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Mycological Profile of Chronic Suppurative Otitis Media in Patients Attending Tertiary Care Hospital, Visakhapatnam

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Abstract

Chronic Suppurative otitis media is characterized by persisting dischargefrom the middle ear through atympanic perforation. It is an important cause of preventable hearing loss. In recent years, the importance of mycological aspects has been increasing due to excessive use of broad -spectrum antibiotics, corticosteroids, and an increase of immunodeficiency conditions.Keeping in view of increasing incidence of fungal agents in CSOM, critical diagnosis of causative agent by employing aseptic culture techniques and susceptibility testing for proper treatment of disease is need of the hour.

Aim:

Aim of the study was to know incidence, type and pattern of fungal floras in the patients with CSOM.

Material and Methods:The study was conducted in the Department of Microbiology, Andhra medical college. 200 clinically diagnosed CSOM patients attending ENT hospital were selected for the study. Samples were processed for fungal culture as per standard protocols.

Results:

The incidence of fungal infection was 17% in the study. The predominant fungal isolates wereCandida albicans and Aspergillusnigerfollowed by Aspergillus flavus, Candida tropicalis, and Candida parapsilosis.

Antifungal susceptibility testing of isolatesshowed that Candida albicans was sensitive to Amphotericin B and Clotrimazole and Aspergillusnigerwas sensitive to Amphotericin B and Nystatin.

Conclusion:

Regular laboratory examination with a definite search for aetiological agents is desirable in all cases of CSOM, since the prolonged use broad spectrum antibiotics or steroid drops may cause suppression of bacterial flora and subsequent emergence of fungal flora

Keywords: Antibiotics, CSOM, Fungal infection

Introduction

Chronic suppurative otitis media (CSOM) is a chronic condition, where there is accumulation of purulent fluid in middle ear in addition to tympanic membrane defect[(1)].Chronic suppurative otitis media is one of the most important causes of preventable hearing loss in India and other developing countries[(2)]. Bacterial flora plays a major role in etiology of chronic suppurative otitis media and to a lesser extent, fungi such as *Aspergillus*species and *Candida* species[(3)].

CSOM has received considerable attention, not only because of its high incidence and chronicity, but also because of issues such as innumerable complications

and high recurrence rate[4]. Higher incidence of otomycosis may be due to high degree of humidity, warm and dusky environment[5]. In recent years, the importance of mycological aspects has been increasing due to excessive use of broad -spectrum antibiotics, corticosteroids, and an increase in the number of immunodeficiency conditions[6].Keeping in view of increasing incidence of fungal agents in CSOM, critical diagnosis of causative agent by employing aseptic culture techniques and susceptibility testing for proper treatment of disease is need of the hour. [5]

Chronicity of ear discharge is an important factor in the cause of fungal infection of otitis media. It causes humid condition in the ear and alters the pH to alkaline. Epithelial debris which eventually helps the growth of fungus. Symptoms of otomycosis include itching, otalgia, aural fullness, hearing loss and tinnitus[7]. Opportunistic fungal infections are gaining greater importance in human disease as a result of increased number of co-morbid conditions and immunocompromised patients[8]. Fungal cultures are essential to confirm diagnosis. It is highly recommended that treatment of fungal infections of middle ear must be started as per antifungal susceptibility reports, to minimize complications such as hearing loss, tympanic membrane perforations and invasive temporal bone infection.

Treatment of fungal infection of middle ear includes microscopic suction clearance of fungal mass, discontinuation of topical antibodies and treatment with local or systemic antifungal agents for three weeks. Ear should be kept dry for three weeks. Small perforations heal spontaneously and larger perforation requires myringoplasty[1]. Recurrences are common in immunocompromised patients than in immunocompetent patients[8].

Aim:

Aim of the study was to isolate and identify the fungal aetiological agents of chronic suppurative otitis media and to determine their antifungal susceptibility patterns.

