

Original Research Article

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A Study on Etiological Agents with Special Reference to Fungal Isolates causing Chronic Suppurative Otitis Media in a Tertiary Care Hospital

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ABSTRACT

Chronic suppurative otitis media is a condition characterized by inflammation of middle ear affecting tympanic membrane, middle ear mucosa and other middle ear structures. To isolate, identify and characterize the fungal organisms and to detect the antifungal susceptibility pattern of the isolates by using conventional microbiological methods from specimens of clinically proved cases of chronic suppurative otitis media. Two hundred and forty five patients with CSOM were studied. Swabs were collected from each patient and cultured for bacteria and fungus. Standard methods for isolation and identification of bacteria and fungus were followed. Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method. Antifungal susceptibility testing was done by Disc Diffusion and Microbroth Dilution method. Out of the 245 samples studied for bacterial and fungal isolates, 225 samples showed growth of pathogens, 20 samples did not show any growth, 201 samples showed bacterial growth, The most predominant organism was *Staphylococcus aureus*, 24 samples showed fungal growth, *Aspergillus* sps being the predominant isolate. Antifungal susceptibility testing was also performed with discs fluconazole (10mcg) and itraconazole (10mcg) 23 isolates showed sensitivity to both and one resistant isolate was subjected to Minimum inhibitory concentration by Microbroth dilution method. Patients would be benefitted by accurate diagnosis of type of infection and the appropriate drug that will be sensitive for the infection can be administered to them.

Keywords

Chronic suppurative otitis media, Minimum inhibitory concentration, *Aspergillus* species.

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Introduction

The global burden of illness from CSOM involves 65-330 million individuals with draining ear. 60% of whom 39-700 million suffer from significant hearing impairment. CSOM accounts for 28000 deaths and a disease burden of over 2 million DALYs. Over 90% of the burden is borne by countries in the South-east Asia and western pacific region, Africa and several ethnic minorities in Pacific rim (Chronic

suppurative otitis media Burden of illness and Management Options). In India chronic suppurative otitis media is one of the most common condition met with in the Ear, Nose & Throat outpatient department. CSOM is still major cause of acquired hearing impairment in children especially in developing countries, Prevalence surveys, which vary widely in disease definitely. The incidence of chronic suppurative otitis

media is higher in developing countries especially among low socio-economic society because of malnutrition, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infection (Prakash *et al.*, 2013).

Micro-organisms found in chronic discharge differ from those found in acute suppurative otitis media. The commonest organisms isolated are *Pseudomonas aeruginosa*, Proteus species (*P. mirabilis* and *P. vulgaris*) and *Staphylococcus aureus*. These organisms are most likely to gain access to the middle ear from the external auditory canal through the tympanic membrane defect. Other organisms found less commonly in chronically discharging ears include *Escherichia coli*, *Streptococcus pneumoniae*, *Diphtheroids*, *Klebsiella* species and the anerobic Bacteroides species (Tony wright *et al.*, 2005).

Aspergillus is a saprophytic mold and is one of the primary colonizers of the manmade substrata. Its rapid growth and production of a large number of small, dry, easily aerosolized conidia make it a significant contaminant with regards to air quality and potential human exposure-related illness. Aspergilli are common in airborne dust, and their growth is aided by cerumen and the slightly acidic pH of the ear canal (Talwar *et al.*, 1988).

This study is carried out to make accurate diagnosis with the type of fungal infection and the appropriate drug showing sensitivity could be administered to them

Materials and Methods

This study was done over a period of one year from April 2015 to April 2016, 245 samples were collected from patients attending the Department of Otorhinolaryngology, Saveetha Medical

college and hospital, Thandalam, Kanchipuram district Tamil Nadu.

All the Ear swab samples of patients diagnosed with tubotympanic type of CSOM sent by the ENT department will be received at the Microbiology Laboratory.

They were processed for fungal examination using 10% KOH mount and Microscopic examination then further inoculated on fungal media for Isolation of fungus such as Sabouraud's dextrose agar and chrome agar. It was incubated at 27° C and 37° C for 2 – 14 days.

The growth was identified based on their morphological and cultural characteristics and microscopic examination was done using lactophenol blue staining technique.

All yeast isolates were subcultured on CHROM agar (HiMedia) and incubated at 37° C for 24 hours and the species were identified by type and colour of the colonies on CHROM agar media where *C.albicans* showed bluish green and *C.tropicalis* showed dark blue gray centre with pink halos.

