftazidime (48.5% each) and lastly Amikacin (41%). The lone isolate of *Pseudomonas aeruginosa*was found resistant to Piperacillin + Tazobactam. On statistical analysis, the difference between resistance pattern against Ceftazidime and Piperacillin + Tazobactam is highly significant (p value < 0.001). However, no statistically signicant difference in the resistance pattern is found between Ciprofloxacin and Gentamicin. No resistance was observed againstImipenem and Meropenem (Table 7).

Table-7: Resistance pattern in *Pseudomonas aeruginosa* (n=66)

Antibiotics	No.	% Resistance
Ciprofloxacin	49	74.2
Ofloxacin	36	54.5
Gentamicin	42	63.6
Netilmicin	42	63.6
Cefotaxime	32	48.5
Ceftazidime	32	48.5
Amikacin	27	41
Piperacillin + Tazobactam	01	1.5
Imipenem	0	0
Meropenem	0	0

On statistical analysis, the difference in resistance pattern between *Escherichia coli* and *Klebsiella pneumonia* for both Ciprofloxacin and Ofloxacin showed p value of 0.010 which is significant (p value < 0.05). No resistance was observed against Amikacin, Imipenem and Meropenem. All the strains of different *Candida species* along with control strains of *C.albicans*and*C.parapsilosis*were found to be sensitive to Fluconazole, Itraconazole and Voriconazole by disk diffusion method (Table 8).

Table-8: Antifungal Susceptibility Testing of Yeast by Disk Diffusion Method.

Antifungal drugs	Pattern	*C.albicans* (n=8) No. (%Susceptibility)	*C.parapsilosis* (n=4) No.% (Susceptibility)	*C.tropicalis* (n=1) No. (%Susceptibility)	Controls	
					C.albicans (n=1) No. %Susceptibility)	*C.parapsilosis* (n=1) No. (%Susceptibility)
Fluconazole 25µg	S≥19mm	8 (100%)	4 (100%)	1 (100%)	1 (100%)	1 (100%)
	SDD 15-18mm	-	-	-	-	-
	R≤14mm	-	-	-	-	-
Itraconazole 10µg	S≥23mm	8 (100%)	4 (100%)	1 (100%)	1 (100%)	1 (100%)
	SDD 14-22mm	-	-	-	-	-
	R≤13mm	-	-	-	-	
Voriconazole 1µg	S≥17mm	8 (100%)	4 (100%)	1 (100%)	1 (100%)	1 (100%)
	SDD 14-16mm	-	-	-	-	-
	R≤13mm	-	-	-	-	-

Discussion

Ear discharge is a common clinical problem presented by patients in the ENT set up, seen in variety of infectious conditions like diffuse otitis externa, otomycosis, and acute and chronic otitis media [18]. The present study included 180 patients with CSOM attending ENT OPD of a tertiary care hospital from 2011 to 2013. The age groups of the patients ranged from 2 years to 78 years. Maximum number of patients (36.7%) belonged to the age group of 11-20 years, similar to a study by Kumar et alwho reported an incidence of35% cases in the age group of 11-20 years [19].

Also, study by Agrawal et al reportingan incidence of 62.4 % patients, a study in (Burdwan) India reporting 31.9% and in Malaysia 69.3% patients, among age group less than 20 years showed similar results [20, 21, 22]. However, a few studies in India have reported that patients with age group less than 10 years, are more affected with an incidence ranging from 30%-50% [23,24,25]. As is evident in literature, CSOM is known to affect all age groups making it evident that the age limit might have no significant role to play in the pathological process of the disease.

Various studies in India as well as abroad have reported males out numbering females, which is in concordance with the present study, explaining the male predominance because of their more exposed way of life [19,22]. Most of the patients (57.8%) in our study belonged to the rural group, the study being conducted in the rural area. Factors like unhygienic conditions overcrowding, ignorance regarding ear disease and lack of medical facilities might have been responsible for the high prevalence in this group of patients. Ear discharge (100%) followed by loss of hearing (53.3%) were the most common symptoms in the present study, similar to other studies in India (Bhopal) and Nigeria in 2008 [26, 27].

In the present study, out of the total 180 cases, bacteria alone were isolated in 68.9% cases, bacteria along with fungi in 15.6% cases and fungi alone in 8.3% cases. Sterile culture was obtained in 7.2% of cases. There werea total of 189 bacterial isolates. Amongst these, *Pseudomonas aeruginosa* was the most common bacterium isolated (34.9%) followed by *Staphylococcus aureus* (24.4%) and *Proteus mirabilis* (6.3%). Out of the 41 fungal isolates obtained, *Aspergillus niger* was the commonest isolate (29.4%) followed by *Candida albicans* (19.5%) and *Aspergillus fumigatus* (12.2%).