Methods:

The study was carried out in the Department of Microbiology, Andhra medical college, Visakhapatnam during the period of June 2018 to May 2019. A total number of 200 Samples were collected from the ears of patients who were clinically diagnosed to have suffered from Chronic suppurative otitis media, before starting treatment either orally or locally.

Inclusion Criteria:

1. Patients who were not on any antibiotic/antifungal treatment for previous 48 hours.
2. Diagnosed Chronic suppurative otitis media cases of all age groups with ear discharge of more than 6 weeks.

Exclusion Criteria:

1. Patients currently on treatment with antibiotics and antifungal drugs.
2. Patients with ear discharge of less than 6 weeks duration.
3. All known HIV or immunosuppression patients

Collection of Samples:

Two pus samples were collected per ear and placed into the sterile container which was labelled.The samples were immediately transported to Microbiology Laboratory for fungal studies.

Processing of Samples:

Processing of samples were carried out in the Department of Microbiology, Andhra medical college, Visakhapatnam.1st swab was used for Gram staining and KOH mount to see the presence of yeast cells and fungal elements.The 2nd swab was inoculated on Sabouraud dextrose agar slope (SDA) with Gentamycin.

For isolation of fungi, samples were inoculated on to Sabouraud dextrose agar (SDA) slopes and incubated in a Biological Oxygen Demand (BOD) incubator at 25°c. The fungal isolates were identified by their colony characteristics, any pigmentation on the observe and reverse (3). Corn meal agar is used for stimulation of chlamydospore formation and CHROM agar Candida medium is used for speciation of Candida species (Fig.1&2)(9). Potato dextrose agar was used for stimulation of sporulation of filamentous fungi(10). (Fig.3)The filamentous fungi were identified by tease mount technique stained with lactophenol cotton blue (LPCB)(11). Further characterization of filamentous fungi was done by slide culture technique (Fig.4). Yeast like isolates

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were identified by gram's staining and germ tube test(10).

Antifungal susceptibility testing of fungi was done by disc diffusion method as per CLSI guidelines M44-A for Candida species and M38A for filamentous fungi(12). Inoculum was prepared from the growth on SDA for 24 hours and adjusted to match the turbidity of 0.5 Mc Farland standards for yeasts and adjust the densities of conidial suspensions to an optical density at 530nm. OD value of Aspergillus ranges from 0.09-0.13(10). The dried surface of a sterile Mueller-Hinton + GMB agar plate is inoculated by evenly streaking the swab over the entire agar surface. Antifungal discs are placed onto the surface of the inoculated agar plate. The plates must be incubated at 35ºc for 24 hrs and measurements of inhibition zone are taken(12,13,14).

The antifungal discs used are Fluconazole (FLC) 25mcg, Ketoconazole (KT) 10mcg, Itraconazole (IT) 10mcg, Amphotericin B (AP) 100units, Clotrimazole (CC) 10mcg, Nystatin (NS) 100units. (Fig. 5, Fig.6, Fig.7

Fig 1- Dalmau plate method of Candida albicans on corn meal

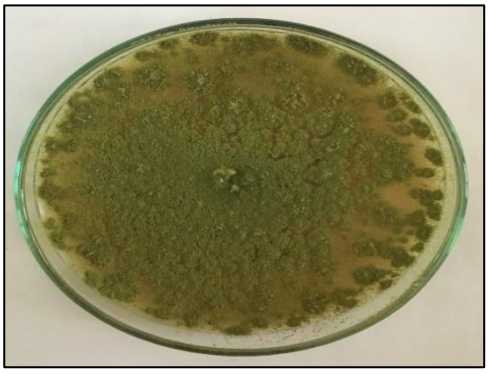
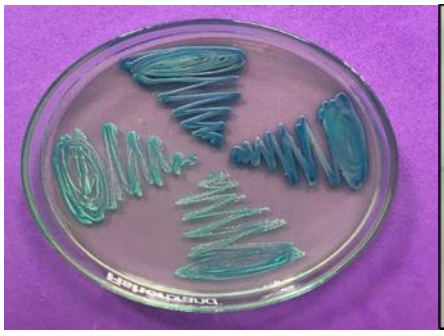


