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Bacterial and fungal profile in Chronic Suppurative Otitis Media in a tertiary care hospital in Uttarakhand

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Abstract:
Introduction: *Chronic Suppurative Otitis Media (CSOM) is one of the most commonly encountered diseases in otorhinolaryngology practice.*
Material and methods: *A total of 70 samples from 64 clinically diagnosed cases of CSOM were included in the study. The bacterial identification and antibiotic sensitivity of the isolates was carried out by automated method. Identification and antifungal susceptibility of yeast was performed using automated method. However identification of moulds was carried out by conventional methods.*
Result: *Out of 70 samples 50% (35/70) showed positive bacterial culture. Among 64 patients, 53.12% (34/64) were males and 46.87% (30/64) were females. The commonest bacteria isolated was Pseudomonas aeruginosa 45.71% (16/35) followed by Staphylococcus aureus 33.42% (11/35), Proteus mirabilis 14.28% (5/35), Enterobacter cloacae 5.71% (2/35) and Sphingomonas paucimobilis 2.8% (1/35). All Pseudomonas isolates obtained in this study were found to be 100% resistant to amoxicillin/clavulanate, cotrimoxazole, and cefixime and 100% sensitive to meropenem and tigecycline. With 36.36% (12/33) Aspergillus fumigatus was found to be the predominant isolate. While 30.30% (10/33) Aspergillus niger, 18.18% (6/33) Candida albicans, 6.06% (2/33) Aspergillus terreus , 3.03% (1/33) Candida tropicalis, 3.03% (1/33) Rhizopus species and 3.03% (1/33) Paecilomyces species were also observed. All the seven strains of yeast were sensitive to fluconazole, amphotericin B, flucytosine and voriconazole.*
Conclusion: *An appropriate knowledge of antibacterial susceptibility of microorganisms would contribute to a rational antibiotic use and the success of treatment for chronic supportive otitis media.*

Keywords*:* *Antibiotics, CSOM, Bacterial infection, Antifungal agents, Fungal infection*

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I. Introduction

Chronic Suppurative Otitis Media (CSOM) is defined as persistent disease of middle ear, which is capable of causing severe destruction sequel with the manifestation of deafness, discharge and a permanent perforation [1]. The duration of the symptoms to define the disease varies according to various otolaryngologists [2]. But according to World Health Organization (WHO) only two weeks of duration is required to define CSOM [3].

CSOM usually develops in the first decade of life but can persist during adulthood. Prevalence of CSOM is more in the developing and underdeveloped countries. According to WHO, the prevalence of CSOM in India is more than 4% and it falls among those countries where urgent attention is needed [3]. The incidence of CSOM is increasing in the developing countries because of low socio-economic status, poor nutrition, poor hygienic practices and lack of health education [4, 5]. Other risk factors involved in pathogenesis of CSOM are recurrent upper respiratory infections, breast-feeding while lying down, craniofacial malformation and Eustachian tube defect [6].

In CSOM, bacteria can reach the middle ear either from the nasopharynx through the Eustachian tube or from the external ear canal through a non-intact tympanic membrane. The aerobic microorganisms most frequently isolated in CSOM are *Pseudomonas aeruginosa*, *Staphylococcus aureus, Proteus species, Klebsiella species*, *Escherichia species*, *Haemophilus influenzae*, and *Moraxella catarrhalis* [7, 8]. Among the aerobic bacteria *Pseudomonas aeruginosa* is particularly involved in deep seated and progressive destruction of middle ear and mastoid structures through its toxins and enzymes [9]. Fungi are also thought to play a role in CSOM. In some populations, especially those residing in hot and humid regions, fungi are isolated from the cases with CSOM as fungus flourish in such environmental conditions. Among the fungus *Candida* species and *Aspergillus* species are the common pathogens involved in CSOM [10, 11]. Recently, concern has risen about

secondary fungal overgrowth as a complication of treatment with topical antibiotics as these drugs not only act against the pathogenic bacteria but these drugs also suppress the normal commensal flora of the ear [10].