Table-9: Shows comparison of various studies done in India in patients of CSOM.

	Nandy A et a; 1991 M angalore [28]	Grewal et al 1996 Ludhiana [29]	Poorey et al 2002 Rewa [30]	Bairy et al 2007 Manipal [31]	Agrawal et al2013 Agra [22]	Present study
Pseudomonas aeruginosa	43.8	37.5	35.2	33.8	32.8	34.9
Staphylococcus aureus	18.2	20.4	14.7	19.9	37.6	24.4
Proteus species	12	10.4	9.8	1	0.8	6.3
Providencia species	-	0.3	-	-	-	2.6
E.coli	-	2.8	5.9	1	3.2	4.8
Klebsiella species	7.3	6	25.4	7.5	4	3.2
Streptococcus pyogenes	-	1.9	3.9	1.6	-	4.2
Streptococcus pneumonae	-	-	-	-	1.6	2.1
Enterococci	-	1.6	-	-	-	1.1
CONS	-	10.7	4.9	19.4	-	4.2
Acinetobacter species	-	1.9	-	5.4	-	1.6
Candida species	-	-	-	2.15	1.6	5.2
Aspergilus species	-	-	-	1	0.8	2.6
Diphtheroids	6.7	1.9	-	4.3	-	7.4

Pseudomonas aeruginosa (33.5%) was the commonest bacteria isolated. A similar varied incidence has been reported ranging from 32.8% in Agra, 33.8% in Manipal and 37.5% in Ludhiana to 43.8% in Mangalore India [22, 25].The percentage of isolation of *Staphylococcus aureus* in the present study was 19.7%. While *Streptococcus pneumoniae, Haemophillus influenzae, Streptococcus pyogenes, Branhamella catarrhalis, Pseudomonas species* and *Entero-bacteriaceae* have been shown to be commonest infectious organisms in CSOM among infants and children from Japan, England and USA (1969), *Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus* species are the common organisms causing CSOM in India [32].

This variation in incidence of various organisms between India and Western countries may be due to geographical changes. It is also possible that the use of antibiotics has changed the picture, eliminating the more susceptible organisms like *Beta hemolytic Streptococci, Pneumococci* and *Alpha hemolytic Streptococci* due to treatment in the acute stage with gradual replacement by hardier and antibiotic resistant organisms like Coagulase positive *Staphylococci, Pseudomonas aeruginosa, Proteus* species, Coliforms etc. in chronic infections [33, 34].

Mixed bacterial infections were caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa* along with either Coagulase negative Staphylococci or Diphtheroids most often. The availability and use of broad spectrum antibiotics in recent times are probably responsible for far lesser incidence of mixed infections and also lesser number of isolates from each specimen as compared to earlier workers [34].

No organisms were isolated in 12 cases (7.8%) compared to sterile culture being reported in 7.29% -16% in other studies; anaerobic bacteria & viral agents being suspected to be the culprits most often in these cases in addition to the prior use of antibiotic coverage in some [5,24,25,33].

Some cases of tubo-tympanic variety of CSOM are seen to keep on discharging despite the use of topical antibiotic ear drops or systemic antibiotics. Sen Gupta et al proposed that this intractable otorrhoea occurs due to superimposed fungal infections over CSOM [35]. Irrational and excessive use of topical antibiotic ear drops, encourages the development of fungal infections in CSOM cases and radical mastoidectomy and fenestration cavities. This happens because of settling of fungal elements e.g. spores from external environment on the moist and alkaline medium of middle ear discharge and debris.

This finally leads to the development of mycotic otitis media causing intractable otorrhoea.In the present study, fungi were isolated along with bacteria in 13% cases while 2.6% cases of CSOM yielded pure cultures of fungi compared to respective figures of 6.1% and 7.6% in a study in Amritsar [29]. Fungal positivity in CSOM has varied from 8.3% to 40.8% in various studies in India [23, 33, 35].

The commonly isolated fungi in CSOM are *Aspergillus* and *Candida species.* The fungal spores found in the atmosphere readily establish themselves in the warm and moist environment of the middle ear, their growth being favoured by the presence of epithelial debris [36]. *Aspergillus species* have always been an important group of pathogens of CSOM ranging from 83.33% in Chandigarh in 1997 and 74.5% in Delhi in 1978 to 40% in Punjab in 1998 and Manipal in 2007, while this study reports an incidence of 25% [8,25,29,35].

