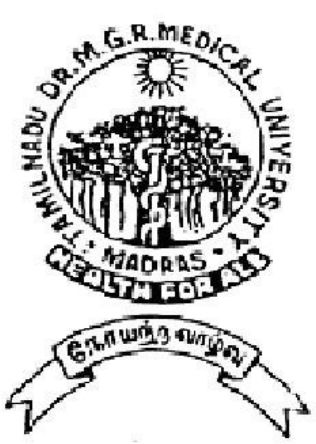
A STUDY OF OTOMYCOSIS IN MADURAI

DISSERTATION SUBMITTED FOR

M.D. (BRANCH – IV)

MICROBIOLOGY



THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

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SEPTEMBER - 2006

CERTIFICATE

This is to certify that the dissertation entitled **" A STUDY OF OTOMYCOSIS IN MADURAI"** by **Dr.B. CINTHUJAH**, for M.D. Microbiology Examination, September 2006 under the Tamil Nadu Dr. M.G.R. University is a bonafide work carried out under my direct supervision and guidance.

Director

Institute of Microbiology
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DECLARATION

I, Dr. B. CINTHUJAH declare that the dissertation titled "**A STUDY OF OTOMYCOSIS IN MADURAI**" has been prepared by me.

This is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment of the requirement for the award of M.D. Degree, Branch IV (MICRO BIOLOGY) degree Examination to be held in SEPTEMBER 2006.

Place : Madurai

Date : **Dr. B. CINTHUJAH**

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CONTENTS

INTRODUCTION

INTRODUCTION

Fungi constitute a large diverse group of heterotrophic organisms, most of which are found as saprophytes in the soil and decaying plant material. They are eukaryotes with nearly 50,000 species. They are essential in breaking down and recycling of organic matter. Some species contribute to the production of food, spirits, antibiotics and an array of enzymes.

Incidence of fungal infections has been grossly underestimated. Fungal infections are on the increase due to improved survival of immuno suppressed patients and better diagnostic facilities. Only 100 to 150 species are generally recognized as a cause of disease in humans.

Fungal infections are not communicable in the usual sense, but humans become an accidental host by inhalation of spores or by their introduction into tissue by trauma. The virulence factors favouring colonization of fungus in a human host are yet to be identified. Ability of the fungus to grow at 37^{o}C and elaboration of a variety of enzymes and toxins are speculated to contribute to virulence.

OTOMYCOSIS

Fungi are ubiquitous in the environment and consequently all are exposed to their infectious element. The external auditory canal is a skin lined cul-de-sac for effective sound transmission, and to protect the middle and inner ears from trauma, infection and environmental extremes. It is constantly exposed to a variety of fungal spores present in the air. Fungi are the causative agents of Otomycosis . They are seen in immuno competent persons

and Aspergillus is the commonest causative organism of this condition.

The external auditory canal (EAC) provides ideal conditions for the growth of micro organisms because of its warmth, darkness, moisture and presence of debris and nutrients. The bacterial flora are normal constituents of external auditory canal. Bacteria frequently and consistently isolated from ear canal are Staphylococcus epidermidis, Micrococcus, Diphtheroids, Enterobacteriaceae and Saprophytic Mycobacteria. Bacteria infrequently and inconsistently isolated from ear canal are Pseudomonas and Streptococcus pneumoniae. Fungi are rarely cultured from healthy ears. The cerumen layer, acidic pH and migrating epithelium of the external auditory canal protects against any infection.

Otomycosis or Fungal otitis externa is a superficial mycotic infection of the outer ear canal. The infection may be either subacute or acute and is characterized by inflammation, pruritis, scaling and intense discomfort. The mycosis results in inflammation, superficial epithelial exfoliation, masses of debris containing hyphae, suppuration and pain.

Primary fungal infection of the ear canal is less frequent than bacterial otitis externa. Fungi have been estimated to play a role in over 20% of all case of otitis externa. The percentage may be even higher in tropical climates. Fungi are found as saprophytes in the external auditory canal super imposed on an underlying bacterial infection. Otomycosis

develops most commonly in a chronically inflamed ear canal that has been treated with a variety of antibiotic or steroid drops.

Predisposing factors to fungal otitis externa are

1. Bacterial otitis externa
2. Increased ear canal moisture resulting from
    a. atmosphere (from a warm humid climate)
    b. ear discharge from Chronic suppurative otitis media (CSOM)
    c. application of ear drops
    d. bathing in contaminated water.
3. Trauma.
4. Warmth
5. Diabetes mellitus or other immuno compromised states

The predisposing factors lead to an absence of the protective cerumen layer, maceration of underlying skin, increase in the ambient pH, and a modification of the microbial flora of the external auditory canal there by favouring fungal growth.

Topical antibiotic-steroid drops used for treatment of chronic suppurative otitis media also increase the likelihood of otomycosis by providing humidity and also by hampering the local immune response.

Bathing removes the protective cerumen layer and also increases the pH.

The presenting symptoms of fungal otitis externa and bacterial otitis externa are often indistinguishable in the beginning. Although pain tends to be the dominant complaint in bacterial infections, the most common complaint in otomycosis is an itching sensation deep inside the canal. Other symptoms include feeling something in the ear, discharge and tinnitus. Scratching the ear canal with a fingertip or instrument may actually facilitate subepidermal invasion of the fungi. The itching generally progresses to a dull and deep seated pain that may be associated with discharge.

The accumulation of fungal debris in the inflamed and narrowed canal frequently produces hearing loss. Actual pain was most common in patients who acknowledged manipulating their ears. The ear becomes more painful as deeper tissues become inflamed.

The early symptoms of Otomycosis tend to become chronic if the disease is unrecognized or treatment is inadequate. In addition the general tendency for patients with Otomycosis to produce further trauma by instrumentation often produces a secondary bacterial infection which further compounds the problem.

Physical examination of the patient with Otomycosis generally demonstrates canal erythema and the presence of white, gray, yellow or black fungal debris within the canal. Oedema of the skin of ear canal is not as severe as in bacterial external otitis.

a. In the earliest stages of Otomycosis the fungal debris may be minimal and difficult to detect.
b. Usually the ***hyphal proliferation*** forms a characteristic membrane

referred to as ***blotting paper or wet newspaper*** like.

c. In more long standing cases, the debris may totally occlude the canal resembling a ***cottony mass.***

d. Trauma from previous instrumentation may produce bleeding and cause the debris to appear dark red or even purplish black.

CAUSATIVE FUNGI

Many fungi have been implicated in the clinical disease process, and as many a 61 different species have been identified in selected studies. Pathogens vary from temperate to tropical climate. The most common organisms are **Aspergillus, Candida** and **Penicillium**.

Aspergillus infection is characterized by a mild moist inflammation of the deep ear canal. The lumen is filled with large shed sheets of keratin, that have a wet tissue paper appearance. With the otoscope, individual colonies of the flowering fungus may be discerned complete with hyphae and condiophores. They may be yellow, grey and black depending on the subspecies.

Candida usually causes greater oedema and maceration of the deep ear canal. The lumen may be filled with a curd like material. Candida may exist in the budding yeast form or the pseudo hyphal form.

Penicillium has also been isolated in significant numbers throughout the world.

Treatment of fungal infection consists of removing the offending agent and altering the environment of the external auditory canal. Thorough cleaning of the external auditory canal

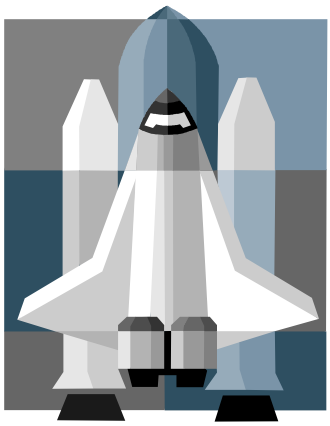
with removal of all fungal debris is essential. Topical Thiomersal, Amphotericin B (3%), Flucytosine(10%), Clotrimazole or Econazole usually brings a cure.

Amphotercin B is a broad spectrum polyene antibiotic obtained from Streptomyces nodosus. It has great affinity for ergosterol found predominantly in fungal cell membrane. It forms pores on the fungal cell and increases the permeability so that the essential molecules of the cell, leak from the cytoplasm and the fungal growth is blocked.Amphotericin B is available both as a parenteral and topical preparation. It‘s systemic use is limited by infusion related reactions and nephrotoxicity .

Itraconazole is a triazole.Triazoles have a five member azole ring that is attached by carbon – nitrogen bonds to other aromatic rings. Triazoles have three nitrogen in the azole ring. The azoles are usually fungistatic drugs and act by inhibition of cytochrome P-450 dependent C-14 demethylation (sterol 14-α-demethylase enzyme) in the biosynthesis of ergosterol found in the fungal cell membrane. It results in impaired ergosterol synthesis, accumulation of 14 methylated sterols, a defective cell membrane and consequently fungal cell death.
Itraconazole has less affinity for mammalian cytochrome P-450 than Ketoconazole and hence less adverse events: No adjustment in dosage is required even with liver or kidney impairment. It is a broad spectrum antifungal acting against most pathogenic fungi except zygomycetes.

The antifungal susceptibility testing is performed to provide information that allows the clinician to select an appropriate antifungal agent useful for treating a particular fungal infection.

In this study an attempt was made to identify the fungi causing Otomycosis from the cases attending GRH and also to carry out antifungal susceptibility testing.



AIM & OBJECTIVES

AIM AND OBJECTIVES

- To study the prevalence of Otomycosis in Madurai.
- To analyse the various predisposing factors responsible for Otomycosis.
- To isolate the fungi causing Otomycosis.
- To see the association of bacterial infections in Otomycosis.
- To find out the anti fungal susceptibility of the isolates.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Otomycosis is a common source of concern for several reasons. Unless manifested in the classical way they tend to be misdiagnosed. They are usually refractory for routine treatment. The condition should be suspected when routine treatment fails to relieve a diffuse otitis externa, when there is continuous irritation in the ear, and when the mass of debris in the meatus rapidly reforms after cleansing or when the fungus can be seen on otoscopy [22]. Mycotic infection of the ear is prevalent in tropical and subtropical countries. The incidence in temperate climates has increased in proportion to the use of topical antibiotics, which leave a medium, sterilized of other organisms in which the fungus may flourish. In severely immunocompromised patients, the external auditory canal can be extensively eroded by fungal invasion to produce a necrotic form of otitis externa. This form may then spread to involve other sites including the middle ear and the mastoids [31].

Meyer first described fungal infections of the external ear in 1844. He also sparked a long lasting debate about whether fungi are the primary infectious agents or are secondary pathogens that affect the skin of the external auditory canal after it has been exposed to bacterial toxins. There is overwhelming evidence to confirm the notion that fungi can be primary pathogens [12].

Beaney and Broughton in their article "Tropical Otomycosis" have attributed the greater frequency of otomycosis in the tropical countries to changes in the composition of cerumen induced by sweating [3].(1967)

Senturia et al in their book "Diseases of the external ear" has described many contributory factors to otitis externa. They are heat, humidity, trauma, absence of the protective coating of cerumen by repeated washing, cleaning or swimming [34].

Mocatela Ruiz E and **Lopez Martinez R** in their article "Clinical diagnosis of Otomycosis" found that out of 163 cases with clinical diagnosis of otomycosis only 72 were confirmed by positive mycological culture. They also noted no differences regarding the age and sex of the patient. They also found that the period of evolution was one year [24] (1980)

Yassin A, Maher A, Moawad M K in their study "Otomycosis – a survey in the eastern province of Saudi Arabia" – subjected 148 clinically suspected cases of otomycosis for culture. The patients were from 13 different countries mostly laborers and people of low socio-economic standard. They got 120 positive fungal cultures. Males were affected more than females and age ranged from 2 to 58 years [43] (1978).

Talwar P, Chakrabarthi A, Kaur, Pahwa R.K., in their article " Fungal infections of

the ear with special reference to CSOM " found fungi to play an important role in all ear infections.Aural swabs from 344 patients, 286 patients having CSOM , 44 patients having Otomycosis and 14 patients having Otitis externa were subjected to fungal culture. Out of 286 CSOM patients 49% had positive fungal culture. Out of 44 patients with Otomycosis , 79.5% had positive fungal culture. Of the 14 patients with Otitis externa, 66.6% had positive fungal culture. Of all these positives, 84.8% were detected both by smear and culture, 14.1% only by culture and 1% by smear preparation only [35].(1988)

Yehia M M, Al Habib H M, Shehab N M in their article "Otomycosis a common problem in North Iraq" found that out of 290 clinically diagnosed cases of Otomycosis only 179 were culture positive. Young adults of the age group 16-30 years were commonly affected. There was a significantly higher prevalence in females (65.4%), mainly in housewives [45].(1990)

Ravinder Kaur , Nalini Mittal and Manish Kakkar of India in their article "Otomycosis a clinico mycological study" found that out of 95 clinically diagnosed cases of otomycosis, only 71 had a positive fungal culture.Two of the samples contained two isolates, bringing the total number of isolates to 73.Despite strong clinical evidence of otomycosis, only 71 were positive. They attributed it to previous treatment.They also found that most culture negative cases of otomycosis were chronic cases with acute exacerbations.Out of the 95 patients 57 were males and 38 were females. The highest number of cases (41.1%) was seen in the age group of16-30 years [30]. (2000)

Garcia Martos et al in their study of 40 cases found a predominantly male population

(60%) infected. They predominantly belonged to two age groups either 31-40 years or over 50 years [13] (1993).

