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TO STUDY THE BACTERIOLOGICAL AND MYCOLOGICAL PROFILE OF CHRONIC SUPPURATIVE OTITIS MEDIA PATIENTS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN

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ABSTRACT

Chronic suppurative otitis media(CSOM) has been a source of a tremendous health predicament since time immemorial and even today it is immensely intricate. It is an important cause of hearing impairment which in turn may cause serious long term effect on language, auditory and cognitive development and on educational development.Knowledge of the prevailing flora and their susceptibility to antimicrobials will guide the clinician as to prescribing an empirical regimen so that a better and more specific management can be provided to the patients. Hence this study is done in present secenario to isolate the organims associated with CSOM and its susceptibility pattern. To identify the bacterial and fungal profile of Chronic Suppurative Otitis Media patients. 120 patients attending ENT OPD in Navodaya Medical College Hospital and Research Centre were analysed from Nov 2009 to Oct 2010. Using predefined inclusion and exclusion criteria, samples were obtained using sterile cotton microswabs. These were cultured for microbial flora and were identified . Drug susceptibility was done using Kirby Bauer disc diffusion method.The most common organism isolated was Pseudomonas aeruginosa 33% followed by Staphylococcus aureus 25.8% among the 91 bacterial isolates. This was followed by Proteus mirabilis 20.6%, Enterobacter aerogenes and Streptococcus spp at 4.1%. The organisms were most prevalant in winter and early spring between Nov- Feb. Also maximum number of cases were seen in age group of 21-30 years.The organisms were sensitive to gatifloxacin, cefoperazone sulbactum and ceftriaxone.Fungal isolates accounted for 6.2% of the organism with Aspergillus flavus 3.1%, Aspergillus niger 2.1% and Candida albicans 1%.The study suggests that Pseudomonas aeruginosa and Staphylococcus aureus are the most common etiological agents of CSOM. Most of the strainwere sensitive to fluoroquinolones and cephalosporins.

KEYWORDS: CSOM, Pseudomonas aeruginosa, Staphylococcus aureus, Antibiotics,Aspergillus flavus, Aspergillus niger, Candida albicans.

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INTRODUCTION

Chronic suppurative otitis media (CSOM) and its complications are the bugbear of the otologists, paediatricians and general practitioners. It is a disease of multiple etiologies and is well known for its persistence and recurrence in spite of treatment[1].CSOM - Chronic inflammation of middle ear and mastoid process with perforated tympanic membrane and ear discharge[2].Destructive and persistent disease with irreversible sequelae and can proceed to serious intra or extra cranial complications.[3]Cholesteatoma is post inflammatory pseudo tumor which is always a consequence of CSOM.[4]Disease of multiple etiologies well known for its persistence and recurrence in spite of treatment.[5]

There are 3 types of CSOM based on perforation of middle ear.[4]

- Tubotympanic
- Atticoantral
- Marginal

It is the single major cause of conductive deafness and 1.5% of speech disorders.[1]
The complications occur frequently because of close the relation of middle ear, cleft to facial nerve, auditory labyrinth, lateral sinus and middle and posterior cranial fossae makes it.[6]

Risk factors for CSOM include-[7]

- Young age.
- Poor nutrition.
- High rate of nasopharyngeal colonization with potentially pathogenic bacteria.

The disease often starts in childhood and is one of the top five common childhood illness.[8]Intracranial complications are abscess, labryinthitis, petrositis and facial nerve paralysis.[9]Situation is more critical in some of the rural areas because of lack of hygiene sense and bathing in stagnant water. This becomes more complicated due to self medication[10]. The consequences are the emergence of resistant strains, super infections, and complications thereby lengthening treatment cost and suffering. [10]

Infection usually results from bacterial causes and fungal causes.[11]

The most common organism isolated nowadays is Pseudomonas aeruginosa, Staphylococcus aureus, Proteus spp, Klebsiella spp among aerobes.[12]Aspergillus flavus and Candida albicans are the most important fungal causes.[12] Fungal infections are generally superimposed.[1]
CSOM has profound impact on society in terms of resources utilized in treatment, multiple OPD visits and surgery as well as impact on hearing.[10]Indiscriminate use of antibiotics and poor follow up of patients resulted in persistence of low grade infections.[3]The study of micro-organisms commonly associated with CSOM and the antibiogram is pertinent to plan a general outline for treatment for patients.[3]The present work deals with the changing flora of CSOM and emergence of strains resistant to the commonly employed antibiotics.