Fig-3 Growth of Aspergillus flavus on Potato dextrose agar



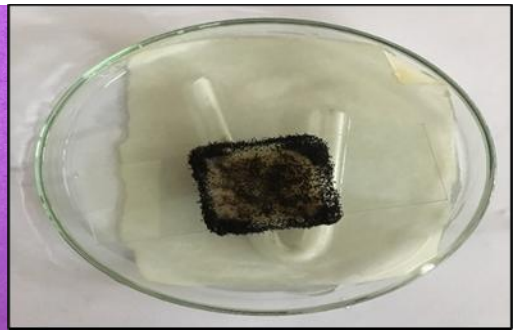


Fig 2- Candida species on chrome agar1 &2 Candida albicans3& 4 Candida

Fig 4 : Slide culture – Aspergillus niger

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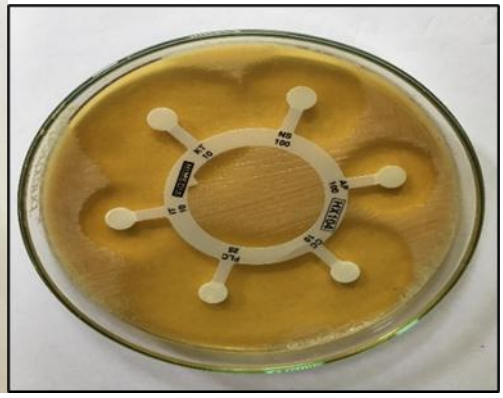
Fig– 6 Antifungal susceptibility of



Fig– 7 Antifungal susceptibility of Candida

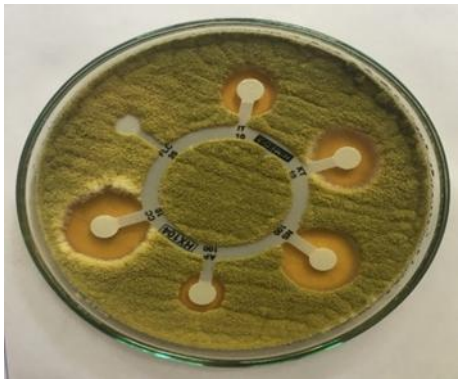


Fig 5 - Antifungal susceptibility of Aspergillus flavus

Results:

Out of total 34 fungal isolates, 30 (88.23%) were obtained in pure growth. The most predominant fungal isolates in pure growth were Candida albicans 10 (29.41%), and Aspergillus niger 10 (29.41%) followed by Aspergillus flavus 6 (17.64%), Candida tropicalis 2 (5.88%) and Candida parapsilosis 2 (5.88%). Out of total 34 (100%) fungal isolates, 4 (11.76%) were obtained from mixed cultures. The organisms isolated in mixed growth was Aspergillus niger 2 (5.88%) and Candida albicans 2 (5.88%)

Distribution of Fungal Isolates (n= 34)

Organism	TOTAL		Pure		Mixed	
	No.	%	No.	%	No.	%
1. Candida albicans	12	35.29%	10	29.41%	2	5.88%
2. Aspergillus niger	12	35.29%	10	29.41%	2	5.88%