II. Material and Methods

This prospective observational study was conducted in the Department of Microbiology and Immunology, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIM&HS) and Microbiology division of Central Laboratory of Shri Mahant Indiresh Hospital (SMIH) in collaboration with E.N.T department of Shri Mahant Indiresh Hospital, Dehradun.

Study Duration: A period of one year from October 2014 to October 2015.

Sample size: A total of 70 samples from 64 clinically diagnosed cases of CSOM of all age groups and both sexes attending outpatient department of ENT as well as those admitted in ENT ward of Shri Mahant Indiresh Hospital were included in this study.

Inclusion criteria:

Patients who were diagnosed as suffering from CSOM after thorough clinical evaluation by an ENT surgeon, patients of all age groups and both sexes, patients who were not on antibiotic both systemically and topically for minimum of 24 hours prior to sample collection were included in this study.

Exclusion criteria:

Patients who were suffering from CSOM and were on systemic antibiotics or who were on topical medication to the ear were excluded from this study.

Study methodology:

An informed consent was taken from the patient or from the guardian (in case of minor) in the prescribed format. At the time of sample collection all demographic details and relevant clinical data was collected in the specially designed Case Recording Form for each patient.

Taking aseptic precautions, the ear discharge was collected using sterile cotton swabs in duplicate prior to instillation of any topical medication. One swab was used for microscopy for Gram staining & KOH mount following standard protocol [12, 13].

The second swab was inoculated on Blood agar (BA), MacConkey agar (MA) and finally in Brain heart infusion (BHI) broth. Blood agar and MacConkey agar plates were incubated at 37°C for 16 -18 hours. In case of no growth, plates were re-incubated for 48 hours and subculture was done from BHI broth on BA and MA and incubated at 37°C. If growth was observed preliminary tests like colony characteristics, Gram stain, catalase, oxidase, coagulase and motility test were carried out as per standard procedures[12, 14]. Final identification of the pathogen and its antimicrobial susceptibility testing was done by automated method (Vitek2 Compact system, bioMèrieux). The second swab was also used for fungal culture following standard protocol [13]. The inoculated media (Sabouraud's Dextrose Agar slant containing 0.05mg/ml of chloramphenicol) were incubated at 37°C and 22°C for 14 days and were checked on 3rd, 7th and 14th day for any evidence of growth. In case of growth of yeast-like colony further identification was done by colony characteristics, Gram staining, germ tube test and finally complete identification and anti fungal susceptibility was performed using Vitek2 Compact system (bioMèrieux). When growth of molds, was observed identification was done by colony characteristics, microscopically by lactophenol cotton blue mount and by performing slide culture[13].

Statistical Analysis:

The results thus obtained were analyzed using Chi square test for their statistical significance. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Results

In this study males were found to be affected more than females with CSOM i.e, out of the 64 patients studied, 53.12% (34/64) were males and 46.87% (30/64) were females this observation was not significant (p=0.733) on statistical analysis. Age wise distribution showed that 50% (32/64) were from age group 0-10 years followed by 20.31% (13/64) in age group 11-20 years (Table 1). This observation when analyzed was found to be statistically significant (p=0.06).

Table 1: Age wise distribution of the cases (N=64)

Age group	Number of cases
0-10 years	32(50)
11-20 years	13(20.31)
21- 30 years	5(7.81)
31-40 years	8(12.5)
41-50 years	3(4.68)
51-60 years	1(1.56)
61-70 years	1(1.56)
71-80 years	1(1.56)
Total	**64(100)**

Figure in parentheses indicates percentage

X2 = 4.54 p = 0.06 significant

With 68.75% (44/64) in low, 28.12% (18/64) in middle and 3.12% (2/64) in high socioeconomic status, preponderance of CSOM was observed in low socioeconomic status. A higher number of CSOM cases as well as higher culture positivity were found in patients who belonged to low socioeconomic class. With p value of 0.00032 this observation was found to be statistically highly significant (Table 2).