MATERIALS AND METHODS

Place of study

The present study was undertaken at Navodaya Medical College Hospital and Research centre, Raichur.

Study Period

The period of study was from November 2009 to October 2010 Sample size 120 cases.

Methodology

The subjects in this study include those who have fulfilled the following inclusion and exclusion criteria.

Inclusion Criteria

The patients diagnosed as suffering from CSOM after thorough clinical evaluation by an ENT surgeon.

Exclusion Criteria

Patients who have taken systemic antibiotics for CSOM.

Study cases

Discharge from ear (Pus) taken from 120 clinically diagnosed cases of chronic suppurative otitis media patients attending Otorhinolaryngology department of NMCH&RC, Raichur.Clinical/demographic data were collected using a prepared questionnaire, which included patient age, and sex, duration of illness (ear disease), previous medications, and patient's ears involved and hearing Status (Annexure).

Collection of discharge

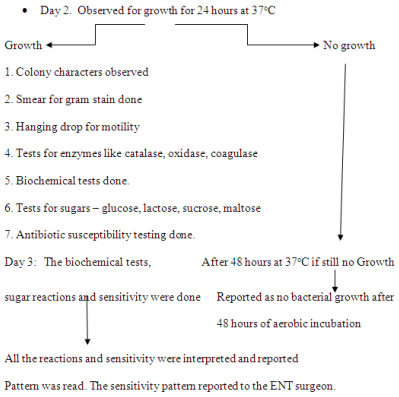
The discharge was collected under aseptic precautions. An external ear canal was cleaned by suction, and then wiped with sterile cotton and with 70% alcohol. This was allowed to dry. Then using a sterile auditory speculum, under aseptic conditions, a sterile cotton swab stick was introduced into the 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. Four specimens from a single ear were collected in this manner, labelled and taken to the laboratory immediately for processing.

For Aerobic growth

Days Procedure

- Day 1. Grams staining done.

Inoculated on BA, MA, CA and incubated for 24 hours at 37^{o}C



Isolation of aerobic bacteria

Four sterile swab sticks were used to collect the discharge from the affected ear.

1. One swab stick used for Grams staining.
2. One swab stick was used for inoculating on MA, BA, and CA
3. One swab stick used for KOH mount.
4. One used to inoculate 2 Sabouraud's Dextrose Agar.

Direct smear examination

With one swab a thin smear is made on a clean glass slide and is fixed by heating. Gram staining is done for the smears so made and is examined under oil immersion, objective to note the various morphological types of bacteria, their number, Gram reaction, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample gram positive and gram-negative organisms were identified and recorded. If more than five epithelial cells/ field were present, the samples were discarded and repeat sample was taken. The second swab stick was used to streak MacConkey agar, Blood agar and Chocolate agar. MacConkey agar & Blood agar plates were incubated at 37^{o}C. Chocolate agar plate was incubated in a candle jar with 5-10% carbon dioxide. The third was used to inoculate 2 Sabouraud Dextrose Agar with chloramphenicol. The fourth was used for KOH mount.Sabouraud dextrose agar was incubated at 37^{o}C and 25^{o}C. After 24 hours of incubation the plates were examined for growth. If there was no growth, the plates were further incubated for 48 hours reported as no growth. If growth was seen then the colony morphology was studied on the MA, BA and CA plates and processed further.

Gram-negative bacilli

From the lactose fermenting and non-lactose fermenting colonies, further Gram staining for

grouping, Hanging drop for motility, and catalase and oxidase tests were done. After which the following biochemical tests were done. They include:

1. Glucose fermentation test.
2. Lactose fermentation test.
3. Sucrose fermentation test.
4. Maltose fermentation test.
5. Indole test.
6. Methyl red.
7. Voges Proskauer test.
8. Citrate utilization test.
9. Urease production test.
10. Nitrate reduction test.
11. Triple sugar iron test
12. Mannitol motility test
13. Lysine decarboxylation test.
14. Arginine dihydrolysis test.
15. Ornithine decarboxylation test.
16. Phenylalanine Deaminase test
17. Malonate utilization test.

These tests were done for gram negative bacteria.

Gram-positive cocci

For golden yellow colour colonies seen on nutrient agar or β hemolytic colonies seen on blood agar Gram stain was done. If Gram positive cocci– Catalase test and modified oxidase test were done. If under gram stain they were Gram positive cocci in clusters, then they were catalase positive and modified oxidase test negative.

