PART - A

SURVEY OF OTOMYCOTIC INFECTION
INTRODUCTION

A number of micro-organisms are known to parasitize man and animals in nature. Depending on their pathogenic potentialities these micro-organisms are causing diseases of various organs of our body. Most fungal diseases of human-beings are termed as mycosis, such as keratomycosis, dermatomycosis, candidiasis and deepmycoses (Sheklakov and Milich, 1974). Amongst fungi, species belonging to genus *Aspergillus* are described as opportunistic pathogens (Cahill et al., 1967; Prystowsky et al., 1976; Carlile et al., 1978; Estes et al., 1980; Grossman et al., 1985, Allo et al., 1987; Googe et al., 1989). Some *Aspergilli* are known to cause Allergic *Aspergillus Sinusitis* (Jonathan et al., 1989), Paranasal Sinus Aspergillosis (Sudhir Bahadur et al., 1983) and Rhinitis and Asthma (Lacey et al., 1972). Schonheyder et al. (1988) reported incidence of pulmonary aspergillosis associated with cystic fibrosis.

The term otomycosis include fungal infection of the external ear, middle ear and open mastoid cavity (Paulose et al., 1989). In the literature the first report of the fungal infection of ear is made in 1843 by Andrai and Gavarret. Since then there were controversies regarding
the prevalence and even existence of the otomycosis. However, it is now considered to be a definite clinical entity and a continuing problem (Sood et al., 1967; Mugliston and O'Donoghue, 1985). Now its world-wide distribution with a higher prevalence in the hot, humid and dusty climates of the tropical and sub tropical regions has been well established (Youssef and Abdou, 1967; Paulose et al., 1989). Conant et al. (1954) expressed the opinion that not more than fifteen to twenty percent of the ear infections are true otomycosis. According to Jones (1965), the patients now had recurrent attacks of otitis externa also had primary fungal infection with a superadded bacterial pathogen and in such cases it becomes difficult to eradicate fungal infection.

Since 1950's, attention has been drawn to the increasing problem of otomycosis and its prevalence. In United Arab Republic it is a very common disease especially in spring, late summer and autumn. Ismail (1962) mentioned that the causal fungus found in all cases of otomycosis was Aspergillus niger, while Yassin et al. (1964) isolated four species of Aspergillus from thirty seven cases of fungal infection. Chisolm and Sutton (1925) studied about sixteen cases of otomycosis and found that the fungi isolated belong mainly to the families Aspergillaceae and
Mucoraceae. Wolf (1947) found that the genus *Aspergillus* was responsible for about ninety percent of the reported cases of otomycosis. Haley (1950) isolated thirty-nine species of fungi in her survey for otomycosis and reported that *Aspergillus* and *Penicillium* were the most common genera of fungi responsible for this disease. The most common *Aspergilli* isolated were *A. niger* and *A. fumigatus*. She also studied some normal cases and found that many species of fungi occur in both the normal and diseased ears. Gregson and La Touche (1961) isolated different species of *Aspergillus* and *Candida* from eighty-three cases of otomycosis. The genera *candida* was also reported by Daggett (1942) and Gordon (1948) as a causative agent of otomycosis. Similar studies were carried out by some other workers to study the fungal infection of external ear (Nelson, 1945; Gordon, 1948; Singer *et al.*, 1952; Youssef and Abdou, 1966). In these studies a number of fungi have been reported to cause ear infection.

The external ear (auris externa) includes the auricle (Pinna) and external aural (auditory) meatus (Fig.1). The auricle is located between the temporomandibular joint anteriorly and the mastoid process posteriorly. The external concave and internal convex
A = Pinna
B = External acoustic meatus
C = Tympanic membrane or ear drum
D = Auditory ossicles

FIG. 1 - EXTERNAL EAR.
PLATE - 1:

A. Fungal infection in external ear

B. Fungal infection in external ear and ear canal
PLATE - 2:

A. Infected ear canal by
   *Penicillium nigricans*

B. Infected ear canal by
   *Candida albicans*
surfaces are distinguished in the auricle. The framework of the auricle is elastic 0.5 to 1 mm thick cartilage, which is covered on both sides with perichondrium and the skin. The auricle consists of a helix which runs by its external margin, and the anthelix located inside the helix. A cartilaginous projection, found anteriorly to the external auditory meatus, is known as tragus. Another projection, located posteriorly to the tragus, is known as antitragus. A notch (incisura inter-tragica) separates the tragus from the antitragus. The auricle ends inferiorly with a lobule, which has no cartilage and is actually connective tissue covered with skin. The auricle is a funnel-shaped structure that is continuous with the external acoustic meatus.