Table 2: Distribution of the cases according to their socio economic status (N=64)

Socio economic status	Culture positive	Culture negative	Number of cases
Low	26(78.78)	18(58.06)	44(68.75)
Middle	6(18.18)	9(29.03)	18(28.12)
High	1(3.03)	1(3.22)	2(3.12)
Total	**33(100)**	**31(100)**	**64(100)**

Figure in parentheses indicates percentage

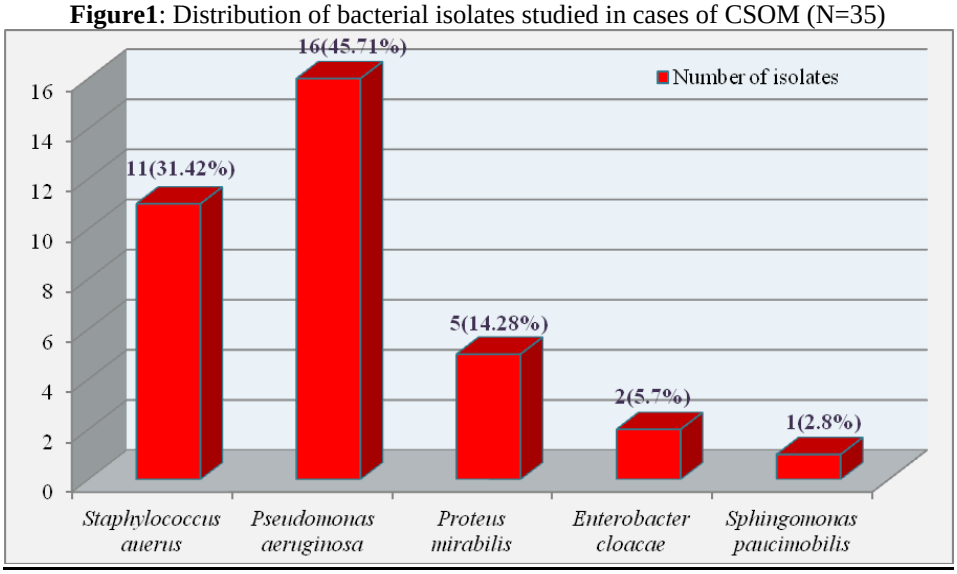
X2=12.90 p=0.00032 highly significant

A total of 70 samples from 64 cases were studied in this study. Out of 70 samples, 80% (56/70) were culture positive and 20% (14/70) were culture negative .With p value of 0.00032 this observation was found to be statistically highly significant. Of the cases studied it was observed that 60.93% (39/64) cases suffered from left ear CSOM, 29.68% (19/64) cases suffered from right ear CSOM and 9.37% (6/64) presented with bilateral CSOM.

In this study, from 32.85% (23/70), 30% (21/70) and 17.14% (12/70) specimen only bacteria, only fungi and multiple isolates were identified respectively. While in 10% (14/70) of the specimens no growth was observed.

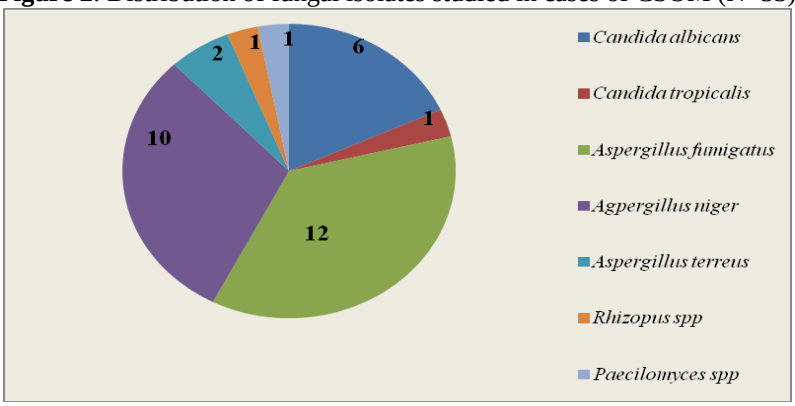
With 45.71% (16/35) *Pseudomonas aeruginosa* was found to be the predominant isolate followed by *Staphylococcus aureus* 31.42% (11/35), *Proteus mirabilis* 14.28% (5/35), *Enterobacter cloacae* 5.71% (2/35) and *Sphingomonas paucimobilis* 2.80% (1/35) (Figure 1).