- Slide and tube coagulase tests were done.
- Urease production test; and
- Anaerobic mannitol fermentation test. For pin point colonies which were β or α-hemolytic or carom coin appearance colonies, gram stain was done. If Gram positive cocci in pairs and short chains, catalase negative –
- Bacitracin and Optochin sensitivity was done on blood agar.

Isolation of fungi

For fungal culture once a clinical diagnosis was made, laboratory examination of the discharge was carried out by direct microscopy and culture.The specimen was spread over a glass slide on a drop of 20% KOH solution and covered with a cover slip. This was examined under the microscope for the presence of fungi. The swab material was inoculated on SDA slant and incubated at room temperature and was observed daily for fungal growth up to two weeks.

1. ***The growth was observed for the following –***Rate of growth
2. Morphology of colony
3. Texture
4. Surface pigmentation
5. Microscopic examination like LPCB mount and slide culture were done to identify the fungi.
6. Gram staining was done for identification of yeast and yeast like fungi.
7. Sugar fermentation and assimilation tests, chlamydospore formation and germ tube tests were done to identify Candida albicans.

For confirmation that the particular fungal isolate was the pathogen, a repeat sample of the discharge was taken and only if the same isolate was repeatedly cultured, only then was it considered as the etiological agent.

The following criteria were followed while making a diagnosis.

1. Growth of the same fungus in more than one medium
2. Same growth is obtained on more than one occasion.
3. Growth of fungus even in one medium with direct examination being positive for fungus.

Antibiogram for bacterial isolates

For antibiotic sensitivity testing, five colonies from the culture plate were inoculated into 2 ml of peptone water and incubated at 37 ^{o}C for 2 hours.Turbidity was compared to that of 0.5 Mc Farland standards. A cotton swab was immersed and rotated in this inoculum, 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 selected antibiotic discs were then placed aseptically on this media 1.5cm apart using sterile forceps. The plates were then incubated 24 hours at 37 ^{o}C. After overnight incubation, the zone size was recorded and reported as sensitive, intermediate sensitive or resistant by comparing the zone size to the Kirby-Bauer chart. If the organisms were not

sensitive to any of the drugs, then a second line of antibiotics were put up using the same procedure as above.

Drugs used for Gram positive organisms were

1. Ampicillin (10 ug)
2. Erythromycin (10 ug)
3. Clindamycin (2 ug)
4. Co-trimoxazole (25 ug)
5. Gentamicin (10 ug)
6. Ceftazidime (30 ug)
7. Oxacillin (1 ug)
8. Tetracycline (10 ug)
9. Gatifloxacin (10 ug)
10. Chloramphenicol (30 ug)
11. Cefoperazone sulbactum (30 ug)
12. Vancomycin (10 ug)

Drugs used for Gram-negative organisms

1. Amoxyclav (30 ug)
2. Co-trimoxazole (25 ug)
3. Gentamicin (10 ug)
4. Tetracycline (10 ug)
5. Ceftriaxone (30 ug)
6. Gatifloxacin (10 ug)
7. Cefotaxime (30 ug)
8. Amikacin (30 ug)
9. Chloramphenicol (30 ug)
10. Ceftazidime (30 ug)
11. Tobramycin (10 ug)
12. Piperacillin tazobactum (100/10 ug)
13. Ciprofloxacin (10 ug)

For Pseudomonas species

1. Co-trimoxazole (25 ug)
2. Gentamicin (10 ug)
3. Ceftriaxone (30 ug)
4. Ticarcillin (75 ug)
5. Tetracycline (10 ug)
6. Cefotaxime (30 ug)
7. Gatifloxacin (10 ug)
8. Ceftazidime (30 ug)
9. Amoxyclav (30 ug)
10. Amikacin (30 ug)
11. Cefuroxime (30 ug)
12. Cefoperazone sulbactum (30 ug)
13. Carbenicillin (100 ug)
14. Piperacillin tazobactum (100/10 ug)
15. Tobramycin (10 ug)

Follow up

On the third day, the antibiogram reports were personally given to the concerned ENT surgeon. During the treatment period follow up examination was performed on the 8th and 9th day from the start of treatment. Patients outcome were evaluated by otoscopic examination. The cessation of otorrhoea post treatment was taken as indicator for clinical success.

RESULTS

One hundred and twenty clinically diagnosed CSOM cases attending ENT OPD of Navodaya Medical College, Raichur during the study period were taken.