All the moulds were subjected to slide culture method. Colonies were identified according to standard mycological methods and identified as *Aspergillus* species

Antifungal susceptibility testing for yeasts was done on Muller Hinton Agar supplemented with glucose and methylene blue with the itraconazole and fluconazole disc as per the reference CLSI M27- A3 and the moulds were tested by CLSI M8-A2 EUCAST Reference method guidelines.

Results and Discussion

Among the 245 samples studied for bacterial and fungal isolates, 225 samples showed

growth of pathogens, 20 samples did not show any growth. Among 201 samples showed bacterial growth, the most predominant organism was *Staphylococcus aureus*, 24 samples showed fungal growth as shown in Figure no. 1. There was a predominance of males (91.6666%) over females (8.333%)

Of the 24 fungal culture positive cases, the predominantly isolated fungi were *Aspergillus* species. Among the *Aspergillus*, the predominant species were *Aspergillus niger* (15 isolates) and *Aspergillus flavus* (5 isolates). *Candida albicans* (2 isolates) and *Candida tropicalis* (2 isolates) were the commonly isolated species of *Candida* and *Candida* species as shown in figure no.1 and table no 1.

Antifungal susceptibility testing was performed with discs fluconazole (10mcg) and itraconazole (10mcg) 23 isolates showed sensitivity to both as shown in Figure no 3 (a-d). The resistant strain was subjected to MIC with microbroth as shown

in the figure no 4. Susceptibility MIC with microbroth dilution method with both the drugs ranged between (0.12– 1) and MIC₅₀ - 0.5 and MIC₉₀ - 1 as shown in figure 5.

In the past, there were controversies regarding the existence of fungal etiology in CSOM but now it is considered to be a definitive clinical entity and an increasing problem as the incidence of the mycotic infections and the diversity of pathogenic fungi have increased dramatically in the recent years due to continuously increasing number of immunocompromised patients (Viswanatha *et al.*, 2011). Among the fungal etiology in CSOM, the most commonly isolated organisms are *Aspergillus* species and *Candida* species (Ibekwe *et al.*, 1997). In our study, *Aspergillus* species comprised of (83.3%) of the total fungal isolates, whereas (16.6%) of the isolates were *Candida* species. A study by Talwar *et al.*, from India reported higher isolation rate of *Aspergillus* species (60.2%) as compared to *Candida* species (17.6%).

Fig.1 Distribution of CSOM Samples

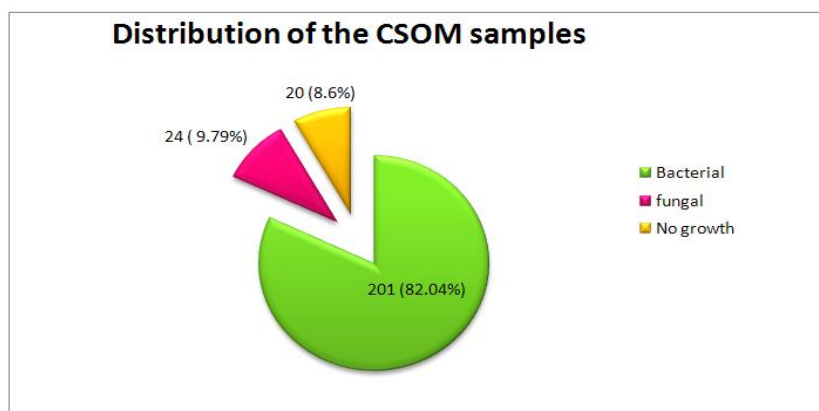


Table.1 Distribution of the *Aspergillus* and *Candida* species

Fungal Isolates	Total
<i>Aspergillus niger</i>	15 (62.5%)
<i>Aspergillus flavus</i>	5 (20.8%)
<i>Candida albicans</i>	2 (8.3%)
<i>Candida tropicalis</i>	2 (8.3%)