Predisposing factors:

In 1961, until **Gregson and La Touche** found fungus infection in 80 out of 180 patients suspected of Otomycosis, the disease was not considered of great importance in temperate climates. They suspected Otomycosis in cases of intractable otorrhoea either due to otitis externa or infection of radical mastoidectomy and fenestration cavities. They proposed it to the increased use of topical antibiotics known to favour growth of fungus [9].

Than K.M. of Burma found that Otomycosis was common in the rainy season [37].(1980)

Oliveri S, Capello G et al in a study of 82 Sicilian patients, in their article "Otomycosis aetiolgy and analysis of predisposing factors" found working in garden and wearing mechanical hearing devices were the common predisposing factors for Otomycosis. Bathing in salt or fresh water was not a significant risk factor [28]. (1984)

Hawke M, Wong J, Krajden in their article "Clinical and microbiological features of otitis externa" studied 40 cases of acute otitis externa and 99 cases of chronic otitis externa. Exposure to water, previous use of ear drops, hearing aid moulds and cotton tipped applicators

predisposed to otitis externa.Antibiotic drops usage predisposed to acute otitis externa of fungal origin.Fungi was commonly isolated from cases of chronic otitis externa. Ear mould induced otitis externa was caused by both bacterial and fungal organisms [17].(1984)

Garcia Martos P. Delgado D, Marin P in a study of 40 patients with Otomycosis found that otomycosis is common during summer (57.5%) and autumn (30%). It was also common after sea bathing (90%), trauma (27.5%) and prior antibiotic treatment (40%) [13].(1993)

Mugliston and O' Donoghue in their article "Otomycosis–a continuing problem" studied the microbiology of 1061 fungal isolates from ears during an 8 year period. They found that the incidence and pattern of the fungi and the accompanying bacteria causing ear infections has not been affected by the widespread use of powerful topically applied, antibiotics / steroid preparations [26]. (1985)

Chander J, Maini S, Subrahmanyan S, Handa A in their article "Otomycosis – a clinicomycological study" found that out of 40 male patients with otomycosis, 21 were Sikhs using turban. Turbans occlude the External auditory canal providing warmth, darkness and moisture favouring fungal growth. 45 out of

80 patients admitted using oil and garlic juice, antibiotics, steroids, antiseptics or wax solvents

as ear drops. Only two patients were diabetics [4].(1996)

Agrawal S R, Jain A K, Goyal R B in their article "A clinico mycological study of Otomycosis with special reference to silent tympanic membrane perforation" stated that Otomycosis is one of the common pathology of the external ear. This is usually associated with the unwanted use of antibiotics and steroids in cases of chronic suppurative otitis media. Humidity during the monsoons favours development of the fungus in the ear especially in nonhygienic conditions.The right ear was more prone to otomycosis [1]. (2001).

Vennewald I, Schonlebe J, Klemm E of Germany in their article "Mycological and histological investigations in humans with middle ear infection" found a strong association between chronic otitis media and Otomycosis .Chronic hyperplastic (polypoid) inflammation of the middle ear lead to increased production of mucus, which probably promotes colonization by pathogenic fungi in the middle ear as well as in the auditory canal [40]. (2003).

Darko E, Jenca A, Orenack M in their article "Otomycosis of Candidal origin in Eastern Slovakia" found that the most common predisposing factors for otomycosis were swimming in public pools, baths, spa or Diabetes mellitus [7]. (2004)

Yavo W, Kasi R.R., Ki Ki Barro of Ivory coast in their article "Prevalence and risk factors for Otomycosis" found that the predisposing factors for Otomycosis were daily ear cleaning, swimming in natural or artificial pools and excessive use of ear drops containing antibiotics and corticosteroids [44].(2004)

Jackman A , Ward R, April M, Bent J in their article "Topical Antibiotic induced Otomycosis" found a sudden increase in incidence of Otomycosis in their outpatient paediatric practice. Retrospectively they linked it to instillation of Ofloxacin ear drops. Each of the twenty six patients who were diagnosed with Otomycosis had used ototopical antibiotic ofloxacin for presumed bacterial otorrhoea [18]. (2005)

Symptoms:

The patient complaints of a feeling of fullness and intensive itching in the ears. The canal is oedematous, erythematous and there are numerous crusts. The inflammation is accompanied by exfoliation of the superficial epithelium and hearing may be impaired by obstruction of the canal with large masses of epithelial debris and mycelial strands. Associated bacterial infection causes marked pain and

suppuration.In prolonged infections eczematoid changes and lichenification may occur. The course is chronic with acute episodes especially in summer and intermittent remissions. The same symptoms occur in many other conditions affecting the external auditory canal including neoplasms. As a result careful physical examination and appropriate cultures are frequently needed to make a definitive diagnosis [12].

When present among debris it is sometimes possible to identify the black headed conidiophores of Aspergillus niger. The conidiophores of Aspergillus fumigatus on the other hand convey an impression of pale blue or green conidiophores. Candida albicans may be seen as deposits, but are difficult to differentiate from squamous debris.

Mocatela Ruiz E of Mexico in their article "Clinical diagnosis of Otomycosis" found secretion of fungal filaments and break in tympanic membrane were the common symptoms [24]. (1976)

Gregoriou et al found the most common symptoms were itching, absence of pain, discharge and no response to antibiotic treatment [14]. (1979)

Thank K.M. et al found itching as the commonest symptom (70%) followed by discomfort (54%) tinnitus (50%) hearing impairment and discharge (35%) [37]. (1980)

Oliveri S et al, in their article " Otomycosis - aetiology and analysis of predisposing factors " found itching was the characteristic symptom followed by pain, conductive hearing loss and tympanic perforation [28]. (1984)

Paulose and associate from Bahrain in their article "Mycotic infections of the ear – a prospective study" reported a series of 193 patients in whom the presenting symptoms noted were as follows: itching 170 patients (88%) ,feeling something in the ear 169 (87.5%), discharge 58 patients (30%) and tinnitus 22 patients (11.4%) [29]. (1989)

Ravinder Kaur et al found the most common symptom was ear block (93.7%) followed by pruritus (71.5%). Other symptoms were otalgia (65.2%), discharge (50.5) and hearing impairment (26.3%) [30] (2000)

Aetiological agents

Pathogens causing Otomycosis vary from temperate to tropical climates. Studies conducted in temperate countries like Sweden and England showed a preponderance of Candida species .Studies conducted in tropical and sub tropical countries like Italy, Nigeria, Egypt and Burma all found Aspergillus niger to be the most common isolate and Candida causing 1-16% of the infections [12].

Yassin A et al of Saudi Arabia found that in 128 fungal isolates Aspergillus niger accounted for 51.15% ,Aspergillus flavus for 18.32%, Penicillium notatum for 5.34%, Candida albicans for 4.58% and Aspergillus terreus for 4.58% [43].(1978)

Oliveri S et al of Italy in a study of 82 cases found Aspergillus niger in 67.1% of cases, Aspergillus flavus in 13.4% of cases, Aspergillus fumigatus in 1.2% of cases and Candida albicans in 11% of cases [28].(1984)

Talwar P et al in their study of fungal infections of ear with special reference to CSOM, found Aspergillus niger to be the most common isolate. Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Candida parapsilosis and Penicillium were also grown. In post antibiotic cases Aspergillus niger and Penicillum were the important isolates [35].(1988)

Gugnami Okafor B C, Nzelibe F, Nyokuub A N in their article "Aetiological agents of Otomycosis in Nigeria" did a study on 67 fungal isolates from Otomycosis and found that 13 were due to Aspergillus niger ,6 were due to Aspergillus flavus,. 6 were due to Candida albicans, 4 were due to Candida parapsilosis, 2 were due to Pseudoallescheria boydii, 1 was due to Candida guillermondi 2 were due to Aspergillus unidentified. and 1 was due to

Candida unidentified. They also did fungal culture from 46 healthy ears, 17 of which showed positive cultures mainly Aspergillus niger [15] . (1989)

Kombila M et al of Liberville in their article " Fungal Otitis in Libreville - a study of 83 cases " found Aspergillus niger in 26%, Aspergillus flavus in 17%, Candida parapsilosis in 18%, Candida albicans in 9%and Fusarium in 1% in 83 cases [21] .(1989)

Yehia M M, Al habib H M, Shehab N M, in their article "Otomycosis - A common problem in Northern Iraq" found Aspergillus was the dominant species isolated, about 92.1% among 179 isolates. Candida accounting for 7% of cases and others 1% [45] **(**1990).

Mohanty J C, Mohanty S K, Sahoo RC, Ghosh S K in their article "Clinico-microbial profile of Otomycosis in Berhampur" carried out fungal culture in 54 clinically diagnosed cases of Otomycosis. The fungi isolated were Aspergillus niger (41.1 %), Aspergillus flavus (24 %), Aspergillus fumigatus (3.7 %), Candida albicans (5.5 %), other Candida species(3.7 %), Mucor (1.89 %) and Pencillium (3.7%). The bacteria isolated were Proteus species, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis and Diphtheroids [25]. (1999)

Ravinder Kaur et al found that out of 73 fungal isolates Aspergillus fumigatus accounted for 41.4% and Aspergillus niger 36.9%.Various Candida species were isolated in the remaining [30].(2000)

Egami et al from Japan found that Aspergillus terreus was the commonest pathogen causing Otomycosis followed by Aspergillus niger and Aspergillus flavus. They also evaluated biological differences between clinical and soil borne strains of Aspergillus terreus. The clinical strains showed slower growth on malt extract agar and different pattern of fingerprinting by Random Amplified Polymorphic DNA analysis [11] (2003)

Jadhav V J, Pal, Mishra G S in their article "Aetiological significance of Candida albicans in Otitis externa" has described a study on 79 patients(42 males, 37 females) with Otomycosis. They found only two cases due to Candida albicans. One was a male aged 18 and the other was a female aged 20 years,. The organism was repeatedly isolated in cultures and also demonstrated by microscopy. Both had

unilateral otomycosis and used antibiotic drops and wooden sticks for wax removal [19].(2003)

Yavo W et al of Ivory coast in a study of 49 fungal isolates from 115 patients found A.flavus in 20.4%,Candida albicans in 16.3%,Candida parapsilosis in 14.3% and A. niger in 12.28% [44].(2004)

Frank E Lucente states that most cases of Otomycosis are actually mixed bacterial and fungal infections, the most common bacterial isolates being Staphylococcus aureus, Pseudomonas species and Proteus species (1993) [12].

Darko E et al isolated Staphylococcus epidermidis (31),Staphylococcus aureus (16) , Alpha – haemolytic streptococci (14), Neisseria species (14), Proteus mirabilis (3),Haemophilus species (1) and Escherichia coli (1) from 40 cases of Candidal Otomycosis, some ears harbouring more than one bacterial isolate usually as commensal. (2004) [7].

Arshad M, Khan N U, Ali . N., Afridi N M in their article " Sensitivity and Spectrum of bacterial isolates in infectious otitis externa" found out that out of 124 samples with pure otitis externa with out CSOM or Otomycosis only 108 were positive for bacterial culture. Staphylococcus aureus was found in 38% and Pseudomonas aeruginosa in another 38%. Other bacteria isolated included Proteus, Enterococci, Klebsiella and Escherichia coli (2004) [2].

Karaarslan A, Arikan S. Ozan M. found MIC – 2 of Itraconazole as 0.125 – 1 mg/l for A.niger (22 strains), 0.06 – 0.25 mg/l for A.flavus (10 strains), 0.125 mg/l for A. terreus (2 strains) for 34 Aspergillus isolates isolated from Otomycosis by Microdilution method (2004) [20].

Nong H, Li J, Huang G, Nong D In their article " The observation of mycology and clinical efficacy in 325 cases with Otomycosis " identified 110 positive fungal cultures of which 79% were Aspergillus which was sensitive to Itraconazole, 8% were Candida albicans which was sensitive to Itraconazole and Amphotericin B and 5% Penicillium citrinum sensitive to Amphotericin B [27]. (1999)

David W Denning W, University of Manchester in his article presented at the "National Workshop of Mycology-2006", Department of Microbiology, National Institute of Medical Sciences, Hyderabad had stated that about 15% of Aspergillus fumigatus isolated are resistant to Itrazonazole. Where as 30% are resistant to Amphotericin B. Itraconazole resistance is seen at 0-3% among primary isolates, but resistance is more from patients already on treatment. Mutations in the target gene 14-alpha demethylase (CYP-51A) is implicated in resistance to Itrazonazole. Alterations at glycine 54(G54) in CYP-51A have been described in both clinical isolates and laboratory mutants of Aspergillus fumigatus [8].

No azole resistance has been described in Aspergillus flavus, but there is a general degree of Amphotericin - B resistance. Aspergillus terreus and Aspergillus nidulans are intrinsically resistant to Amphotericin B, but no azole resistance has

been described in these strains. Eventhough there is no described resistance to Itraconazole by Aspergillus niger 31% of their clinical culture collection were resistant. [8]

Edward J Bottone et al in their article "Basic mycology underscoring medically important fungi" in the "Otolaryngologic clinics of North America" give the reasons for the frequency of recovery of A. flavus, A. fumigatus and Candida from human infection mainly due to the presence of virulence factors, exoenzymes and glycoproteins [10].