Table 1
Distribution of isolation of organisms from CSOM cases

Organism isolated	Number of isolates (Frequency)	Percentage (%)
Acinetobacter spp	1	1
Aspergillus flavus	3	3.1
Aspergillus niger	2	2.1
Candida albicans	1	1
Enterobacter aerogenes	4	4.1
Klebsiella oxytoca	2	2.1
Klebsiella pneumonia	2	2.1
Streptococcus pneumonia	1	1
Proteus mirabilis	20	20.6
Providencia stuartii	1	1
Pseudomonas aeruginosa	32	33
Staphylococcus aureus	25	25.8
Streptococcus pyogenes	3	3.1
Total	**97**	**100.0**

The above table shows that out of 100 organisms isolated 97 were pathogenic.

Out of 97 pathogenic organisms most common organism isolated Pseudomonas aeruginosa 32(33%) followed by Staphylococcus aureus 25 (25.8%) and Proteus mirabilis 20(20.6%). This was followed by Enterobacter aerogenes 4 (4.1%), Streptococcus pyogenes 3(3.1%), Klebsiella pneumoniae 2(2.1%) and Klebsiella oxytoca 2(2.1%), Acinetobacter spp 1(1%), Streptococcus pneumoniae 1(1%) and Providencia Stuartii 1(1%).The fungal isolates were followed by Aspergillus flavus 3 (3.1%) Aspergillus niger 2(2.1%), cases and 1 (1%) case of Candida albicans isolated.

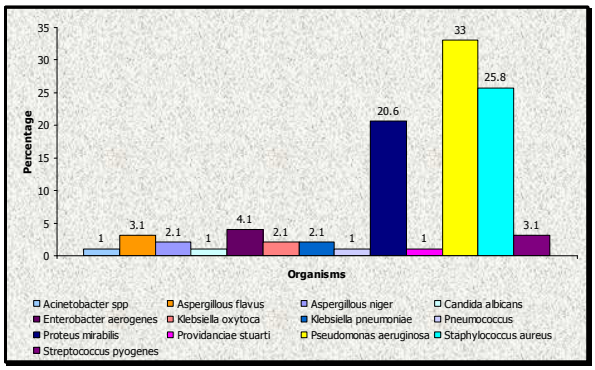


Figure-18
Organisms isolated from CSOM

Table 2
Showing antibiotic sensitivity pattern of aerobic bacterial isolates isolated

Organism	Total	AM	AC	GF	G	C	T	CIP	Cu	CA	CFS	Co	Tb	Ak	CE	Va	Ox	Ti	Cl	Cb	E	CD	Pt
Pseudomonas aeruginosa	32	0	0	30	22	24	1	10	11	26	29	1	29	21	22	0	0	15	30	5	0	0	30
Staphylococcus aureus	25	1	11	24	15	7	7	15	13	9	18	18	0	15	16	25	13	0	17	0	15	18	0
Proteus mirabilis	20	5	13	17	19	18	1	11	7	13	17	16	18	15	18	0	0	0	18	0	0	0	17
Enterobacter aerogenes	4	0	0	4	1	0	2	2	1	1	4	1	2	2	4	0	0	0	3	0	0	0	4
Klebsiella oxytoca	2	0	1	2	2	2	0	1	1	1	0	2	0	0	1	0	0	0	0	0	0	0	2
Klebsiella pneumoniae	2	0	0	2	2	0	0	1	2	2	2	2	2	2	2	0	0	0	0	0	0	0	2
Streptococcus pyogenes	3	3	0	0	0	3	0	3	0	0	3	0	0	0	0	3	0	0	3	0	3	0	0
Acinetobacter spp	1	0	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1
Streptococcus pneumoniae	1	1	1	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
Providencia stuartii	1	0	1	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1
Total	91	10	27	83	62	56	11	42	35	54	74	44	53	55	65	29	13	15	72	5	20	19	62

Out of the 97 culture positives, 91 were bacterial and their antibiotic sensitivity was tested. The bacterial isolates were studied for antibiotic sensitivities by Kirby Bauer disc diffusion method. The organisms were subjected to antibiotic susceptibility test using the following antibiotics i.e., Ampicillin, Amoxycillin Clavulanic acid, Gatifloxacin, Gentamicin, Chloramphenicol, Tetracycline, Ciprofloxacin, Cefuroxime, Ceftazidime, Cefoperazone sulbactum, Co-trimoxazole, Tobramycin, Amikacin, Cefotaxime, Vancomycin, Oxacillin, Ticarcillin, Ceftriaxone, Carbenicillin, Erythromycin, Clindamycin, and Piperacillin tazobactum.