Figure1: Distribution of bacterial isolates studied in cases of CSOM (N=35)



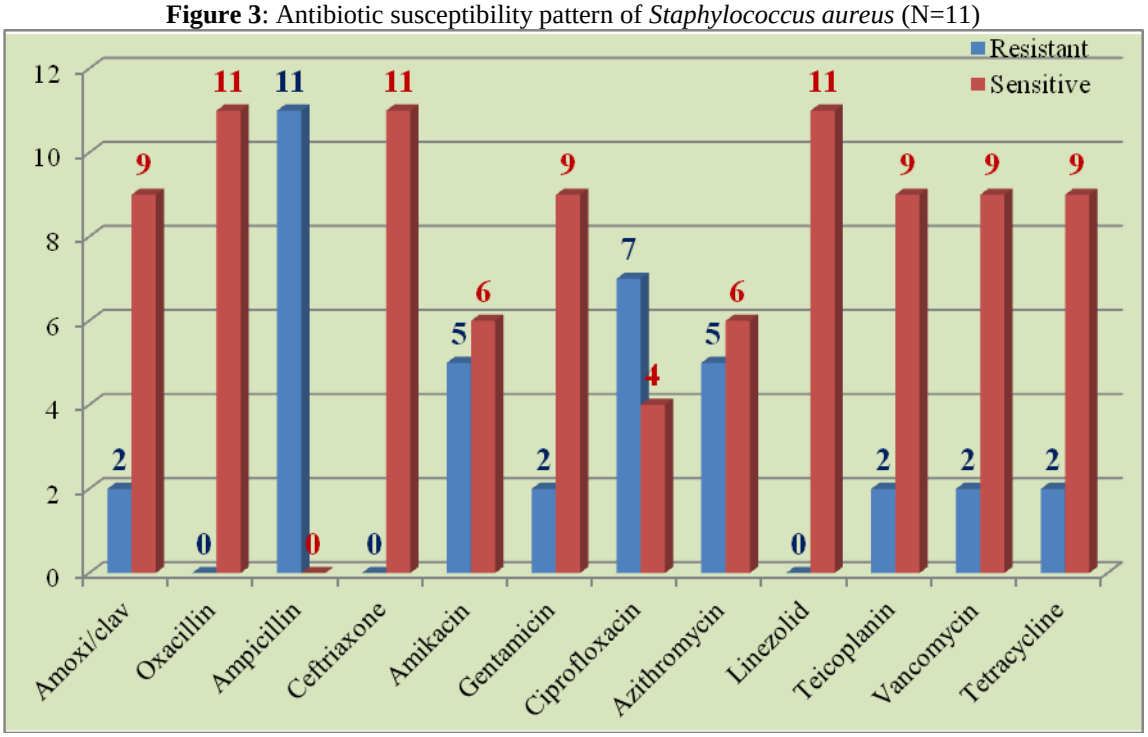
With 36.36% (12/33) *Aspergillus fumigatus* was found to be predominant fungal isolate. While, 30.30% (10/33) *Aspergillus niger*, 18.18% (6/33) *Candida albicans*, 6.06% (2/33) *Aspergillus terreus.* A few i.e., 3.03% (1/33) each of *Candida tropicalis*, *Rhizopus species* and *Paecilomyces species* were also observed (Figure 2). Among 12 samples that showed multiple isolates, predominant combinations were *Pseudomonas aeruginosa* with *Aspergillus fumigatus* followed by *Enterobacter cloacae* with *Aspergillus fumigatus.*

Figure 2: Distribution of fungal isolates studied in cases of CSOM (N=33)