The manual of the Aspergilli by **Charles Thom and Kenneth Rapper** explains that Aspergillus flavus with brighter shades of green, when subjected to a vapour of ammonia would lose the green color and assume the somber yellow shades of variant members of the same group and that this reaction was reversible, since the vapour of acetic acid would restore or even intensify an original green shade. The experiments pointed to the hypothesis that the wide range of shades produced by mixtures of yellow and green in the A flavus group may be attributed to racial limitations, strain variations and the range of hydrogen-ion concentration produced by metabolism.

Czapek's solution agar has a mixture approximately neutral in reaction, which is readily made in any laboratory in fairly uniform manner and permits moderately vigorous growth of nearly all of the saprophytic Aspergilli. The quantity of mycelium and conidia produced by many forms are much greater upon other media, but for comparative study a moderate growth of the majority of the species is more useful than the great mass of mycelium and conidia which are readily obtained by using enriched substrata. So Czapek's agar is used as a routine medium for culture work [23].

Ruchel R.Margraf S. in their article "Rapid microscopical diagnosis of deep seated mycosis following maceration of fresh specimen and staining with optical brightners" state that mycology laboratories traditionally use the potassium hydroxide method, which gradually dissolves human material and makes fungal cells easier to see. But even for experienced personnel, the sensitivity of this method is relatively low. Using phase contrast microscopy with the KOH procedure is an alternative method that facilitates detection of hyphae provided that the preparation is of thin consistency. Thick areas of a preparation cannot be examined by phase contrast microscopy. The ideal technique for use in mycology and bacteriology laboratory is the KOH –calcofluor white method. This technique uses KOH to dissolve human material and an optical brightener called calcofluor white that binds to the cell wall of the hyphae. Fungal cell wall including septations fluoresce intensely when viewed using a fluorescence microscope equipped with correct filters [32] **(**1993).

MATERIALS & METHODS

MATERIALS AND METHODS

This study was conducted at the Government Rajaji Hospital attached to Madurai Medical College for a period of one year from January 2005 to December 2005.Patients presenting with symptoms of Otomycosis were selected as the study population.

The Otomycosis study group consisted of 150 patients (80 males and 70 females) aged between 7 and 80 years who had been clinically diagnosed as Otomycosis. Their infection was diagnosed clinically on the basis of symptoms, pruritis, otalgia, blockage, hearing impairment and the presence of fungal debris in the external ear.

The following patients were included in the study

1. Patients with otitis externa and otoscopic evidence of otomycosis.
2. Patients with CSOM and with otoscopic evidence of Otomycosis.

Exclusion criteria

1. Patients with otitis externa and without otoscopic evidence of fungi.

For patients presenting with Otomycosis with strict aseptic precautions, after swabbing the pinna and adjacent area of the ear with antiseptic (Dettol), the debris in the external auditory canal was collected by moist sterile swabs or by sterile forceps. The collected specimen was promptly transported to the lab and processed on the same day

10% POTTASIUM HYDROXIDE MOUNT

Direct microscopy with 10% Potassium hydroxide was done on the specimen.

Procedure:

a. The material to be examined was placed on a clean glass slide.
b. A drop of 10% KOH, was added to the material and mixed.
c. A cover slip was placed over the preparation with out any air bubbles
d. The KOH preparation was kept at room temperature until the material was cleared. The slide was warmed at times to speed the clearing process.
e. The preparation was examined by bright field microscopy.

The presence of fungal elements (hyphal elements, Conidiophore, spores) was looked for.

The specimen was subjected to both fungal and bacterial culture.

Fungal culture

The specimen was inoculated on Sabourauds dextrose agar & Czapek's agar on the same

day. Cycloheximide / Actidione was not added to the Sabouraud's dextrose medium, as they inhibit a large number of saprophytes. However Gentamicin 80mg was added to one litre of the medium to minimize bacterial contamination. Czapek's medium with Gentamicin was also prepared as it was the standard medium based on which all Aspergilli are speciated.

All inoculated media were incubated both at 25^{O}C and 37^{O}C and were observed daily for a week and twice weekly for another one week. The use of a variety of culture media at incubation temperatures 25^{O}C and 37^{O}C increased the chance of recovery of fungal pathogens.

GROSS MORPHOLOGY

The following factors were noted in the growth of the fungus

1. **RATE OF GROWTH OF THE FUNGUS**
    a. rapid grower grew within 2-5 days
    b. intermediate grower grew within 6 – 10 days
    c. slow grower grew within 2 – 3 weeks
2. **SURFACE**
    a. flat
    b. hemispherical
    c. raised
    d. folded
    e. verrucose
    f. cerebriform
3. **TEXTURE**
    a. yeast like
    b. glabrous

c. powdery

d. granular

e. velvety

f. cottony

4. PIGMENTATION

The pigmentation on the surface of the colony was dependent on the color of the spores and did not appear until significant spore formation.

The pigmentation on the reverse of the colony was formed by soluble pigments in the medium.

The surface of the colony was looked for blue-green, black, cinnamon brown, yellow green, blue gray or white pigmentation.

The reverse was looked for white, golden, red brown, olive, yellow or purplish red pigmentation

Lactophenol cotton blue mount (Wet mount)

On a clean glass slide, a drop of lacto phenol cotton blue was kept. A small piece of the fungal growth was kept on it and teased using teasing needle. A cover slip was applied over it without any air bubbles. It was then viewed under light microscope for microscopic morphological features, first under low power objective and then under high power objective.

MICROSCOPY

The basic microscopic morphology of **Aspergillus** species were looked for. The following features common to all Aspergilli were noted.

1. Presence of septate and hyaline hyphae.
2. Presence of basal foot cell terminating in a vesicle at the apex.
3. Presence of phialides which are uniseriate or biseriate.
4. Arrangement of phialides in a radiate or columnar fashion.
5. Presence of spherical conidia arising from the phialides forming radial chain.

Features which are unique to certain species of Aspergillus like **sclerotia, cleistothecia, aleuriconidia** and **hulle cells** were also noted.

Cleistothecium: A round closed structure enclosing the asci, which carry the ascospores. They are produced during the sexual reproductive stage of some Aspergillus species like Aspergillus nidulans and Aspergillus glaucus.

Sclerotia: Hard masses with characteristic surface marking and coloration and consisting of thick walled parenchyma like cells and occur in several groups of Aspergillus like Aspergillus flavus, Aspergillus niger, Aspergillus candidus.

Aleuriconidia: A type of conidium produced by lysis of the cell that supports it. The base is usually truncate and carries marks of the lysed supporting cell.It is seen mainly in Aspergillus terreus.

Hulle cell: A large sterile cell bearing a small lumen. Similar to cleistothecium it is associated with the sexual stage of some Aspergillus species.

Penicillium

When the growth on SDA showed shades of green or blue green with velvety to powdery surface, wet mount was put by teasing the growth. Penicillium was confirmed by the following microscopic features:-

1 .Septate hyaline hyphae

2 .Brush like conidiophore

3 .Conidiophore branching into metulae

4 .Metulae dividing into phialides

5 .Phialides having chains of conidia

Candida

If the growth was creamy white with curdy odour, Grams staining was done, if gram positive budding cells were seen Candida was confirmed. It was further speciated by germ tube test, Chlamydospore formation test, sugar fermentation test and sugar assimilation test.

Germ Tube Test

The production of germ tube as a method for presumptive identification of Candida albicans is known as **"Reynolds Braude"** phenomenon. The culture of

Candida was lightly touched with a loop and added to 0.5ml of normal human serum and incubated at 37°C for 2-4 hours. A drop of the suspension was taken on a slide and examined under the microscope. The germ tube was seen as a long tube like projections extending from yeast cells. They grew at the distal end. There was no constriction at the point of attachment to yeast cells .

Chlamydospore formation

The suspected strain of Candida was streaked across the **Corn meal agar** ploughing the medium .A cover slip was placed over the streak taking care that the streak was seen to project beyond the cover slip. The plate was incubated for 24 to 48 hours at 25°C. The edge of the cover slip was focused under the low power of the microscope as the chlamydospores were found best in that area. There was formation of large, highly refractile thick walled chlamydospores which confirmed Candida albicans.

Sugar Fermentation Test

Sugar fermentation medium contains peptone 1%, NaCl 0.5%, Andrade's indicator 0.005%. Different sugars like dextrose, lactose, maltose, sucrose in a concentration of 2% were taken, in test tubes. Inverted Durham's tube was placed to note the collection of gas. The test tubes were inoculated by adding 1 drop of the Candida suspension. The sugar tubes were incubated for 48 – 72 hours at 30^0C. If the sugar was fermented the colour of medium was changed to pink. C. albicans fermented glucose and maltose and did not ferment sucrose and lactose. C. parapsilosis fermented

glucose only and did not ferment sucrose, lactose and maltose.

Sugar Assimilation Test

The yeast like fungi were further tested for carbohydrate assimilation. Suspension of the fungi in saline was prepared to a density, equivalent to Mc Farland No 4. **Yeast nitrogen base agar** plate containing bromo cresol purple was prepared. The suspension of the yeast was poured over it. The excess inoculum was removed and the **Yeast nitrogen base agar** medium was allowed to dry for 10 minutes. With sterile forceps the carbohydrate discs, dextrose, lactose, maltose, sucrose were placed on the surface of the medium; 30 mm apart from each other. The plate was incubated at 30^{o}C for 24-48 hours. The plates were removed and observed for colour changes around the carbohydrate disc and for presence of growth surrounding them. For control, test control yeasts were used. Growth of the yeast produced an opacity in the medium. It indicated the ability of the isolates to assimilate the sugar. C.albicans assimilated glucose, galatose, maltose, sucrose, cellobiose, lactose and trehalose but did not assimilate raffinose. C. parapsilosis assimilated glucose, galatose, sucrose and maltose but did not assimilate lactose, cellobiose and raffinose.

SLIDE CULTURE

Slide culture was done wherever necessary.

Riddles slide culture A sterile Petri dish was taken and it was duely labeled with the specimen number and date of inoculation. A grease free sterile glass slide was placed in the sterile Petri dish and from the already prepared Sabouraud's dextrose agar, a one

centimeter square block of agar was cut and placed in the centre of the glass slide. From the primary growth of the concerned fungi the four corners of the agar block were inoculated. This was covered with a sterile cover slip. 20% glycerol was kept in a sterile screw cap inside the Petri dish to keep the atmosphere humid. This was incubated at room temperature till adequate growth of the fungus was obtained. When spores were evident a Lacto phenol cotton blue mount was made by placing the cover slip from the slide culture to a glass slide. A second preparation can be made by removing the agar block from the slide culture and doing a lacto phenol cotton blue preparation on the slide.

The microscopic morphological features like hyphae, Conidiophores and Conidia were noted.

BACTERIAL CULTURE

Similarly bacterial culture was put up from the aural swabs in Nutrient agar, Mac conkey and Blood agar plate. The inoculated plates were incubated at 37°C for 18 – 24 hours. The next day it was examined for growth of the organisms. Colony morphology was noted. Grams staining, motility and biochemical reactions were put up. The bio chemical reactions used for identification were:

- Catalase production,
- Oxidase production,
- Triple sugar iron fermentation,

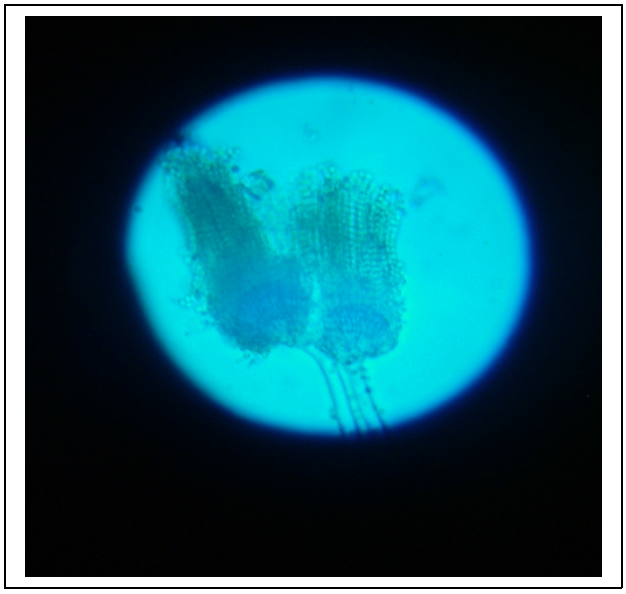
- Indole production,
- Citrate utilization,
- Urease production,
- Coagulase production.

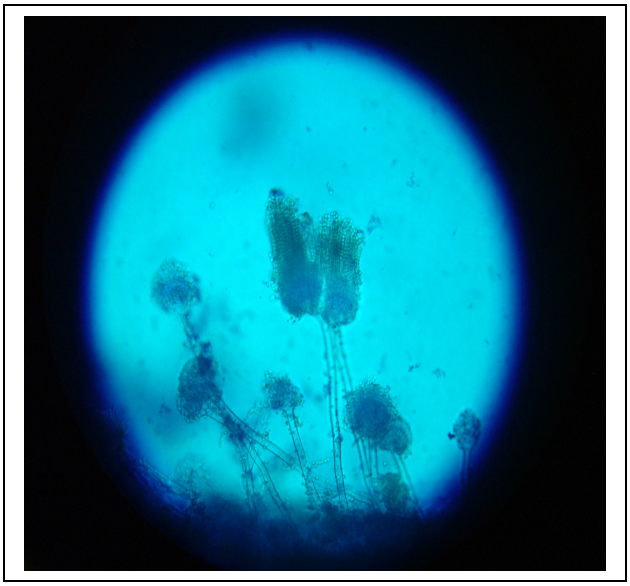
If no growth had occurred by 72 hours the samples were considered negative for bacterial culture. Anaerobic culture was not put up.