Fig.2 Distribution of fungal isolates

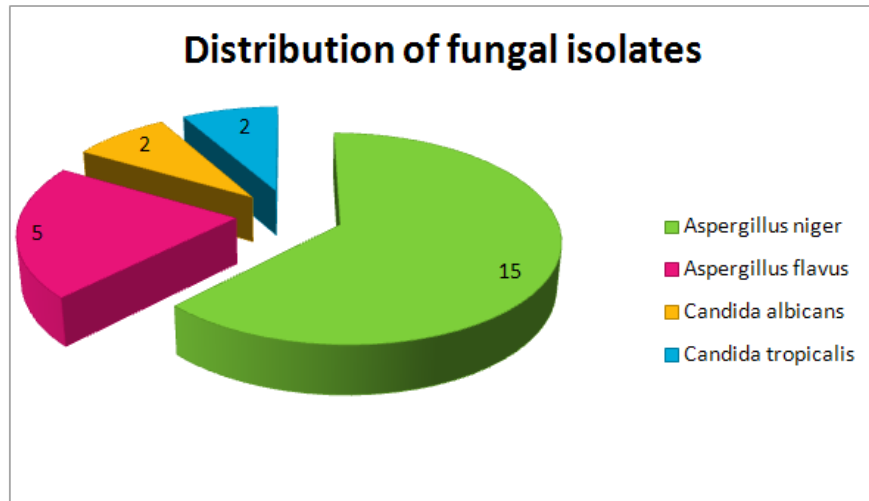
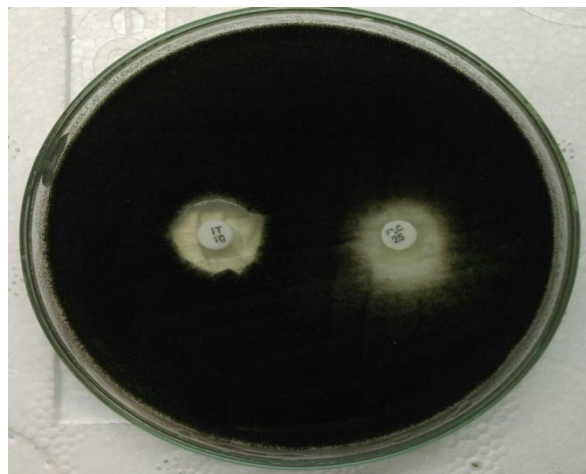
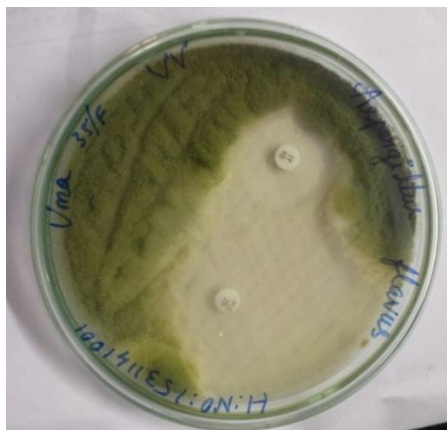


Fig.3 3 (a) , (b), (c) and (d) Antifungal susceptibility by Disc Diffusion Method



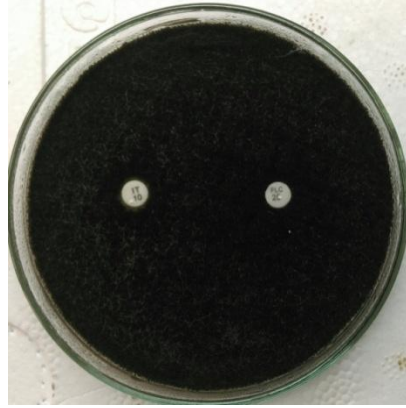
(a) Sensitive strain of *Aspergillus niger*