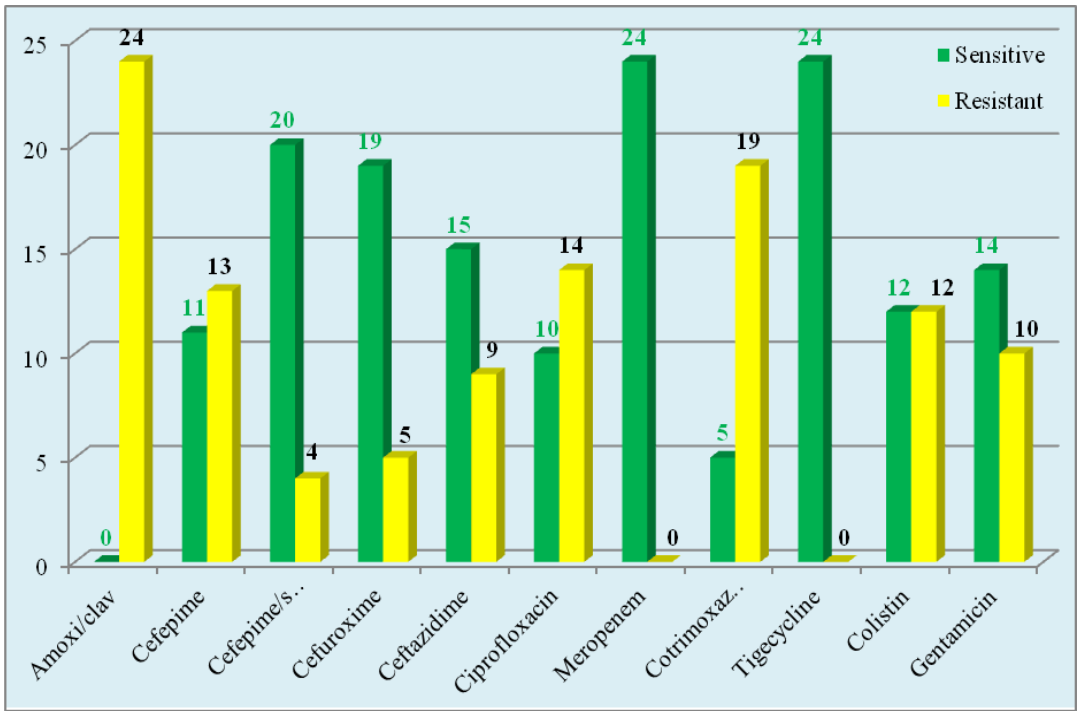
On analyzing the antibiotic susceptibility pattern it was observed that all the 11 isolates of *Staphylococcus aureus* were sensitive to oxacillin, ceftriaxone and linezolid while 81.81% (9/11) were sensitive to amoxicillin/clavulanic acid, teicoplanin, vancomycin, tetracycline and gentamicin. Sensitivity of isolates for amikacin, azithromycin & ciprofloxacin was 54.54% (6/11), 54.54% (6/11) and 45.45% (5/11) respectively. All the isolates studied were found to be resistant to ampicillin (Figure 3).

Figure 3: Antibiotic susceptibility pattern of *Staphylococcus aureus* (N=11)



The antibiotic susceptibility pattern of Gram negative bacterial isolates from CSOM showed 100% sensitivity towards tigecycline & meropenem. While sensitivity to cefepime/sulbactam, cefuroxime, ceftazidime, gentamicin, colistin, cefepime, ciprofloxacin and cotrimoxazole were 83.33% (20/24), 79.16% (19/24), 62.5% (15/24), 58.33% (14/24), 50% (12/24), 45.83% (11/24), 41.66% (10/24) and 20.83% (5/24) respectively. All the isolates studied were found to be resistant to amoxicillin-clavulanic acid (Figure 4).

Figure 4: Antibiotic susceptibility pattern of Gram negative bacterial isolates (N=24)



In the present study an attempt was made to analyse the type of phenotypic resistance of the isolates. Among the *Staphylococcus aureus* strains isolated from cases of CSOM, 27.27% (3/11) were phenotypically found to express macrolide-lincosamide-streptogramin B (MLSB) resistance, only 18.18% (2/11) isolates were found to express both vancomycin resistance i.e., VRSA and MLSB resistance phenotypically.

Among the yeast, *Candida albicans* was the predominant species isolated from CSOM cases followed by *Candida tropicalis*. All the seven strains of yeast were sensitive to fluconazole, amphotericin B, flucytosine and voriconazole.