- *Pseudomonas aeruginosa* was sensitive to Piperacillin tazobactum and Gatifloxacin 93.75% followed by Cefoperazone sulbactum at 90.5%.
- *Staphylococcus aureus* was sensitive to Gatifloxacin at 96% followed by Cefoperazone sulbactum and Co-trimoxazole at 72%.
- *Proteus mirabilis* was sensitive to Gentamicin at 95% and then to Chloramphenicol and Cefotaxime at 90%.
- *Klebsiella spp* showed 100% susceptibility to Gentamicin, Co-trimoxazole and Gatifloxacin.
- *Streptococcus pyogenes* showed 100% susceptibility to Ampicillin and Cefoperazone sulbactum.

Overall, the antibiotic susceptibility for the 91 isolates showed that 83 (91.2%) were sensitive to Gatifloxacin followed by 74 (81.3%) to Cefoperazone sulbactum, 72 (79.1%) to Ceftriaxone. The lowest sensitivities were seen to be for Ampicillin, Tetracycline and Carbenicillin.

Table 3
Incidence of CSOM according seasonal variation

Organism isolated	Nov-Feb	Mar-Jun	July-Oct	Total
Acinetobacter spp	1 (100)	0 (0)	0 (0)	1 (0.8)
Aspergillus flavus	1 (33.3)	2 (66.7)	0 (0)	3 (2.5)
Aspergillus niger	1 (50)	1 (50)	0 (0)	2 (1.7)
Candida albicans	1 (100)	0 (0)	0 (0)	1 (0.8)
Enterobacter aerogenes	4 (100)	0 (0)	0 (0)	4 (3.3)
Klebsiella oxytoca	2 (100)	0 (0)	0 (0)	2 (1.7)
Klebsiella pneumonia	2 (100)	0 (0)	0 (0)	2 (1.7)
Streptococcus pneumoniae	1 (100)	0 (0)	0 (0)	1 (0.8)
Proteus mirabilis	7 (35)	4 (20)	9 (45)	20 (16.5)
Providencia stuartii	1 (100)	0 (0)	0 (0)	1 (0.8)
Pseudomonas aeruginosa	19 (59.4)	5 (15.6)	8 (25)	32 (26.5)
Staphylococcus aureus	22 (88)	2 (8)	1 (4)	25 (20.8)
Streptococcus pyogenes	3 (100)	0 (0)	0 (0)	3 (2.5)
CONS	1 (100)	0 (0)	0 (0)	1 (0.8)
Diphtheroids	5 (100)	0 (0)	0 (0)	5 (4.2)
No growth	14 (87.5)	0 (0)	2 (12.5)	16 (13.3)
Non pathogenic Neisseria	1 (100)	0 (0)	0 (0)	1 (0.8)
Total	**86 (71.7)**	**14 (11.6)**	**20 (16.7)**	**120 (100)**

χ^2 =16.37, df=4, p<0.003

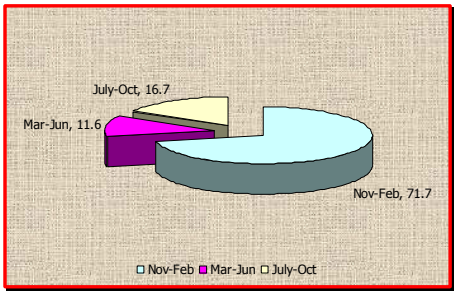


Figure 19
Incidence according to seasonal variation

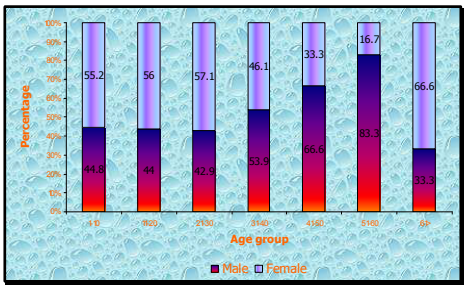


Figure 20
Age group affected along with sex distribution

Out of 120 cases, 86 (71.7%) cases were observed during November-February 2009-2010, 14 (11.6%) cases were observed during March-June and 20 (16.7%) cases during July-October. Out of 25 cases of *Staphylococcus aureus* 88% were isolated during November- February and in case of *Pseudomonas aeruginosa* 59.4% of cases out of 32 cases were isolated during the same time frame. Out of 20 cases of *Proteus mirabilis* 45% of cases were isolated in July-October There was a significant association between seasonal variation and organism ($p<0.003$). The above results indicate that the incidence of CSOM was more during winter season and in early spring.