ASPERGILLUS TERREUS ON SDA SLOPES

ASPERGILLUS TERREUS ON CZAPEK

LPCB MOUNT OF ASPERGILLUS TERREUS





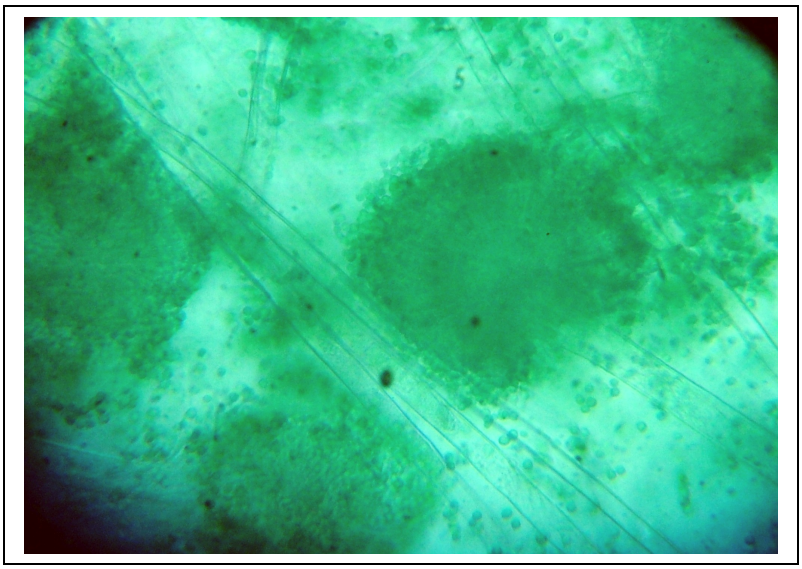
ASPERGILLUS FLAVUS ON SDA SLOPES

ASPERGILLUS FLAVUS ON CZAPEK'S MEDIUM

BLACK SCREW CAPPED BOTTLE SHOWING SCLEROTIA OF ASPERGILLUS FLAVUS

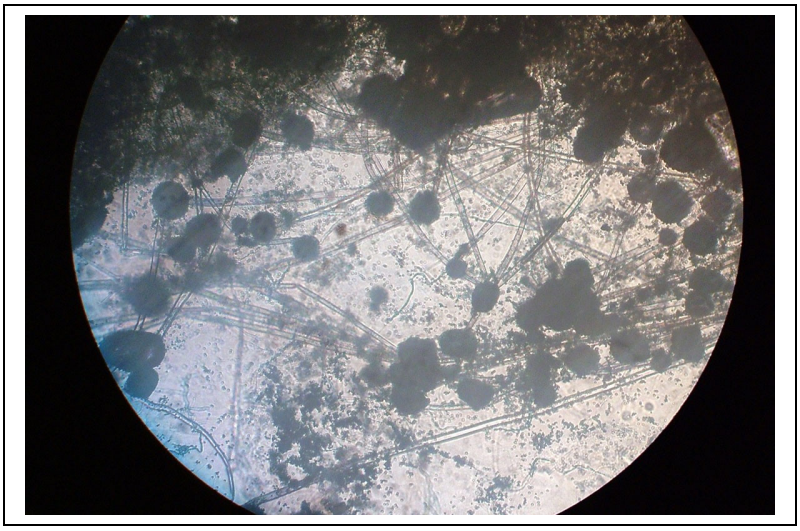
LACTOPHENOL COTTON BLUE MOUNT OF

ASPERGILLUS FLAVUS

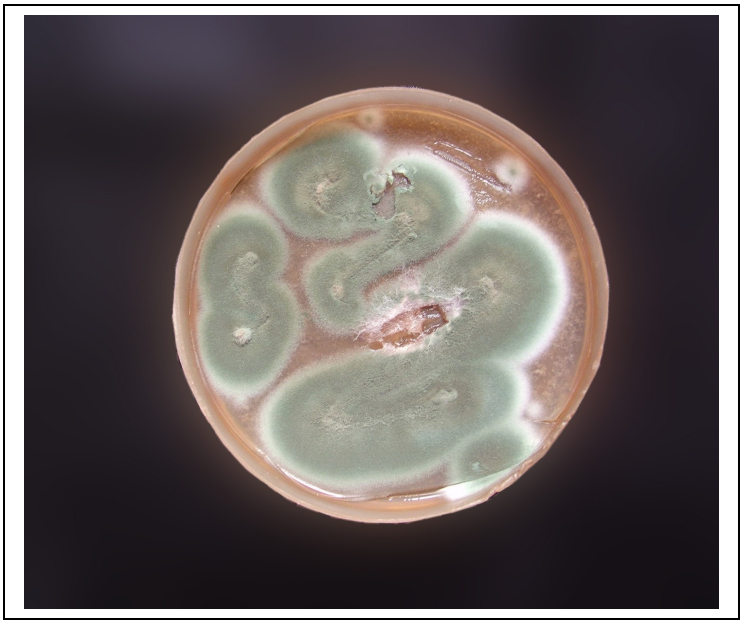


GROWTH OF ASPERGILLUS NIGER – SDA SLOPE
GROWTH OF ASPERGILLUS NIGER ON CZAPEK MEDIUM

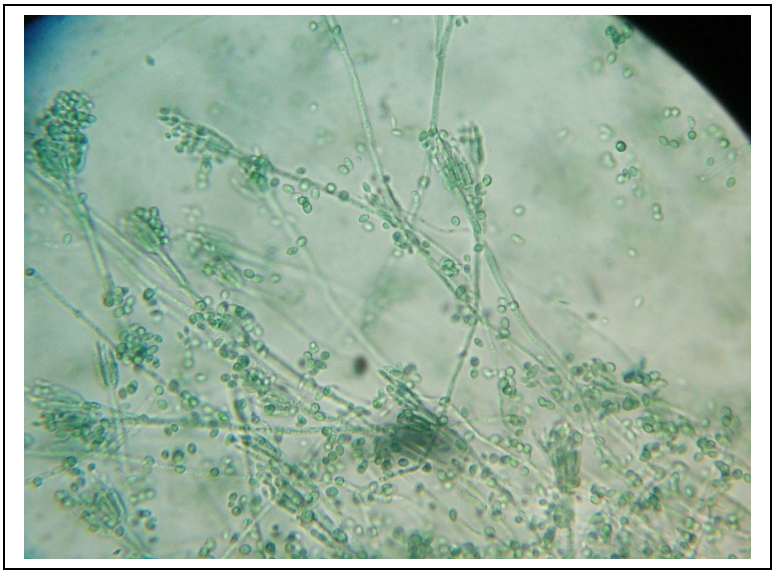
LACTOPHENOL COTTON BLUE MOUNT OF ASPERGILLUS NIGER