IV. Discussion

Chronic Suppurative Otitis Media is a major health problem and occurs with a high incidence and prevalence in both developed and developing countries. In India, due to temperate climate with heavy monsoons, CSOM is a major complaint encountered in ENT clinics. In the present study an attempt was made to know the microbiological profile of CSOM (both bacterial and fungal)along with their antimicrobial susceptibility patterns from the cases of CSOM in the population attending our hospital.

In this study, CSOM in males with 53.12% (34/64) dominated that in females 46.87% (30/64), this is in accordance with the other studies Kumar S.A. Jagdish et al and Arun Ghosh et al [15, 16].The higher incidence in males was probably due to the fact that males are more involved in outdoor activities making them more prone to develop infection and exposed to moisture. Dominance of CSOM in the age group of 0-10 years i.e., 50% (32/64) of the cases followed by 20.31% (13/64) cases falling in age group of 11-20 years has also been observed by several workers [17, 18, 19] which was found to be statistically significant (p value < 0.001). The possible reasons for greater tendency of young children to CSOM is anatomical, due to shorter and horizontal course of Eustachian tube and increased frequency of respiratory infections causing AOM due to reduced physiological and immunological defense pathogens.

The CSOM cases in our study were found highest among individuals belonging to low socioeconomic status with 68.75% (44/64), it was statistically found to be highly significant (p value 0.00032). Thus role of improving the socioeconomic status of the general population will have a direct impact on reducing the prevalence of CSOM, especially in developing countries. Such observations have also been reported from most national and international studies on CSOM [20, 21, 22]. Poverty, overcrowding, lack of education, poor hygiene, malnutrition and lack of medical facilities have been suggested as a basis for the widespread prevalence of CSOM in people with low socioeconomic status.

In our study, *Pseudomonas aeruginosa* with 45.71% (16/35) was the commonest bacteria isolated, followed by *Staphylococcus aureus* with 31.42 % (11/35) from the cases of CSOM. Studies carried out in the past under similar conditions have also reported *Pseudomonas* and *Staphylococcus aureus* as the dominant

pathogen in CSOM [23, 24, 25, 26, 27]. Antimicrobial susceptibility profile of *Staphylococcus aureus* was found to be similar to the observations reported by Orji F.T. et al [28] and Vishwanath S. et al [27]. All the Gram negative bacilli were found to be completely sensitive to tigecycline & meropenem. While complete resistance was found against amoxicillin-clavulanic acid. Analysis of the phenotypic resistance of Gram negative bacilli reveals a low level of resistance probably due to the fact that most patients attended the out-patient clinic and were not exposed to highly resistant strains of hospital flora.

Aspergillus fumigatus as the commonest fungal isolate has also been observed in various studies [29, 30]. It has been suggested that the prolong use of topical broad spectrum antibiotics leads to suppression of bacterial flora and subsequent emergence of opportunistic fungal flora in the ear. In our study, antifungal susceptibility for *Aspergillus species* and other molds were not performed as it is still not standardized universally.

Since specimen were processed only for aerobic bacteria in this study therefore it is possible that this may not be a true reflection of polymicrobial etiologies of CSOM as the specimens were not processed for anaerobic pathogens and therefore incidence of anaerobes in CSOM cannot be commented upon. When results of our study were compared with the findings of other researches, it was clear that bacterial profile and antibiotic susceptibility pattern of CSOM has been changing over a period of time. Geographical variation and difference in patient population studied could also be the possible factor for variability. Moreover in developing country like India, self–treatment, instillation of oil for temporary relief and seeking treatment from the quacks further lead to chronicity of the disease.

V. Conclusion

Chronic Suppurative Otitis Media has become a matter of public health importance in the present days in developing countries like India. It is well known disease of multiple etiologies and its recurrence and persistence.

Irregular, haphazard and indiscriminate use of antibiotics not only precipitates the emergence of multi-resistant bacteria but also suppress normal bacterial flora of ear leading to fungal infection. Therefore it is strongly recommended to use carefully selected local or systemic antibiotics guided by culture and sensitivity, along with the use of frequent ear toilet as an effective treatment modality. This will prevent development of drug resistance and administration of unwanted antibiotics and in return would prevent complications related to CSOM.

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