Table 4
Incidence of CSOM according age

Age group	Aspergillus flavus	Aspergillus niger	Candida albicans	CONS	Diphtheroids	Acinetobacter spp	Enterobacter aerogenes	Klebsiella oxytoca	Klebsiella pneumonia	No growth	Neisseria pathogenic	Non pathogenic Neisseria	Streptococcus mirabilis	Proteus stuartii	Providencia aeruginosa	Pseudomonas aureus	Staphylococcus pyogenes	Streptococcus	Total
1-10					3		1		1	5	1	1	4	1	5	7	0		29 (24.2)
11-20				1	2	1		1		5			3		7	4	1		25 (20.8)
`21-30	2	2	1				2	1	1	3			3		9	5	2		31 (25.8)
31-40	1									3			4		5	4			17 (14.2)
41-50							1						4		3	1			9 (7.5)
51-60													1		2	3			6 (5)
61>													1		1	1			3 (2.5)
Total	3	2	1	1	5	1	4	2	2	16	1	1	20	1	32	25	3		120

$\chi^2 = 10.61$, df=9, p=0.303

The mean age (SD) of patients was 24.55 ± 15.83 years, with the range 1-72 years. The majority of them were in the age group of 21-30 years (25.8%) followed by 1-10 years (24.1%). There was an equal distribution between sexes (male (48.3%) and females (51.7%). Out of 120 cases, 29 (24.2%) cases were observed in 1-10 years age group. Out of these 29 cases, 13 (44.8%) cases were males and 16 (55.2%) cases were females. 25 (20.8%) cases were observed in the age group between 11-20 years where 11(44%) cases were females and 14(56%) cases were males. 31(25.8%) cases were observed between 21-30 years out of which 18 (57.1%) cases were females and 13 (42.9%) cases were males. 17 (14.2%) cases were observed between the age group 31-40 years of age of which 8(46.1%) cases were females while 9 (53.9%) cases were males. 9(7.5%) cases were observed between age group 41-50 years were in 3 (33.3%) cases were females while 6(66.6%) cases were males. 6(5%) cases were seen in the age group 51-60 years of which 5(83.3%) cases were males while 1(16.7%) case was female. 3 (2.5%) cases were seen in age group >61 years where 2(66.6%) cases were females and 1(33.3%) were male. The above table shows that highest incidence of CSOM was observed between 21-30 years There was no statistically significant association between

age and organism (p>0.05). *Pseudomonas aeruginosa* was the predominant organism seen in the age group 21-30 years while *Staphylococcus aureus* was the predominant causative organism in the first decade of life.

Table 7
Distribution of presence and absence of Cholesteatoma according to sex

Sex	Cholesteatoma		
	Present	Absent	Total
Male	7 (11.5)	54 (88.5)	61 (50.8)
Female	4 (6.8)	55 (93.2)	59 (49.2)
Total	11 (9.2)	109 (90.8)	120 (100)

$\chi^2 = 0.794$, df=1, p=0.373

Cholesteatoma was present in 11 (9.2%) cases and absent in 109 (90.8%) cases. Majority of cholesteatoma was present in males 7 (11.5%) where as in females it was 4(6.8%). Hence the result shows that there was no association between cholesteatoma and sex (p>0.05)

Table 9
Culture results of cases studied

Sl. No.	Culture Result	Number of subjects	%
1	Positive culture	97	80.8
2	No growth	16	13.3
3	Non-pathogenic organism	7	5.9
	Total	120	100

The table depicted that, out of 120 ears examined, majority of the cases show positive culture (80.8%) followed by no growth (13.3%) and non-pathogenic organism (5.9%)

DISCUSSION

In the present study an attempt is made to find out aerobic bacterial and fungal profile of CSOM with antimicrobial susceptibility testing of the bacterial isolates. The results are compared with other studies.