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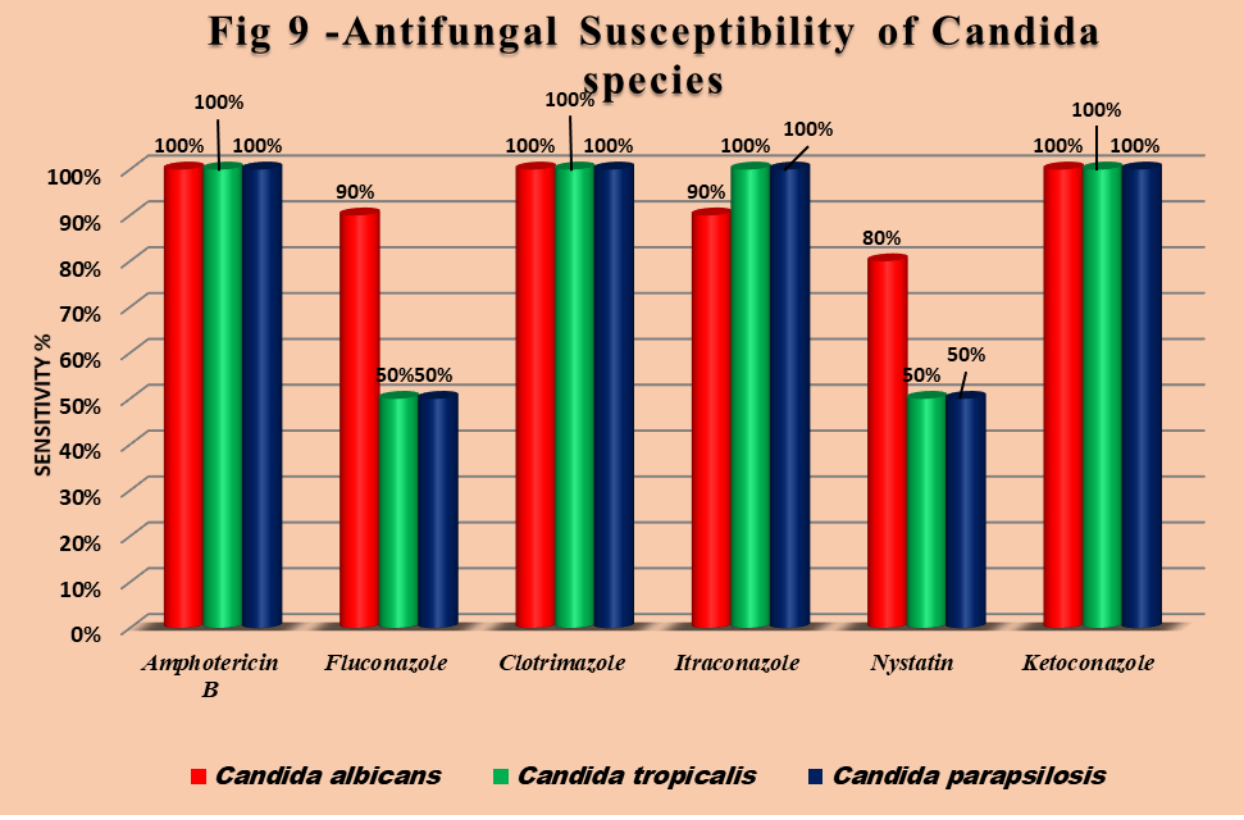
3. Aspergillus flavus	**6**	**17.64%**	**6**	**17.64%**	**-**	**-**
4. Candida tropicalis	**2**	**5.88%**	**2**	**5.88%**	**-**	**-**
5.Candida parapsilosis	**2**	**5.88%**	**2**	**5.88%**	**-**	**-**
TOTAL	**34**	**100%**	**30**	**88.23%**	**4**	**11.76%**

In vitro susceptibility data of antifungal agents is required to guide the selection of antifungal chemotherapy in the face of increasing fungal infections in immunocompromised as well as immunocompetent hosts. Routine susceptibility is advocated only in Candida isolates from deep sites or multi drug resistant infection.

In the study, all 12 (100%) isolates of Candida albicans showed sensitivity to Amphotericin B, Clotrimazole and Ketoconazole. Out of 12 isolates of Candida albicans, 10(90%) were sensitive to Fluconazole and 2(10%) were resistant. 10(90%) were sensitive to Itraconazole, and 9(80%) to Nystatin.

A total of 2 isolates of Candida tropicalis were isolated. Both the isolates (100%) showed sensitivity to Amphotericin B, Clotrimazole, Itraconazole and Ketoconazole. 1 (50%) was sensitive to Fluconazole and Nystatin and 1 (50%) was resistant.