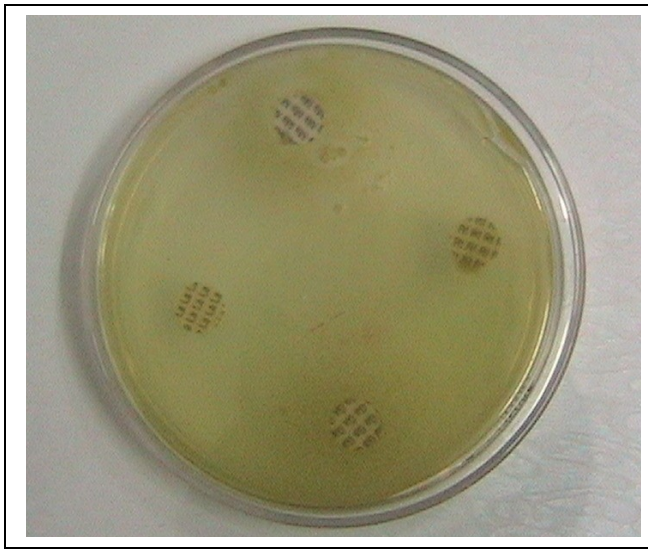
PENICILLIUM ON CZAPEK



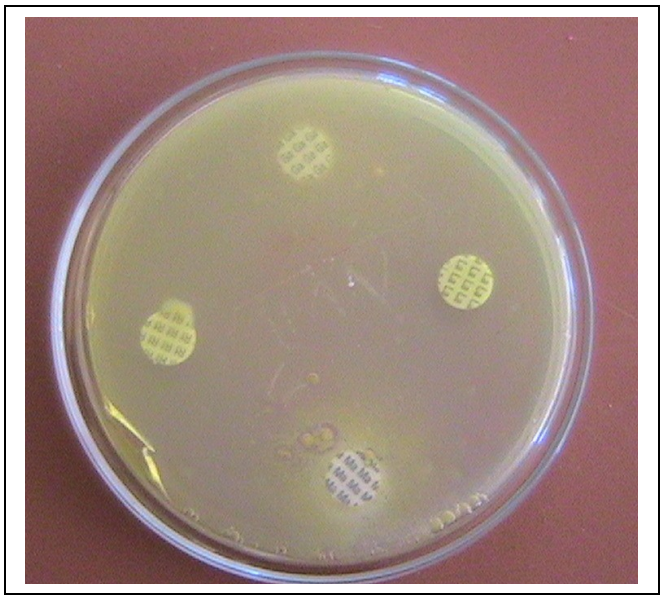
LPCB MOUNT OF PENICILLIUM



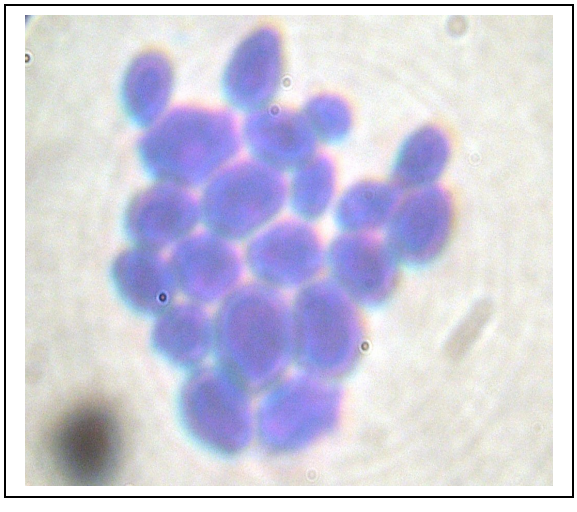
SUGAR ASSIMILATION CANDIDA PARAPSILOSIS
LACTOSE NOT ASSIMILATED



SUGAR ASSIMILATION – CANDIDA ALBICANS
LACTOSE, SUCROSE ASSIMILATED

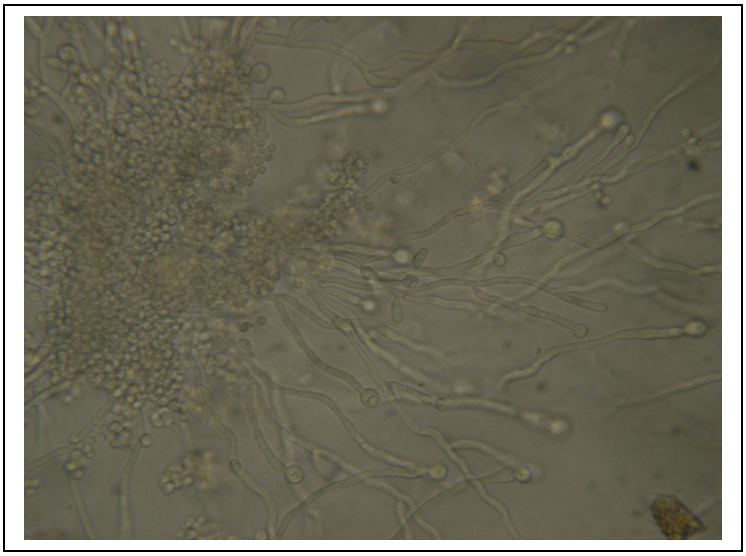


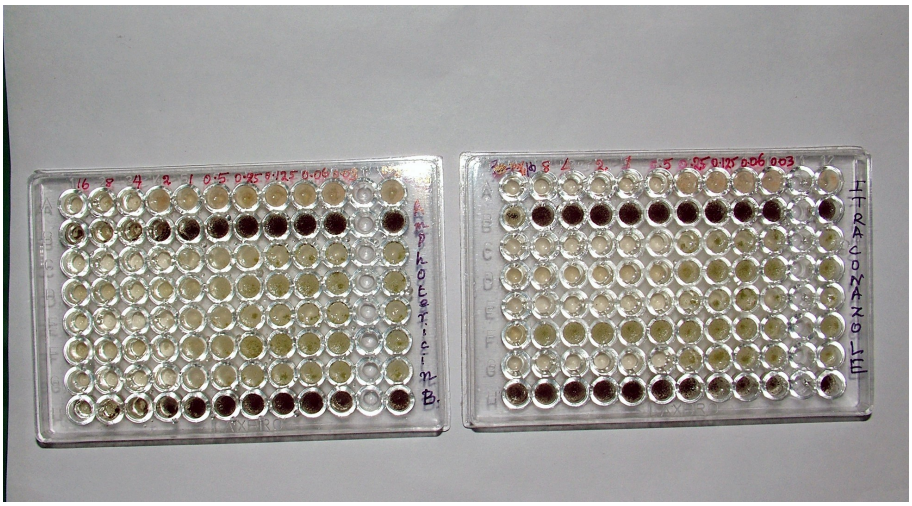
GRAMS STAINING – CANDIDA



GERM TUBE C. ALBICANS

CHLAMYDOSPORES – CANDIDA ALBICANS





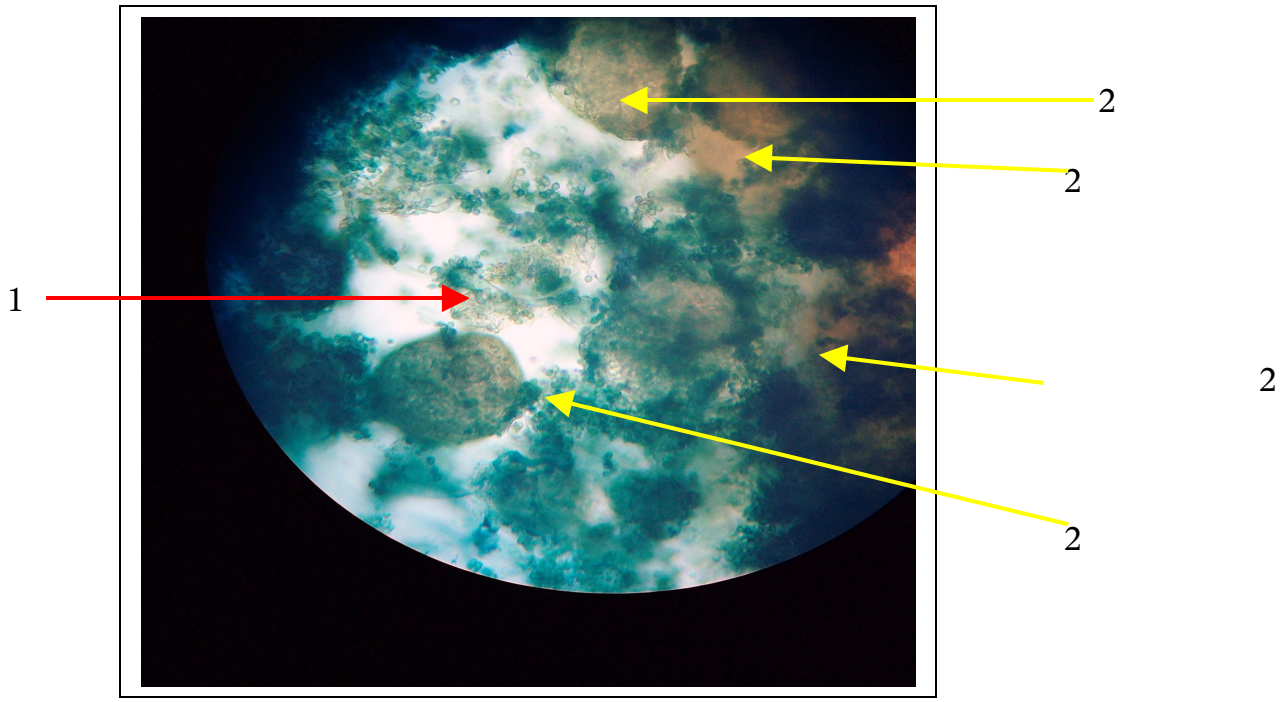
I

ANTIFUNGAL SUSCEPTIBILITY – MICRO DILUTION METHOD

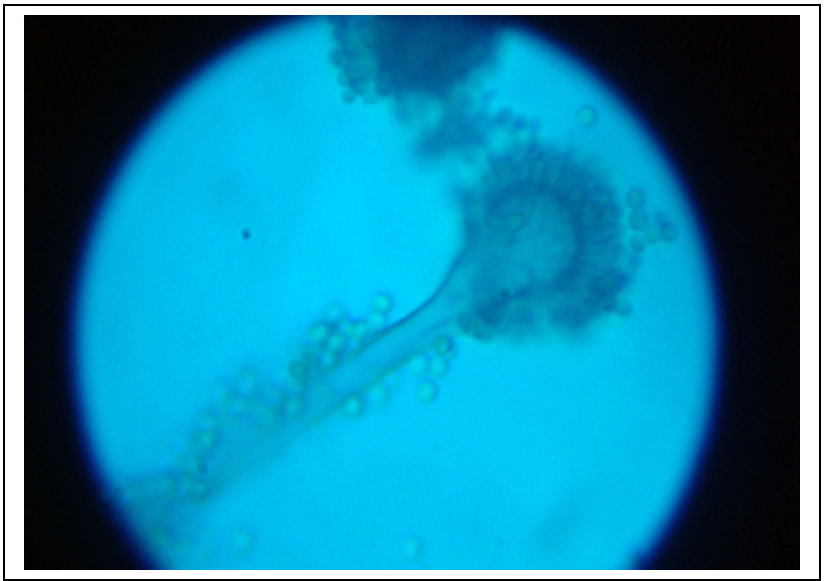
AMPHOTERICIN - B **ITRACONAZOLE**

GILLUS GLAUCUS LPCB SHOWING

1. CONIDIOPHORE, VESICLE
2. CLEISTOTHECIA



ASPERGILLUS FUMIGATUS LPCB MOUNT



ANTI FUNGAL SUSCEPTIBILITY TESTING

Broth microdilution method was used for the determination of minimum inhibitory concentration (MIC) for Amphotericin B and Itraconazole against Aspergillus species.

PROCEDURE:

"Roswell Park Memorial Institute Medium" (RPMI 1640) with glutamine and without sodium bicarbonate to which 2% glucose was added was used for doing anti fungal susceptibility testing. It was buffered with **Morpholinopropanesulfonic acid (MOPS)** as it was did not inhibit the action of anti fungal drugs.

Preparation of Drug Dilutions: AMPHOTERICIN B

Stock solution of Amphotericin B (1600 mg/l) was prepared by dissolving 0.035 g in 10 ml of sterile distilled water. This solution was dispensed into 0.6 ml amounts and stored at -20° C protected from light. 0.6 ml of the prepared stock solution of Amphotericin B was diluted in sterile water by 1/5 to give a solution of Amphotericin B at 320mg/l. The start solution of Amphotericin B was prepared at 32mg/l in RPMI medium by diluting the 320mg/l solution by 1/10. (200µl needed per isolate).

1. A sterile micro titre plate was taken.

2. To the wells in columns 2-12 (excluding 11), 100 µl of RPMI medium in solution was added .

3. To the first well in each row of the microtitre plate 200 µl of the Amphotericin B start solution (32mg/l) was added .

4. Using the micro pipette 100 µl volumes from columns 1-10, were double diluted discarding the extra 100 µl from wells in column 10. This yielded a final range of drug dilutions from 16-0.032 mg/l.

5. Column 12 was the **drug-free positive control.** A separate well was inoculated with 200 µl RPMI medium. This acted as the **medium sterility control.**

Preparation of Drug Dilutions:- ITRACONAZOLE

Stock solution of Itraconazole (3200mg/l) was prepared. 0.032g of pure Itraconazole powder was weighed out into a glass universal and 5ml of acetone was added. After adding the acetone, 5ml of 0.2M HCl was added.It was vortexed vigorously and placed in a water bath at 60^{o}C until it was completely dissolved. It was dispensed into 1ml amounts and stored at -20^{o}C. This stock solution of Itraconazole was

diluted in sterile water by 1/10 to give a solution of Itraconazole at 320mg/l.The start solution of Itraconazole was prepared at 32mg/l in RPMI medium by diluting the 320mg/l solution by 1/10 (200µl needed per isolate).

1. A sterile microtitre plate was taken.
2. To wells in columns 2-12 (excluding 11) of the micro titre plate 100µl of RPMI medium.was added.
3. To the first well in each row of the microtitre plate 200µl of the Itraconazole start solution. (32mg/l) was added
4. Using the micro pipette 100µl volumes from columns 1-10, was double diluted discarding the extra 100µl from wells in column 10. This yielded a final range of drug dilutions from 16-0.03mg/l
5. Column 11 was the **solvent control.** 100µl of the solvent solution equivalent to the solvent contained in the 32mg/l drug dilution was added to this well. Column 12 served as the **drug-free positive control**. A separate well was inoculated with 200µl RPMI medium. This acted as the **medium sterility control**.

Inoculum Preparation:

In a class I safety cabinet, a sterile loop was moistened in phosphate buffered saline with Tween -80 (PBS with Tween) and gently moved over the surface of the *Aspergillus* spores.The loopful of spores was transfered into 5ml of PBS with Tween and vortexed well. The number of spores was counted using a haemocytometer. The number of spores present per ml of

PBS with Tween was calculated and adjusted to $1x10^6$ conidia/ml using Roswell Park Memorial Institute medium (RPMI).

Inoculation of assay plates:

1. Two micro titre plates one having doubling dilutions of Amphotericin B and the other having doubling dilutions of Itraconazole were taken
2. For each isolate 100µl of the fungal spore in PBS with Tween was added into all wells in the appropriate row for both Amphotericin B and Itraconazole (One row for each isolate). The final inoculum was $5x10^5$ conidia/ml.
3. The microtitre plate was covered with a sterile lid and incubated for 48 hours at 37°C in a moist chamber.

Interpretations

The MIC was read visually. The concentration of drug in the first well in which there was no growth gave the MIC value.

MIC for Amphotericin B

MIC= 2 mg/l or less **SENSITIVE**
MIC=>2mg/l **RESISTANT**

MIC for Itraconazole

MIC = 4 or <4mg/l **SENSITIVE**
MIC = 8 or >8mg/l **RESISTANT**
MIC was recorded in mg/l.

The procedure was Caroline Moore's procedure and it was taken from

aspergillus.man.ac.uk.

The same procedure was done with Penicillium spores for determining MIC.

INVITRO ANTIFUNGAL SUSCEPTIBILITY TESTING FOR CANDIDA

Five to eight isolated colonies of Candida were picked up from Sabouraud's dextrose agar and added to 5 ml of sterile 0.85% saline and vortexed for 15 to 20 seconds. The number of yeast cells were counted using a haemocytometer. It was finally adjusted to 10^6 using Roswell Park Memorial Institute medium. Inoculation of assay plates was done as for Aspergillus.

MIC for Candida

Amphotericin B

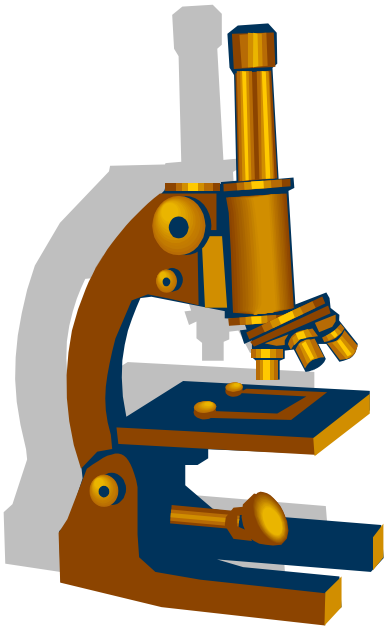
MIC = 0.125 mg/l to 1.0 mg/l **SENSITIVE**

MIC = >1 mg/l **RESISTANT**

Itraconazole

MIC = 0.125 mg/l to 0.5 mg/l **SENSITIVE**

MIC = 1 mg/l **RESISTANT**



RESULTS

RESULTS

A total of 150 cases of Otomycosis were selected for this study, from the ENT department of Government Rajaji Hospital, Madurai for a period of one year from January 2005 to December 2006.

Month wise distribution of the 150 Otomycosis cases was made. It was found that there were 10 cases in January (6.67%), 5 cases in February (3.33%), 7 cases in March (4.67%), 9 cases in April (6%), 9 cases in May (6%), 14 cases in June (9.33%), 13 cases in July (8.67%), 16 cases in August (10.67%), 18 cases in September (12%), 19 cases in October (12.67%), 18 cases in November (12%) and 12 cases in December (8%). Table No. 1.