Different aerobic bacteria isolated

The bacteria were Pseudomonas aeruginosa 32(33%), Staphylococcus aureus 25 (25.8%), Proteus mirabilis 20 (20.6%), Enterobacter aerogenes 4 (4%), Streptococcus pyogenes 3(3%), Klebsiella oxytoca 2 (2.1%), Klebsiella pneumonia 2(2%), Acinetobacter spp 1(1%), Providencia stuartii 1 (1%), and Streptococcus pneumoniae 1(1%).The fungi were Aspergillus flavus 3 (3.1%), Aspergillus niger 2 (2.1%) and Candida albicans 1 (1%). Present study showed Pseudomonas aeruginosa (33%) highest number of isolate in cases afflicted with CSOM. This was same as the study done by Chandrashekhar M. R et al[13] where Pseudomonas aeruginosa (46.76%) was the predominant organism. Another study by Mandana J et al [14] showed that Pseudomonas aeruginosa was the predominant organism. Other studies done which correlated with above were Osazuva Favour et al [15] Maji P.K et al[10], Loy AHC et al[16], and. O O Oguntibeju.

Organism	Present study	Mandana J et al (2011)	Srivatsava A et al (2010)	Nikakhlagh S et al (2007)	Maji P.K et al (2007)	Chandrashekar M.R et al (2004)	Vijaya. D & S.H. Geetha (2002)	O. O. Oguntibeju (2002)	Loy AHC et al (2002)	Hiremath et al (2001)	Varshney S et al (1999)	Ballal M. et al (1992).	Rama Rao M.V. et al. (1980)	Srivatsava V.K. et al (1979)
Pseudomonas aeruginosa	33%	32%	28.3%	21.6%	63.8%	46.6%	19.3%	31%	33.3%	40.4%	35.71%	31.9%	23%	4.2%
Staphylococcus aureus	25.8%	21%	29.2%	32.4%	33.8%	17.98%	23.65%	17.2%	33.3%	30.1%	19.52%	30.6%	31.7%	44%
Proteus mirabilis	20.6%	20%	7.5%	8%	0	0	3.23%	18.9%	2.2%	15.04%	21.9%	11.5%	25.9%	8.4%
Enterobacter aerogenes	4.1%	0	2.8%	21.6%	0	0	0	0	0	0	0	0	0	0
Klebsiella spp	4.1%	0	3.7%	5%	0	12.23%	33.33%	13.8%	7.8%	6.34%	6.19%	13.4%	13.7%	4.2%
Streptococcus spp	4.1%	0	3.7%	0	0	0	0	0	3.3%	0	1.7%	0	0	0
Acinetobacter spp	1%	0	1.9%	0	0	0	0	0	0	0	0	0.3%	0	0
Providencia stuartii	1%	0	0	0	0	0	0	0	1.1%	0	0	0	0	0
Candida albicans	1%	4%	1.8%	0	0	0	2.15%	5.2%	2.2%	0	0	0	0	0
Aspergillus spp	5.2%	0	0	0	0	0	0	0	3.3%	0	0	0	0	0

In studies by Bairy I et al[17] and Gulati SK[18], Pseudomonas aeruginosa was the highest isolate obtained. Studies done by Erkan Mustafa et al[19] and Kenna Margaret et al[20] showed that Psedomonas aerugniosa followed by Staphylococcus aureus, were commonest aerobes.

The occurrence of *Pseudomonas aeruginosa* as prime offender can be attributed to:

- Pseudomonas survives competition with other pathogens due to minimal nutritional requirement and its armamentarium of antibacterial products like pyocyanin and bacteriocin.[26]
- Vartiainen and Vartiainen postulated Pseudomonas has ability to carve a niche for itself in local infection through necrotizing activities of its extracellular enzymes.[27]
- In addition, the organism acts as an opportunistic pathogen, flourishes in the external auditory canal and may cause suppurative disease in contiguous sites.[26]

Staphylococcus aureus was second common organism at 25.8% in present study. This compares with studies like Maji P.K et al[10] ,Varshney S et al [28] and Hiremath et al[25] in contrast to Srivatsava A et al[26] and Ettehad GH et al[9] where Staphylococcus aureus was primary organism. Proteus mirabilis in present study was third with 20.6% and in concordance with Mandana J et al[14] in which it was isolated in 20% of cases while VK Poorey and Arati Iyer[3] isolated Proteus in 9.08% cases. Studies done by Nikakhlagh et al[12], Rao R et al,[1]Grewal et al[23] show that Proteus species was third highest isolate in CSOM cases. Study done by Osazuva F et al[15] showed that Proteus mirabilis (8.9%) was the fourth common organism. This was not in accordance with the present study.Enterobacter aerogenes (4%) marked as the fourth most common organism isolated. Nikakhlagh et al[12] showed that Enterobacter spp was the third most common.Klebsiella spp (4.2%) was the next organism isolated in present study. This was in accordance with study done by Nikakhlagh S et al[12] and Chandrasekhar MR[13] showed Klebsiella spp to be the third highest with 12.23%, Klebsiella spp. becomes opportunistic pathogens in the middle ear when resistance is low.Streptococcus spp (4%) was the next common organism isolated.