(b) Sensitive strain of *Aspergillus flavus*



(c) Sensitive strain of *Candida* spp



(d) Resistant strain of *Aspergillus niger*

Fig.4 Minimum inhibitory concentration by microbroth dilution method

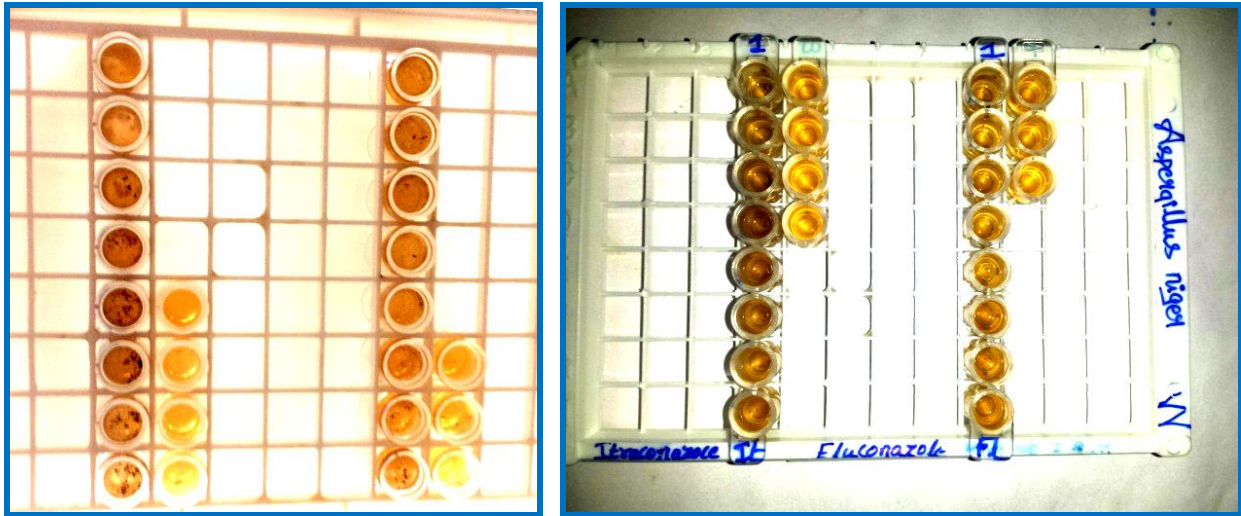
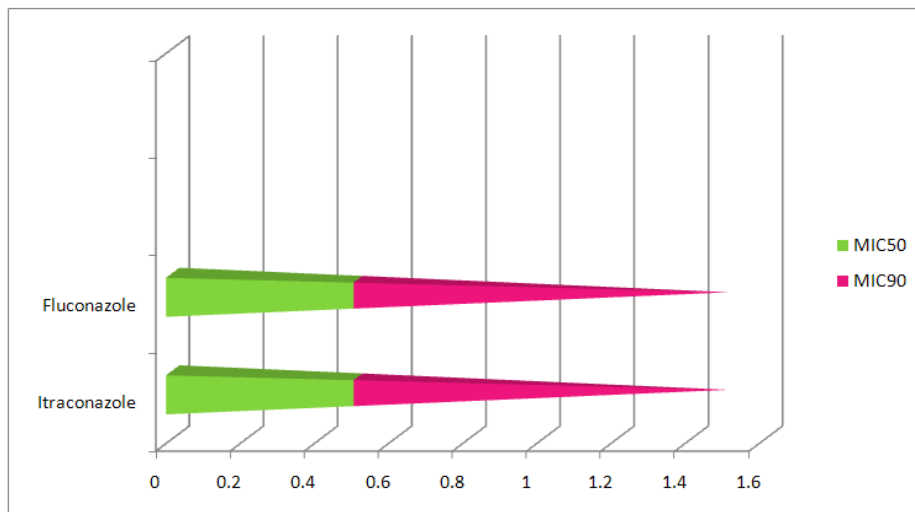


Fig.5 Distribution of Minimum Inhibitory concentration range



A recent study by Aneja *et al.*, from India reported *Aspergillus* in 86.8% of patients with *A. niger* (39.8%) and *A. flavus* (16.6%) to be the most common species which is almost similar to this study. In our study majority of the patients were in second and third decades of life. There was a predominance of males (91.6666%) over females (8.3333%) which is almost similar to a study done in Uttarakhand by Jugal *et al.*, showed predominance of males (59.03%) and females (40.9%) (Juyal *et al.*, 2014).

Patel *et al.*, have reported that Azole group showed sensitive among *C.albicans* and sensitive among *C. tropicalis* to fluconazole which is almost similar to our study. The limitation of the study is that a large number of isolates are required to find out the frequency of itraconazole resistance for *Aspergillus* spp. Although some studies do suggest that *Aspergillus* spp can be resistant to itraconazole. Large number of isolates are required for significant results.

The distribution of fungi is not only affected geographically but also changes with time and season. In the present study, higher incidence of the fungal infections was seen during the rainy season followed by summer season. This could be attributed to the hot and humid climate and the presence of dust in the environment which facilitates the fungal growth.

Prevalence of fungal infections of the ear during moist and humid conditions has been reported previously by several authors (7th Annual workshop on basic and molecular Diagnostics in mycology; Ozcan *et al.*, 2003).

In conclusion, chronic otitis media is an important cause of morbidity in very large group of Indian population in the form of preventable hearing loss. This morbidity is severe as duration of disease progresses.

From this study, patients would be benefited by accurate diagnosis and the appropriate drug that will be sensitive for the infection can be administered to them.

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