A total of 2 isolates of Candida parapsilosis were obtained. They showed 100% sensitivity to Amphotericin B, Clotrimazole, Itraconazole and Ketoconazole. 1 (50%) was sensitive to Fluconazole and Nystatin and 1(50%) was resistant.



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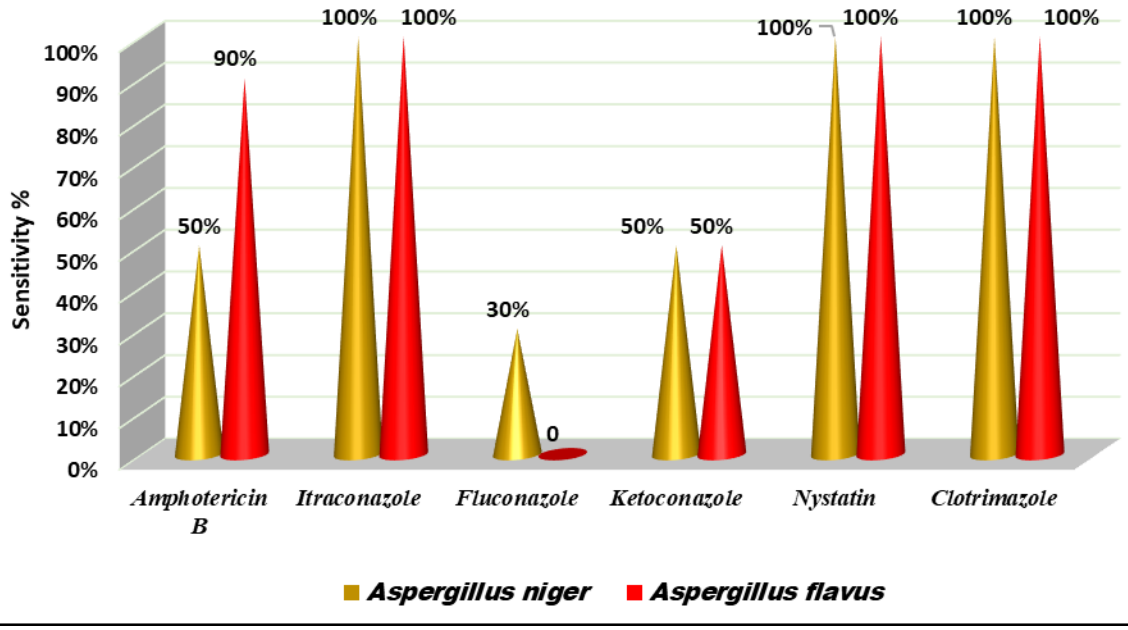
In the present study, a total of 18 Aspergillus species were isolated. Out of which 12 were Aspergillus niger and 6 were Aspergillus flavus. Antifungal susceptibility testing was done by disc diffusion method. Aspergillus niger showed 100% sensitivity to Itraconazole, Nystatin and Clotrimazole. 8 were resistant to Fluconazole. 6 were resistant to Amphotericin B and Ketoconazole.

In the study, a total of 6 isolates of Aspergillus flavus were isolated. They showed 100% sensitivity to Itraconazole, Nystatin and Clotrimazole.90% were sensitive to Amphotericin B and 10% was resistant. 50% were sensitive to Ketoconazole, and 50% were resistant. 100% isolates were resistant to Fluconazole

Fig 10 -Antifungal Susceptibility Pattern of Aspergillus species (n=18)



Discussion:

Chronic suppurative otitis media is one of the major health issues, mainly in developing countries. If left untreated, it causes destruction of middle ear structures leading to hearing loss and complications like mastoiditis, periostitis, facial paralysis, labyrinthitis, brain abscess, meningitis, and lateral sinus thrombophlebitis etc[(1)]. In CSOM, microorganisms reach middle ear either through Eustachian tube from nasopharynx or through perforated tympanic membrane from the external auditory canal[(2)].