A higher incidence of Candida isolates in this study could be explained by the increase in the incidence of non albicans*Candida species* over the years due to increased antibiotic use and abuse and the development of local immunocompromised states that facilitate the proliferation, colonization and transformation of commensals to pathogens over the period of time. In this study all the isolates of *Staphylococcus aureus* were resistant to penicillin G and ampicillin. Similar high resistant rates to these antibiotics have also been reported by others [25,33].

Resistance of 67.4% was encountered against ciprofloxacin in this study which is also similar to the value of 71.5% reported from Manipal [25]. In this study all the isolates of *Staphylococcus aureus* were sensitive to antibiotics like

Amikacin, Netilmicin, Fusidic acid, Vancomycin and Teicoplanin. Surprisingly, a resistance rate of 26.5% has been reported for Teicoplanin by workers Manipal [25].

In this study, 17 strains of MRSA (37%) were isolated.A recent study from Manipal reports that 27.3% of their isolates of *Staphylococcus aureus* were MRSA, which appears similar to our figure of 37%. Among MRSA, maximum resistance was observed for Ofloxacin (64.7%) and clindamycin (53%). In the present study, isolates of *Pseudomonas aeruginosa* exhibited a maximum resistance of 74.2% to Ciprofloxacin which is some what similar to figure of 53.4% reported from Burdwan in 2007 [21].

While workers from abroad have either recorded no resistance at all or very low rates of resistance [20]. For the anti-Pseudomonal cephalosporin Ceftazidime, a resistance of 48.5% was observed. The relatively high resistance rate reported in this study correlates with a study by Prakash et al who reported 100% resistance to Ceftazidime [37].

The frequency of serious fungal infections is on the rise because of increasing use of cytotoxic immunosupressive drugs and newer antibacterial agents. Fortunately, this increase in fungal infections has been accompanied by the development of new, less toxic, systemically active alternates to Amphotericin B, such as Fluconazole, Itraconazole and various Amphotericin B lipid formulations. Resistance to antifungals agents is emerging and in vitro susceptibility data might be required to guide the selection of antifungal chemotherapy in the face of increasing immunocompromised states and increasing fungal infections in immunocompromised as well as immunocompetent hosts.Antifungal susceptibility testing has evolved during the last decade and has now become a relevant clinical tool.

However, susceptibility testing is not an infallible answer to questions about treatment of fungal diseases. Routine susceptibility testing is only appropriate for Candida isolates (esspecially non-albicans species) from deep sites but not at present for other fungi or settings and importantly knowledge of the species of Candida is still a useful guide to the predictability of the probable pattern of susceptibility [38].

On doing disc diffusion susceptibility test in this study, all Candida isolates (i.e. 8 strains of C. *albicans,* 4 strains of *C.parapsilosis,* and one strain of C. *tropicalis)* were all sensitive (100%) to Fluconazole (25mcg), Itraconazole (10mcg) and Voriconazole (1mcg). Pfaller et al (2007) reported that fluconazole was active (>90%) against *C.albicans*(97.9%), *C. Parapsilosis* (93.3%), C. *Tropicalis* (90.4%), *C. keftr*(95.6%)and C. *Dublinnensis* (97.6%).

Decreased susceptibility to Fluconazole (<75%) has been seen with *C. glabrata* (68.9%) and C. *krusei*(9.2%) [39]. While Matar et al reported 3.8% resistance by disk diffusion method, none of our *Candida species* were found to be resistant to Fluconazole [40].

Conclusion

CSOM remains the common & important disease responsible for chronic ear discharge and a common source of misery for patients and frustration for doctors. With the advent of antibiotics, it is seen that there has been a rapid disappearance of active cases of otitis media and a change in microbiological profile of the disease, with elimination of the more susceptible organisms like *Streptococcus pyogenes, Streptococcus pneumonia, Haemophillusinfluenza,* and emergence of more resistant microbial flora. Otorrhoea due to fungal infection is seen to occur in the setting of refractory infection and is often discovered after multiple oral and ototopical anti bacterialmedications.

It is emphasized that early diagnosis and proper antimicrobial treatment in addition to the patient education is mandatory to avoid complications and in decreasing morbidity and mortality.

What's New in this study: This study provides information regarding the prevalence and antimicrobial susceptibility pattern of isolates found in patients of CSOM. Knowledge about the etiologic agents and their resistance pattern in specific geographical locations may help the clinicians to choose appropriate drugs for empirical therapy. This study emphasises the need for close monitoring and appropriate prescription of drugs after culture and sensitivity results.

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