Table - 1

Monthwise Prevalence of Otomycosis

Sl.No.	Month	Number
1	January	10 (6.67%)
2	February	5 (3.33%)
3	March	7 (4.67%)
4	April	9 (6%)
5	May	9 (6%)
6	June	14 (9.33%)
7	July	13 (8.67%)
8	August	16 (10.67%)
9	September	18 (12%)
10	October	19 (12.67%)
11	November	18 (12%)

12	**December**	**12 (8%)**

Among the 150 cases of Otomycosis **80 were males** (53.3%) and **70 were females** (46.6%). Among the **males** 3 were in the age group 0-15 year (2%), 33 were in the age group 16-30 years (22%), 24 were in the age group 31-45 years (12%), 17 were in the age group 46-60 years (11.3%) and 3 were in the age group above 60 years(2%). Similarly among the **females** 2 were in the age group 0-15 years (1.33), 22 were in the age group 16-30 years (14.67%), 26 were in the age group 31-45 years (17.3%), 14 were in the age group 46-60 years (9.3%) and 6 were in the age group above 60 years (6%). Table No. 2

Table - 2

Agewise and Sexwise Prevalence of Otomycosis

Sl.No.	Age Group	Male	Female	Total
1	**0-15**	3(2%)	2 (1.33%)	5 (3.33%)
2	**16-30**	**33(22%)**	**22 (14.67)**	**55 (36.67%)**
3	**31-45**	24(16%)	26 (17.3%)	50 (33.3%)
4	**46-60**	17(11.3%)	14 (9.3%)	31 (20.6%)
5	**Above 60**	3(2%)	6 (4%)	9 (6%)
Total		80 (53.3%)	70 (46.7%)	150 (100%)

The samples from Otomycosis, were collected either from single or both ears. Thus 69 samples were collected from Otomycosis right ear (46%), 71 samples were from Otomycosis left ear (47.3%) and 10 samples were from cases with bilateral Otomycosis(6.6%). There were 37 males and 32 females who had right ear Otomycosis, 38 males and 33 females who had left ear Otomycosis and 5 males and females who had bilateral Otomycosis. Table No. 3

Table - 3

Side predilection for Otomycosis

Sl.No.	Side	Male	Female	Total
1	**Right**	37	32	69 (46%)
2	**Left**	38	33	**71 (47.3%)**
3	**Bilateral**	5	5	10 (6.6%)

Among the 150 Otomycosis patients, 39 were house wives and house maids (26%), 38 were persons doing miscellaneous work mainly indoors (25.33%) like retired persons, weavers, electricians etc., 29 were agriculturists (19.3%), 23 were students. (15.3%) and 21 were labourers mainly doing outdoor work (14%). Table No. 4

Table No - 4

Occupation wise distribution of cases

Sl.No.	**Occupation**	Number
1	**Housewife and house maid**	**39 (26%)**
2	**Miscellaneous indoor worker**	38 (25.3%)
3	**Agriculturist**	29 (19.3%)
4	**Student**	23 (15.33%)
5	**Labourer**	21 (14%)
	Total	150 (100%)

The predisposing factors for Otomycosis were analyzed and it was found that 61 cases had only CSOM (40.6%), 11 cases had both CSOM and ear drops (7.3%) usage, 20 cases had instillation of ear drops only (13.3%), 5 had trauma to the external auditory canal (3.3%). 2 had diabetes mellitus with CSOM (1.33%), 1 used hearing aid (0.66%) and 4 used to bath in ponds (2.66%). There was no predisposing factor for 46 cases (30.6%). There were a total of 74 **cases of CSOM (61 of pure CSOM + 11 CSOM with ear drops + 2 DM WITH CSOM) mounting to 49%.** There was a total of 31 cases (11 cases of CSOM using ear drops + 20 cases using only ear drops) using ear drops (20.67%). Table No. 5**.**

Table - 5

Distribution of Predisposing factors

Sl. No.	Predisposing factor	Total Number
1	**CSOM**	**61 (40.6%)**
2	**CSOM with ear drops (Antibiotic/Steroids)**	**11(7.3%)**
3	**DM with CSOM**	**2 (1.33%)**
4	**Ear drops only (Antibiotic/Steroids)**	20 (13.3%)
5	**Trauma**	5 (3.3%)
6	**Hearing aid**	1 (0.66%)
7	**Bathing in pond**	4 (2.66%)
8	**No predisposing factor**	46 (30.6)%

The patients presenting with Otomycosis were analyzed symptom wise and it showed that 105 cases presented with itching. (70%), 95 presented with pain (63.3%), 67 with ear block (44.67%) and 45 with discharge (30%). Table No. 6.

Table - 6

Symptom wise distribution of cases

Sl. No.	Symptom	Number
1	**Itching**	**105 (70%)**
2	**Pain**	95 (63.3%)
3	**Ear block**	67 (44.67%)
4	**Discharge**	45 (30%)

The 150 Otomycosis samples were processed for fungal culture and it was found that 141 out of 150 samples gave positive results. (94%). Among the 141 positive fungal isolates 62 were Aspergillus flavus (43.9%), 48 were A.niger (34%), 13 were A. terreus (9.2%), 5 were A. fumigatus (3.54%), 1 was A. glaucus (0.07%), 3 were Candida albicans(2.12%),1 was Candida parapsilosis (0.07%) and 8 were Penicillium (5.7%). Table Nos. 7 & 8.

Table No - 7

Fungal Culture Positivity in Otomycosis

Sl. No.	Number Tested	Positive Culture	Negative Culture
1	150	141(94%)	9 (6%)

Table - 8

Fungal Isolates in Otomycosis

Sl. No.	Fungal Isolate	Number
1	**Aspergillus flavus**	**62 (43.9%)**
2	**Aspergillus niger**	48 (34%)
3	**Aspergillus terreus**	13 (9.2%)
4	**Aspergillus fumigatus**	5 (3.54%)
5	**Aspergillus glaucus**	1 (0.7%)

6	**Candida albicans**	3 (2.12%)
7	**Candida parapsilosis**	1(0.7%)
8	**Penicillium**	8 (5.7%)

The fungal isolates isolated from Otomycosis were analysed sex wise and age group wise. In males out of the 80 clinical cases only 75 were positive by fungal culture. In **males** of the age group **0 – 15** years there were 2 isolates of A. flavus (1.42%) and 1 case of A. niger (0.71%), in the **16 – 30 age group 15 males had A. flavus,** 7 had A. niger (4.96%), 2 had A. terreus (1.42%), 2 had A. fumigatus (1.42%) and 3 had Penicillium (2.13%). In the age group of **31 – 45** years 11 males had A. flavus (7.81%), 8 had A. niger (5.67%) and 3 had A. terreus (2.13%) and 1 had Penicillium (0.71%). In the age group of **46 – 60** years 10 males had A. flavus (7.1%), 6 had A. niger (4.26%) and 1 had Penicillium (0.71%). In the **above 60** years age group 2 males had A. flavus (1.42%) and 1 had A. niger (0.71%).

Out of the 70 specimens from female cases only 66 grew fungi. In the age group of **0 – 15** years there was 1 case of Penicillium (0.71%) and another 1 case of A. glaucus (0.71%). In the age group of **16 – 30** years there were 7 females with A. flavus (4.96%), 9 due to A. niger (6.38%), 3 due to A. terreus (2.13%), 1 due to A. fumigatus (0.71%) and 1 due to Penicillium (0.71%). In the age group **31 – 45** years .

6 females had A. flavus (4.26%) 11 had A. niger (7.81%), 4 had A. terreus (2.84%), 1 had A. fumigatus (0.71%), and 1 had Penicillium (0.71%). In the age group **46 – 60** years 7 females had A. flavus (4.96%), 4 had A. niger (2.84%), 1 had A. terreus (0.71%) and 2 had Candida albicans (1.41%). In females above 60 years there were 2 isoaltes of A. flavus (1.42%), 1 isolate each of A. niger, A. fumigatus, Candida albicans and Candida parapsilosis (0.71% each)

Among the 75 positive fungal isolates in **males, 40 were Aspergillus flavus (28.37%),** 23 were A. niger (16.31%) , 5 were A. terreus (3.55%), 2 were A. fumigatus (1.42%) and 5 were Penicillium (3.55%). Among the 66 positive fungal isolates in **females** there were 22 isolates of A.flavus (15.6%), **25 isolates of A. niger (17.73 %),** 8 isolates of A.terreus

(5.67%), 3 isolates of A.fumigatus (2.13%), 1 isolate of A. glaucus (0.71%), 3 isolates of Candida albicans (2.13%), one isolate of Candida parapsilosis (0.71%) and 3 isolates of Penicillium (2.13%). Table Nos. 9 & 10.

Table - 9

Agewise analysis of fungal isolates in Males

Sl. No.	Fungal isolate	0-15 years	16-30 years	31-45 years	46-60 years	Above 60 years	Total
1	A. flavus	2 (1.42%)	15 (10.64%)	11 (7.8%)	10 (7.1%)	2 (1.42%)	40 (28.37%)
2	A. niger	1 (0.71%)	7 (4.96%)	8 (5.67%)	6 (4.26%)	1 (0.71%)	23 (16.31%)
3	A. terreus	0	2 (1.42%)	3 (2.13%)	0	0	5 (3.55%)
4	A. fumigatus	0	2 (1.42%)	0	0	0	2 (1.42%)
5	A. glaucus	0	0	0	0	0	0
6	C. albicans	0	0	0	0	0	0
7	C.parapsilosis	0	0	0	0	0	0
8	Penicillium	0	3 (2.13%)	1 (0.71%)	1 (0.71%)	0	5 (3.55%)

Table - 10

Agewise analysis of fungal isolates in Females

Sl. No.	Fungal isolate	0-15 years	16-30 years	31-45 years	46-60 years	Above 60 years	Total
1	A. flavus	0	7 (4.96%)	6 (4.26%)	7 (4.96%)	2 (1.42%)	22 (15.6%)
2	A. niger	0	9 (6.39%)	11 (7.81%)	4 (2.84%)	1 (0.71%)	25 (17.73%)
3	A. terreus	0	3 (2.13%)	4 (2.84%)	1 (0.71%)	0	8 (5.67%)
4	A. fumigatus	0	1 (0.71%)	1 (0.71%)	0	1 (0.71%)	3 (2.13%)
5	A. glaucus	1 (0.71%)	0	0	0	0	1 (0.71%)
6	C. albicans	0	0	0	2 (1.42%)	1 (0.71%)	3 (2.13%)

7	C.parapsilosis	0	0	0	0	1 (0.71%)	1 (0.71%)
8	Penicillium	1 (0.71%)	1 (0.71%)	1 (0.71%)	0	0	3 (2.13%)

The fungal isolates were analysed according to occupation. It was found that among the 39 house wives and maids 35 had fungal isolates. Among this 9 were A. flavus (25.71%), 14 were A.niger (40%), 6 were A. terreus (17.14%), 2 were A. fumigatus (5.71%), 3 were Candida albicans (8.57%) and 1 was Candida parapsilosis (2.85%).

Among the 38 indoor workers 37 were positive for fungal culture. Out of this 37 isolates there were 13 isolates of A. flavus (35.14 %), 15 isolates of A. niger (40.54 %), 4 isolates of A. terreus (10.81 %), 1 isolate of A.fumigatus (2.7%) and 4 isolates of Penicillium (10.81 %). Among the 29 agriculturist all had fungal isolates. Among this 18 were positive for A. flavus (62.07 %), 10 for A. niger (34.48 %) and 1 for A. terreus (3.45 %).

Among the 23 students, 19 were positive by culture. Among these 9 were A. flavus (47.37%), 3 were A. niger (15.79 %), 2 were A. terreus (10.53%), 2 were A. fumigatus (10.53%), 1 was A. glaucus (5.26%) and 2 were Penicillium (10.53 %). Among the 21 labourers all were positive for fungal culture. Among this 13 had A. flavus (61.90 %), 6 had A. niger (28.57 %) and 2 had Penicillium (9.52%).Table11.

Occupationwise distribution of Fungal Isolates

Occupation	No. Tested	A. flavus	A. niger	A. terreus	A. fumigatus	A. glaucus	C. albicans	C. parapsilosis	Penicillium	
Housewife and Maid	39	9 (25.17%)	14 (40.0%)	6 (17.14%)	2 (5.71%)	0	3 (8.57%)	1 (2.85%)	0	3
Miscellaneous indoor workers	38	13 (35.14%)	15 (40.54%)	4 (10.81%)	1 (2.70%)	0	0	0	4 (10.81%)	3
Agriculturist	29	18 (62.07%)	10 (34.48%)	1 (3.45%)	0	0	0	0	0	2
Students	23	9 (47.37%)	3 (15.79%)	2 (10.53%)	2 (10.53%)	1 (5.26%)	0	0	2 (10.53%)	1
Labourers	21	13 (61.90%)	6 (28.57%)	0	0	0	0	0	2 (9.52%)	2

In the analysis of predisposing factor wise distribution of isolates, out of the 74 isolates from **CSOM** only 72 were positive for fungal culture. Of this **A. flavus was seen in 37 patients (51.39%)**, A. niger was seen in 26 patients (36.11%), A. terreus in 3 patients (4.17%), A. fumigatus, C. albicans, C. parapsilosis in 1 patient each (1.39%) and Penicillium in 3 patients (4.17%).

Of the 33 patients using **ear drops** only 29 had fungi. Out of this 7 were by A. niger (24.14%), **19 were by A. flavus (65.52%)**, 2 was by A. terreus (6.90%) and 1 was by A. fumigatus (3.45%).

Of the DM with CSOM there was 1 case of Candida albicans (50%) and 1 case of Candida parapsilosis (50%).

The sole case of hearing aid user had A. niger (100%). Of the 4 cases with history of bathing in ponds 3 had A. flavus (75%) and 1 had A. niger (25%). Of the 5 cases with trauma, 3 were by A. flavus (60%), 1 was by A. niger (20%) and one was by Penicillium (20%).

Out of the 46 patients with no predisposing factors 41 had positive fungal culture. Out of this 41, 7 had A. flavus (17.07%), 16 had A. niger (39.02%), 8 had A. terreus (19.51%), 3

had A. fumigatus (7.32%), 1 had A. glaucus (2.44%), 2 had Candida albicans (4.88%) and 4 had Penicillium (9.76%). Table 12.