Fungal infections of the middle ear are common as fungi thrive well in moist pus. The most commonly found fungi in CSOM are *Candida species* and *Aspergillus species*[(15)]. The indiscriminate use of broad- spectrum antibiotics, corticosteroids, cytotoxic chemotherapy as well as the increased incidence of conditions accounting for immune deficiency has led to the increase prevalence of superadded fungal infection in CSOM[(16)].It has been suggested that the irrational and excessive use of topical antibiotic and steroids for all cases with CSOM, promotes the growth of fungus[(17)]. Fungal infection of ear cannot be diagnosed clinically. The co- administration of topical antifungal with antibacterial agent is greatly recommended[(18)].

In the present study, culture positivity was seen in 94% of the cases where 17% were fungal isolates and the remaining being bacterial isolates. This correlates with studies of Loy AH et.al[(19)] PajorA.et.al.[(20)]. In the present study, males were59% and females were 41% whichcorrelates with the studies of Narayana R. S. et. al.[(21)] who reported males (59%), Saranya SK et al (61.42%)[(22)] respectively. In the present study, the disease was more prevalent in age group of 1- 10 years (42.5%)which correlates with the study of Ibekwe et al (55.2%)[(23)].In the Present study, 78.40% of bacterial isolates were reported which coincides with the studies of Fatima G et. al.[(24)]who reported 80.07%, Bhumbla. U. et. al.[(25)](79%) Whereas,

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Narayana R. S. et. al.[21](62.4%) and Attalah MS. et. al.[6](48%)reported relatively lower incidence. A higher incidence was reported by Sharma M. et.al[26] (89.6%), PajorA. et. al.[20](88.6%).

In the present study,fungal isolates were 17%, which coincides with Attallah MS. et. Al[6].who reported 17%, Kumar H et. al.[27](15%), and Pajor A et. al.[20] (11.4%). Whereas, Fathy Mohamed E. S. et. al.[15](21.67%) and Vaidya K et. al.[28](21.98%)reported comparatively higher incidence. Loy AH et. al.[19](8.8%), GH. Ettehad et. al.[29](6.56%) and Fatima G et. al.[24](2%)reported relatively lower incidence.

Candida isolates were 100% sensitive to Amphotericin B, Clotrimazole and Ketoconazole, 90% sensitive to Fluconazole and Itraconazole and 80% sensitive to Nystatin in our study which corelates with the studies of., Kaur P. et. al.[30], Jahn S. et. al.[31]and Pfaller et. al.[16] Whereas, Jyoti R. et.al.[32]reported higher incidence and Ramesh Agarwal et.al.[33]reported lower incidence.

In present study, *Aspergillus* isolates showed 100% sensitivity to Itraconazole, Nystatin and Clotrimazole and 50% sensitive to Amphotericin B, Ketoconazole and showed maximum resistance to Fluconazole (100% in *Aspergillus flavus* and 70% in *Aspergillus niger*) which correlates with the studies of RameshA. et. al.[33], Fathy Mohamed ES. et.al,[15]

Summary & Conclusion:

The frequency of serious fungal infections is on the rise because of increasing use of cytotoxic immunosuppressive drugs and newer antibacterial agents. A regular laboratory examination with a definite search for aetiological agents is desirable in all cases of CSOM, since the prolonged use broad spectrum antibiotics and/or steroid drops may cause suppression of bacterial flora and the subsequent emergence of fungal flora . Fortunately, the increase in fungal infections has been accompanied by the development of new, less toxic, systemically active alternates to Amphotericin B, such as Fluconazole, Itraconazole etc. Resistance to antifungal agents is emerging and in-vitro susceptibility data might be required to guide the selection of antifungal chemotherapy. Antifungal susceptibility testing has evolved during the last decade and has now become a relevant clinical tool.

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Disclosure:

All the authors listed have made a substantial direct and intellectual contribution to the work, and approved it for the publication.

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2. Dr. Nunsavathu Lakshmi*
3. Dr. Reddi Sankara Rao

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