Antibiotic sensitivity pattern of isolates

Pseudomonas aeruginosa was highly sensitive to Piperacillin tazobactum, (93.7%) Gatifloxacin, (93.7%) and Ceftriaxone (93.7%), followed by Cefoperazone sulbactum (90.6%), Tobramycin (90.6%), Chloramphenicol (75%) and Gentamicin (68.7). Maji P.K et al[10] showed 46% susceptibility to Ciprofloxacin and 100% susceptibility to Amikacin followed by Gentamicin and Cefotaxime. The decreased sensitivity of Pseudomonas aeruginosa to the quinolone family, is indicative of rapid appearance of antibiotic resistant strains.Staphylococcus aureus showed sensitivity to Vancomycin (100%), Gatifloxacin (96%), Clindamycin (72%), Ceftriaxone (68%), Erythromycin and Gentamicin (60%). Ciprofloxacin sensitive to 60% strains. Lowest sensitivity seen in Ampicillin and Amoxycillin Clavulanic acid. Mandana J et al[14] Staphylococcus aureus showed highest sensitivity to Vancomycin, followed by Fluroquinolones. Varshney S et al[28] showed sensitivity pattern as follows: Out of 41 isolates of Staphylococcus, 22 were sensitive to Ciprofloxacin, 12 to Norfloxacin, 10 to Gentamicin, 7 to Erythromycin and 6 to Ampicillin. Poorey V K et al [3] showed maximum sensitivity to Fluoroquinolones and Macrolides.Proteus mirabilis was sensitive to Gentamicin (95%), Chloramphenicol (90%), Tobramycin (90%), Cefotaxime (90%), Ceftriaxone (90%), Piperacillin tazobactum (85%), and Gatifloxacin (85%). 100% Proteus mirabilis isolated showed sensitivity to Ceftazidime and Ciprofloxacin. This was seen in study done by Mandana J et al[14] and Osazuva F et al[15] with similar sensitivity to Gentamicin.Streptococcus spp was sensitive to Ampicillin (100%), Vancomycin (100%), and Erythromycin (75%). This is in comparison with study done by Poorey V. K et al[3] .Overall, the antibiotic susceptibility for the 91 isolates showed that 83 (91.2%) were sensitive to Gatifloxacin, followed by 74 (81.3%) to Cefoperazone sulbactum, 72 (79.1%) to Ceftriaxone and lowest sensitivities were seen for Ampicillin, Tetracycline and Carbenicillin.

Similar to the study done by, Hiremath S.L. et al[25] Saurabh V. et al [28] and Nandy A. et al. [22]

Season wise distribution

CSOM cases were more prevalent during winter and early spring Nov2009-Feb2010 with almost 71.7% of patients. This was followed by cases in July-Oct with 16.7%. While Maji P.K[10] showed considerable aggregation of cases in months from July to September.

Distribution of organisms depending on season

Cluster of Staphylococcus aureus (88%) during the period between Nov2009 –Feb2010 and only 3 cases were seen subsequently. Pseudomonas aeruginosa (59.3%) was isolated maximum during the Nov2009 – Feb2010 season and subsequently highest during July-Oct (25%) and lowest during the summer months of April, May and June (15.7%). Proteus mirabilis was seen to be distributed equally with maximum number of cases (9) seen in July-Oct, followed by Nov2009 –Feb2010 (7) and lowest again in summer months of April, May and June (4). This is in comparison to Maji P. K et al[10] study.

Age distribution of CSOM

Present study the age ranged from less than 1 year to 80 years. Maximum numbers in age group of 21-30 years (25.8%) followed by 1-10 years (20%). This is in concordance with the study of Erkan Mustafa [19], and Ettehad GH[9].

Culture results of cases studied

The culture results are variable in other studies. In the present study 104(86.6%) were found to be culture positive while 16(13.4%) were negative for culture.

Negative culture can be attributed to: [22, 25]

- Anaerobic growth
- Prior treatment
- Presence of antimicrobial enzyme

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