TABLE NO. 12
PREDISPOSING FACTOR WISE ANALYSIS OF FUNGAL ISOLATES

g	NUMBER	A. flavus	A. niger	A. terreus	A. fumigatus	A. glaucus	C. albicans	C. parapsilosis	Penicillium
	61	**30 (50.8%)**	22 (37.3%)	3 (5.08%)	1 (1.69%)	0	0	0	3 (5.08%)
	11	**7 (63.64%)**	4 (36.36%)	0	0	0	0	0	0
only	20	**12 (66.6%)**	3 (16.66%)	2 (11.11%)	1 (5.56%)	0	0	0	0
OM	2	0	0	0	0	0	**1 (50.0%)**	**1 (50.0%)**	0
	1	0	1 (100%)	0	0	0	0	0	0
	4	3 (75.1%)	1 (25.0%)	0	0	0	0	0	0
	5	3 (60.0%)	1 (20.0%)	0	0	0	0	0	1 (20.0%)
sing	46	7 (17.07%)	**16 (39.02**)	8 (19.51%)	3 (7.32%)	1 (2.44%)	2 (4.88%)	0	4 (9.76%)

Ab/st -- antibiotic / steroids

The Bacterial isolates isolated from Otomycosis cases were as follows:

Out of 150 specimens only 135 gave positive bacterial culture. There were 40 (29.63%)

isolates of coagulase negative Staphylococcus (CONS), 32 (23.70%) of Micrococci, 13 (9.63%) of Staph aureus, 16 (11.85%) of Pseudomonas aeruginosa, 13 (9.63%) of Proteus mirabilis, 8 (5.93%) of E. coli, 9 (6.67%) of Klebsiella, 3 (2.72%) of Proteus vulgaris and 1 (1.11%) of Coliforms. Table 13.

Table No. 13

Bacterial Isolates in Otomycosis

Sl. No.	Bacterial Isolates	Number
1	**CONS**	**40 (29.63%)**
2	**Micrococci**	32 (23.7%)
3	**Staph aureus**	13(9.63%)
4	**Pseudomonas aeruginosa**	16(11.85%)
5	**Proteus mirabilis**	13 (9.63%)
6	**Escherichia coli**	8 (5.93%)
7	**Klebsiella aerogenes**	9 (6.67%)
8	**Proteus vulgaris**	3 (2.22%)
9	**Coliforms**	1 (0.74%)
10	**Total**	135 (100%)

Out of the 80 males 68 were positive for both bacterial and fungal culture (45.33%), 7 were positive only for fungal culture (4.67%) and 5 were positive only for bacterial culture (3.33%). Out of the 70 females 58 were positive for both bacterial and fungal culture (38.67%). 8 were positive only for fungal culture (5.33%) and 4 were positive only for bacterial culture (2.6%). Table No. 14.

Table 14

Culture positivity in Otomycosis

Sl.No.	Culture	Male	Female	Total
1	**Both**	68 (45.33%)	58 (38.67%)	126 (84%)
2	**Fungus only**	7 (4.67%)	8 (5.33%)	15 (10%)
3	**Bacteria only**	5 (3.33%)	4 (2.67%)	9 (6%)

The various bacterial and fungal isolates were analysed according to the predisposing factors. Among the 74 CSOM cases there were 64 with both fungal and bacterial cultures positive (86.49%), 7 with positive fungal culture (9.46%) and 3 with positive bacterial culture (4.05%). Among the 31 cases using ear drops 22 had both cultures positive (70.97%), 7 had positive fungal culture (22.58%) and 2 had positive bacterial culture (6.45%). The 2 diabetics had fungal culture only (100%). All the 5 cases with trauma, 4 cases with history of bathing in ponds and 1 case of hearing aid usage had positive fungal and bacterial cultures (100%). Among the 46 patients with no predisposing factors 39 were positive for both cultures (84.78%), 3 were positive for fungal culture (6.52%) and 4 were positive for bacterial culture (8.70%). Table No. 15.

Table - 15

Predisposing factors Vs Culture isolation in Otomycosis

Sl. No.	Predisposing factor	Both	Fungus only	Bacteria only
1	**CSOM**	**55**	**3**	**3**

		(90.16%)	**(4.92%)**	**(4.92%)**
2	**CSOM with ear drops (Antibiotic/Steroids)**	**9 (81.82%)**	**2 (18.8%)**	**0**
3	**DM with CSOM**	**0**	**2 (100%)**	**0**
4	**Ear drops only (Antibiotic/ Steroids)**	13 (65%)	5 (25%)	2 (10%)
5	**Trauma**	5 (100%)	0	0
6	**Hearing aid**	1 (100%)	0	0
7	**Bathing in pond**	4 (100%)	0	0
8	**No predisposing factor**	39 (84.78)%	3 (6.52%)	4 (8.7%)

The various fungal isolates were subjected to **Antifungal susceptibility by Microdilution Method**.

For Amphotericin B

Out of the 62 isolates of A. flavus, 57 (91.9%) were sensitive and 5 (8.1%) were resistant. Out of the 48 A. niger isolates, 41 (85.4%) were sensitive and 7 (14.6%) were resistant. **All the 13 isolates of A. terreus were resistant (100%).** Out of the 5 A. fumigatus isolates, 4 (80%) were sensitive and 1 (20%) resistant. The single A. glaucus isolate was sensitive (100%). All the four Candida isolates were sensitive (100%). All the 8 Penicillium isolates were sensitive (100%). Table 16.

Table 16

Sensitivity to Amphotericin – B

Sl. No.	Isolate	No. of Isolate	Sensitive	Resistance > 2 mg/l
1	A. flavus	62	57 (91.9%)	5 (8.1%)

2.	A. niger	48	41 (85.4%)	7 (14.6%)
3.	A. terreus	13	0	13 (100%)
4.	A. fumigatus	5	4 (80%)	1 (20%)
5.	A. glaucus	1	1 (100%)	0
6.	Candida albicans	3	3 (100%)	0
7	Candida parapsilosis	1	1 (100%)	0
8	Penicillium	8	8 (100%)	0

For Itraconazole

Out of the 62 isolates of A. flavus, 61 (98.4%) were sensitive and 1 (1.6%) was resistant, out of 48 A. niger isolates, 44 (91.6%) were sensitive and 4 (8.3%) were resistant. All the 13 (100%) isolates of A. terreus were sensitive. Out of the 5 A. fumigatus isolates, 4 (80%) were sensitive and 1 (20%) resistant. The single A. glaucus isolate was sensitive (100%). **All the four Candida** isolates were sensitive. **All the 8 Penicillium** isolates were sensitive. Table 17.

Table 17

Sensitivity to Itraconazole

Sl. No.	Isolate	No. of Isolate	Sensitive	Resistance
1	A. flavus	62	61 (98.4%)	1 (1.6%)
2.	A. niger	48	44 (91.6%)	4 (8.3%)
3.	A. terreus	13	13 (100%)	0
4.	A. fumigatus	5	4 (80%)	1 (20%)
5.	A. glaucus	1	1 (100%)	0
6.	Candida albicans	3	3 (100%)	0
7	Candida parapsilosis	1	1 (100%)	0
8	Penicillium	8	8 (100%)	0

Colonies on Czapek formed spores more readily than on Sabourauds Dextrose Agar. The characteristic cinnamon brown coloured conidio spores of A. terreus was brought out only in Czepek agar. Similarly the same strain of A. flavus produced yellow conidio spores on SDA and green conidio spores on Czapeck.

DISCUSSION

150 cases of clinically diagnosed Otomycosis were selected as the study population.

Otomycosis is a common pathology of the external ear. It is common in India. The high incidence can be attributed to the increased heat and humidity, the dusty environment and the fact that a large proportion of the population is constituted by outdoor labourers and persons of low socio economic status.

The study was conducted for a period of one year. The incidence was more during the **North East monsoon** from October to January 59 (39.3%) and **South West monsoon** from June to September 61 (40.7%). This correlates with the study of Than K M. et al who found increased incidence of Otomycosis during the rainy seasons [37]. Agrawal S K et al also proved that high humidity during the monsoon months favoured the development of Otomycosis [1].

In this study, it was observed that **males were more commonly affected** than females, 80 (53.3%) males had Otomycosis against 70 females (46.6%). This is in accordance with the study of Ravinder Kaur et al who had shown a high incidence in males [30]. Our study is in accordance with the study of Yassin A et al who showed higher incidence in males mainly because of occupation and cleaning the ear with matchsticks [43]. This is in contrast to the study of Yehia et al, who showed that Otomycosis was common among females in Northern Iraq (65.44%) [45].

The present study revealed higher incidence of disease among the age group of 16 -30

years 55 (36.67%) compared to other age groups. This is in favour of the study of Yehia M M et al, showing that young adult of the age group 16 – 30 years were commonly affected [45]. The study of Ravinder Kaur et al is in support of our study, showing 41.1% of incidence in the age group 16 – 30 years [30]. But Garcia Martos et al in their study showed that Otomycosis was seen predominantly in the age groups of 31 – 40 years and over 50 years [13]. In contrast Mocatela Ruiz et al in their study showed no difference regarding age and sex of the patients [24].

Our observation showed that Otomycosis was unilateral in 140 (93.3%) cases in spite of various predisposing factors. This Observation is supported by the study of Yehia et al and Ravinder Kaur et al [45, 30]. The unilateral occurrence of disease support the fact that the disease is not highly infectious and other local factors play important role in the occurrence of Otomycosis.. In our study there was no predominance between the either sides of the ear. But Agrawal et al showed that the right ear was found to be more prone to Otomycosis than the left ear [1].

Predisposing factors

In this study, presence of CSOM was the main predisposing factor for the occurrence of Otomycosis 74 (49%). Our study is favoured by Vennewald I et al who showed 89.84% of his patients with Otomycosis had CSOM as the predisposing factor [40]. Talwar P et al showed that 49% of their CSOM cases had positive fungal culture [35]. Our study is also supported by Agrawal et al [1].

Antibiotic / Steroid ear drops

In our study the second common predisposing factor for Otomycosis was repeated usage of Antibiotic / Steroid drops 31 (20.6%). This correlates with the study of Chander et al showing 56.25% for ear drops [4]. Jackman et al showed that all 26 patients with acute onset Otomycosis when enquired gave history of using Ofloxacin ear drops for otorrhoea (100%) [18]. Garcia et al showed that Antibiotic / Steroid drop usage was the causative factor for 40% of Otomycosis [13].

It was observed that there was no predisposing factors in 46 (30.6%) of cases of clinically diagnosed Otomycosis. This is supported by the study of Agrawal et al, who had done a study on Otomycosis in 70 cases showed no predisposing factor and showed 42% of fungal isolates [1].

Symptoms

In our study the commonest symptom was itching 105 (70%) followed by pain 95 (63.3%), ear block 67 (44.67%) and ear discharge 45 (30%). Paulose et al found that itching was the most common symptom (88%) followed by ear block (87.5%), discharge (30%) and tinnitus (22%) [29]. Oliveri S et al also found itching as the characteristic symptom of Otomycosis followed by pain and loss of hearing [28]. In contrast Ravinder et al found ear block as the commonest symptom (93.7%) followed by itching (71.5%) [30].

Fungal isolation

In this study, 141 out of a total 150 cases were positive by fungal culture giving an isolation rate of 94%. Similarly Ravinder Kaur et al had 74.74% positive fungal culture [30]. Yassin et al also had 81.08% culture positivity from clinically diagnosed cases [43].

In this study there was no fungal growth in 6% of clinically diagnosed cases of Otomycosis. This may be due to previous treatment or acute exacerbation of chronic disease.

In this study among 141 patients positive for fungal growth, **Aspergillus** was the most common isolate 129 (91.48%) followed by **Penicillium** 8 (5.7%), and **Candida** 4 (2.91%). This is supported by Yehia M M et al who had 92.1% Aspergillus isolates in his study [45]. Our study correlates with the findings of Than K M et al who also found Aspergillus as the commonest species isolated in their study [37]. Oliveri S et al also had an isolation rate of 81.7% for Aspergillus [28]. Nong H et al also had an isolation rate of 79% for Aspergillus which supports our study [27].

Among the 129 Aspergillus isolates, 62 (43.9%) were **A. flavus,** 48 (34.04%) were **A. niger,** 13 (9.2%) were **A.terreus**, 5 (3.54%)were A.fumigatus and 1(0.7%) was A.glaucus. **Aspergillus flavus** being the most common isolate 62(43.9%) in our study. This is supported by Yavo W et al showing A. flavus 20.4% [44], Oliveri et al showing 13.4% of A.flavus [28] and Chander et al showing 33.7% A. flavus [4].

In our study A. niger was isolated as the second common fungus 48 (34%) and this is supported by Oliveri S et al showing 67% of A.niger [28]; Talwar showing 52% [35], Gugnami Okafor et al showing 52% of A. niger [15], Mohanty J C et al showing 41.1%of A niger [25] and Ravinder kaur showing 36.9% of A. niger [30]. But Yavo W et al and Kombila M et al showed

only 26% and 12.28% isolation rates for A.niger [44,21]. It is comparatively less than our study. The increased incidence of A. flavus and A. niger may be due to the spores, that are found profusely in the atmosphere during the rainy season, because of the abundance of the dead organic matter on which they grow. They thrive on fallen leaves and in compost heaps and may be found throughout vegetation materials [6].

Among 141 culture positive Otomycosis 13 (9.2%) were due to **A. terreus**. It was the third commonest isolate. But Egami et al of Japan found A. terreus (46%) as the common pathogen followed by A. niger and A. flavus [11] Our study is supported by Yassin A. et al showing 4.6% A. terreus [43].

Among the 141 positive isolates 5 were due to A. fumigatus (3.55%). This is supported by Oliveri S. et al showing 1.2% of A. fumigatus [28] and Chander et al showing 3.7% [4]. But this is in contrast to the study of Ravinder Kaur et al who showed an isolation rate of 41.4% for A. fumigatus [30].

In our study, **Candida** species was isolated only from 4 patients (2.92%) among this 3 (2.12%) were Candida albicans and 1 (0.7%) was Candida parapsilosis. This is supported by Yassin et al showing 4.6% [43], Jadhav et al showing 2.53% [19]

and Nong H et al showing 8% [27]as the isolation rate for Candida in their studies. But Kombila M et al Gugnami B C et al had shown 11% Candida species isolation in their study [21,15]. Ravinder Kaur et al isolated 21.7% of Candida species in their study [30]. Darko E et al of Eastern Slovakia isolated Candida in all 40 cases of Otomycosis (100%) [7].

Among 141fungal isolates 8 were due to **Penicillium** (5.68%). This correlates with the study of J. C. Mohanthy et al showing 3.7% [25] , Tinser J et al showing 4.8% [39] and Yasin et al showing 5.34% [43] as the isolation rate for Penicillium in their study.

When the fungal isolates were analyzed sex wise it was found that **A.flavus** was the commonest isolate among **males** 40 (28.37%) and **A. niger** the commonest isolate in **females** 25 (17.73%). This can be due to the fact that A. niger could be cultured from house dust and females spend more time indoors [12]. Candida albicans 3 (2.13%), Candida parapsilosis 1 (0.71%) and A.glaucus 1 (0.71%) all were exclusively isolated from females. A.terreus was isolated in higher numbers in females 8 (5.67%) and Penicillium in males 5 (3.55%).

When fungal isolates were analysed occupation wise it was found that **A. flavus** was the most common fungus isolated in **agriculturists** and **labourers** (62%) followed by students (47.37%). This is in accordance with the study of Ravinder Kaur et al that fungal spores are more abundant in the outdoor atmosphere and these people spend more time outdoors [30].

A.niger was the most common isolate among **housewives** (40%) and **indoor workers** (40.54%). They spend more time indoors and are more exposed to house dust. This is in favour of Frank E. Lucente's statement that A. niger could be cultured from house dust [12].

When the isolates were analyzed on basis of predisposing factors it was found that **A.niger** was the commonest isolate in the group that had no predisposing factors (39.02%). It is in accordance with the study of Agrawal S R et al who predominantly isolated A. niger from

Otomycosis cases not having CSOM or other predisposing factors [1] .

A. flavus was the commonest isolate among the groups using ear drops (65.52%). But Talwar P et al isolated mainly **Penicillium** and A. niger as the important isolates in Otomycosis occurring after using antibiotic drops [35].

Bacterial Isolates from Otomycosis

In this present study coagulase negative Staphylococci was isolated in 40 (29.63%), Micrococci in 32 (23%). Staph aureus 13 (9.63%), Pseudomonas 16 (11.85%), Proteus species 16 (11.85%) and Klebsiella 9 (6.67%). Similar bacterial isolates were seen in the study of Darko E et al for coagulase negative Staphylococci (CONS) and Staph aureus [7]. But in contrast Arshad M et al have shown 38% isolation rate for Staph aureus, 38% for Pseudomonas aeruginosa and the 24% Proteus, Klebsiella, E. coli and Enterococci [2].

When both bacterial and fungal cultures were done for 150 cases of Otomycosis only fungi was isolated from 10% of cases, only bacteria was isolated from 6% of cases and mixed growth of both bacterial and fungi was seen in 84% of cases. More number of **mixed organisms** 84% grown in Otomycosis is supported by Frank E. Lucente [12] who observed that most cases of Otomycosis were actually mixed bacterial and fungal infections. Our study is further supported by Darko E et al who showed mixed isolates in 77% of Otomycosis cases. Isolation of fungi alone was more in cases using ear drops (25%). This is supported by Garcia et al showing 40% [13], Chander et al showing 54% [4] and Yavo et al showing 40% [44].

Mixed growth of both bacteria and fungi was seen in 100% cases in trauma, hearing aid usage and bathing in ponds 90.16% in CSOM cases. This is supported by Frank E Lucente's observation that most cases of Otomycosis are mixed infections. It is also supported by Hawke et al who showed "ear mould induced otitis externa" was caused by both bacterial and fungal organisms [17].

Antifungal susceptibility testing

Among the 62 isolates of A. flavus 57 (91.9%) were sensitive to Amphotericin B and 5 (8.1%) were resistant. This correlates with the finding of David W Denning who showed Amphotericin B resistance [8].

Among the 48 isolates of A. niger 41 were sensitive (85.41%) and 7 were resistant 14.6% to Amphotericin B.

Among the 13 isolates of A. terreus all the 13 (100%) were resistant to Amphotericin B. This also correlates with David W Denning's statement that A. terreus is intrinsically resistant to Amphotericin B [8].

Among the 5 isolates of A. fumigatus 4 (80%) were sensitive to Amphotericin B and 1 was resistant (20%). This correlates with David W Denning who showed 30% resistance in A.fumigatus to Amphotericin B [8].

In our study, it was found the minimum inhibitory concentration of Itraconazole as

0.125 to 4 mg/l for A. flavus (61 isolates), 8 mg for 1 isolate of A. flavus, 0.125 to 16 mg/l for A. niger (48 isolates), 0.125 to 8 mg/l for A. fumigatus (5 isolates) and 0.25 to 2 mg/l for A. terreus (13 isolates). This is in contrast with the study of Karaarslan A et al showing MIC - 2 of 0.06 to 0.25 mg/l for A. flavus (10 isolates), 0.125 to 1 mg/l for A. niger (22 isolates) and 0.25 mg/l for A. terreus (2 isolates) for Itraconazole [20].

In the present study, among 62 A. flavus isolated 61 (98.4%) were susceptible to Itraconazole and 1 (1.6%) was resistant. This supports the study of Karaarslan A et al [20] and David W Denning et al [8] who showed 100% sensitivity and no resistance to Itraconazole by A. flavus.

In our study among the 48 isolates of A. niger 44 (91.6%) were sensitive and 8 were resistant (8.3%) to Itraconazole. This is in accordance to David W Denning who showed 31% resistance to Itraconazole in A. niger [8].

In our study all the 13 isolates of A. terreus were sensitive to Itraconazole. This correlates with Karaarslan A et al and David W Denning showing 100% sensitive to Itraconazole [20, 8]. In the present study among the 5 A. fumigatus isolates 4 (80%) were susceptible to Itraconazole and 1 (20%) was resistant. This is in accordance with David W Denning showing 15% resistance to A. fumigatus [8].

Among the 4 Candida species isolated 3 were Candida albicans and 1 was Candida

parapsilosis. All the 4 were sensitive to Amphotericin B and Itraconazole (100%). Our study correlates with the study of Nong H et al who showed all Candida species susceptible to Amphotericin B and Itraconazole [27].

Among the 8 isolates of Penicillium all were sensitive to Amphotericin B and Itraconazole (100%). This is supported by the study of Nong H. et al showing all the isolates of Penicillium as sensitive to Amphotericin B [27].

SUMMARY

The present study was aimed at finding out the prevalence, predisposing factors for Otomycosis and doing drug susceptibility of the fungi isolated for Amphotericin B and Itraconazole. This was done at Government Rajaji Hospital attached to Madurai Medical College for a period of one year from January 2005 to December 2005.

150 patients clinically diagnosed as Otomycosis were selected for this study and the different predisposing factors were analysed.

- The incidence was more during the monsoon period (74.6%) and this was due to increased humidity in the atmosphere.

- Males were more affected (53.3%) than females. This may be due to the fact, that men are more exposed to fungal spores, as they spend more time outdoors than females.

- The outdoor air is an important vehicle for locally prevalent fungal flora.

- Most of the cases were **unilateral (93.3%)**.

- There was no predilection for either side of the ear.

- The most common predisposing factor was **CSOM (49%)**.

- The second common predisposing factor was repeated use of **ear drops**. (antibiotic / steroids) (20.6%).

- **Itching** was the most common symptom (70%).

- **Pain** was the second commonest symptom (63.3%).

- **Aspergillus flavus** was the most common fungal isolate (43.9%).

- **Aspergillus flavus** was the commonest isolate in patients with CSOM as the predisposing factor (51.39%).

- **Aspergillus flavus** was the commonest isolate in patients with Otomycosis using ear drops (65.52%).

- **Aspergillus niger** was the second common fungal isolate (34%).

- **Aspergillus niger** was the commonest isolate in females (17.73%).

- **Aspergillus terreus, Aspergillus fumigatus, Aspergillus glaucus** were also isolated.

- Different species of **Aspergillus** may be the dominant organism in different climates.

- **Aspergillus** needs dead organic matter as a substrate for their growth.

- **Penicillium** was isolated in 8 cases (5.7%).

- **Candida** was isolated in 4 cases (2.82%).

- **Candida** was the commonest isolate in **diabetes mellitus** patients having Otomycosis (50%).

- **Coagulase negative Staphylococci** was the most common bacterial isolate (29.68%).

- Proteus, Pseudomonas and Klebsiella were the other common isolates.

Aspergillus

- In **A. flavus** 91.9% were sensitive to Amphotericin B and 98.4% were sensitive to Itraconazole.
- In **A. niger** 85.4% were sensitive to Amphotericin B and 91.6% were sensitive to Itraconazole.
- In **A. terreus** 100% were resistant to Amphotericin B and 100% were sensitive to Itraconazole.
- **A. fumigatus** was 80% sensitive to Amphotericin B and Itraconazole.
- **A. glaucus** was 100% sensitive to Amphotericin B and Itraconazole.
- Czapek medium favoured the development of more spores than Saborauds dextrose agar.

Candida

- **Candida albicans** was 100% sensitive to Amphotericin B and Itraconazole.
- **Candida parapsilosis** was 100% sensitive to Amphotericin B and Itraconazole.

- **Penicillium** was 100% sensitive to Amphotericin B and Itraconazole.

CONCLUSION

CONCLUSION

* The incidence of Otomycosis is more during monsoon period.
* Males are more affected.
* Unilateral incidence of disease is common.
* CSOM is the most common predisposing factor.
* Aspergillus flavus is the most common fungal isolate.
* Aspergillus flavus is the most common isolate in agriculturists and labourers. (outdoor workers)
* Aspergillus niger is the common fungal isolate in females.
* Candida is the commonest isolate in Diabetes mellitus.
* A. flavus, A. niger, A. fumigatus and A. glaucus are sensitive to Amphotericin B and Itraconazole.
* A. terreus is 100% resistant to Amphotericin B.
* Candida species and Penicillium are 100% susceptible to Itraconazole.

The frequency of recovery of certain fungal species such as A. flavus, A. niger, A. terreus and Candida albicans from Otomycosis reflects the great ubiquity of the distribution of their spores and hence exposure to them.

Particular virulence factors also attribute to the increased frequency. A. flavus and A. fumigatus elaborate 2 exo enzymes (i) elastate – which degrades elastin, Scleroprotein and enhances invasion of elastin containing tissues like skin and ear. (ii) Alkaline protease which degrades collagen, elastin and enhances invasion.

Candida has cell wall glycoprotein that helps in adherence to epithelial surface. Candida albicans has in addition epithelial and monocyte cytocidal activity.

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PROFORMA

PROFORMA

Otomycosis

Name:

Age:

Sex:

Address:

OP/IP:

Occupation: agriculturists / labourer / student / house wife and maids / indoorworker

Duration of symptoms:

C/o. itching -
pain -
earblock -
hearingloss -
tinnitus -
discharge -

Predisposing factors:

1. CSOM
2. antibiotic / steroid drops
3. bathing in contaminated water
4. trauma
5. occlusive hearing aids
6. Diabetes Mellitus or any other immuno suppression
7. habit of cleaning ear with stick, feather, ear buds

Clinical examination:

Otoscope

Clinical Diagnosis:

Fungus isolated
Sensitivity to Amphotericin B
Itraconazole
Bacterial isolate

APPENDIX

Potassium hydroxide mounting fluid

The Potassium hydroxide mounting fluid contains 10 gms of potassium hydroxide, 20 ml of glycerol and 80 ml of distilled water.

CZAPEK'S SOLUTION Agar

Water	-	1000 cc
$NaNO_3$	-	3.0 gms
K_2HPO_4	-	1.0 gm
$MgSO_4.7H_2O$	-	0.5 gm
KCL	-	0.5 gm
$FeSO_4.7H_2O$	-	0.01 gm
Sucrose	-	30 gm
Agar	-	15 gm

Add dry ingredients except sucrose to water and boil to dissolve. Add sucrose and sterilize by autoclave.

Sabourauds Dextrose Agar has

Peptone – 10gm
Dextrose – 40 gm
Agar – 20 gm
Distilled water – 1000 ml

The pH of the medium is adjusted to 5.6. The prepared media is autoclaved at 121°C for 15 minutes.

Preparation of RPMI-1640 Medium with 2% Glucose (RPMI medium)

Place 900ml of RPMI-1640 medium into a conical flask.

Add 34.5g of morpholinopropanesulfonic acid powder.

Stir to dissolve.

Add 20g of glucose and continue stirring. Ensure that all powder has completely dissolved.

Adjust the pH to 7.0 using 10M NaOH.

Make up to 1 litre with RPMI-1640 medium and re-check the pH.

Filter sterilise.

Store in sterile medical flats at 4^{o}C.

Preparation of PBS-Tween

Prepared Phosphate buffered saline as per standard procedure.

Add 0.05% Tween 80 before sterilisation.

Aliquot into 20ml amounts in universal bottles.

Sterilise in an autoclave for 20 minutes at 121^{o}C (15psi).

Tighten the lids and store at room temperature.