The external acoustic includes two parts, namely the external one, which is cartilage and membranous tissue, and the internal, the body structure. In order to inspect the bony structure and the tympanic membrane, the auricle should be pulled up and back. The auditory meatus is thus made straight.

The external acoustic meatus is covered with skin which is continuous with the skin of the auricle. In the membranous cartilaginous part of the acoustic meatus, the skin is 1 to 2 mm thick and is covered with hairs.
sebaceous and ceruminous glands. The ceruminous glands are modified sebaceous glands, they secrete a brown substance, cerumen, which mixes with the secretion of the sebaceous glands and desquamated epithelium to form earwax.

The earwax provides a protective sleeve to the external ear canal. The external factors such as humidity, temperature, dust particles and pathogenic propagules are some of the important factors which determine the occurrence and development of infection in external ear canal. The sweat has normally acid reaction due to the presence of acid sodium phosphate which maintain a pH level of 4.7 to 7.5 but under excessive sweating this pH drift towards alkalinity (Macleod and Muende, 1946). This is the reason why tropical earwax fails to provide the protective sleeve normally attributed to it in temperate conditions. In addition, the more dilute oliferous form of wax is readily removed by irritation.

As we know a great variety of micro-organisms are surviving in almost all natural habitats. These micro-organisms grow and sporulate under favorable environmental conditions in their respective habitats. The dust from these habitats serve as a source of inoculum for a number of human diseases of fungal origin.
As reported earlier in this chapter the incidence of otomycosis is accounts only fifteen to twenty percent of the total ear diseases reported time to time by various workers. But is a painic problem to present day medicine as there are not many antifungal drugs known to cure fungal infection. Very few and fragmentary reports are made by some Indian workers on the incidence of otomycosis in our country (Sood et al., 1967; Jaiswal, 1990). As indicated by Youssef and Abdou, 1966 the incidence of fungal infection of the external ear are quite high in tropical and subtropical countries, the chances of such infections may be quite high in India also. However, is a subject of great enquiry before generalisation of above statement for all countries to tropic and subtropic region.

Keeping above facts in mind the present investigation is undertaken to study the cases of ear diseases in district Sagar (M.P.) and to findout frequency of otomycosis in the area.
MATERIALS AND METHODS

The purpose of the study was to investigate the frequency of fungal infections of external ear canal or mastoid cavity in the patients suffering from ear diseases (Plate 1 & 2) and to characterize the symptoms developing during for this establishment of otomycosis. The general practitioners and medical officers of district hospital Sagar and the nearby health centers were also informed about the study and were requested to refer all the patients complaining diseases of external ear canal. All such patients attending the E.N.T. out patient clinics of district hospital and the Govt. health centers situated in the rural areas of Sagar district where attended to obtain samples from them. Samples of some normal persons were also obtained in the similar manner for comparison.

COLLECTION OF SAMPLES:

Three sterile swabs with a tight cotton plug were used for every patient to pickup the debris (Plate 3) from the infected ear. The first was used to swab the outer most part of the ear canal to remove the superficial debris or the mycologic plug. This swab was always discarded. The other two swabs were then used to obtain samples from the deep end of the meatal wall and/or from the mycologic plug. So obtained swab were kept in sterile
screw cap tubes and stored at 2° ± 1° C if not processed immediately for mycological examinations.

(1) CLINICAL INVESTIGATIONS:

Clinical investigations includes the otoscopic picture of each patient which includes the mycologic appearance and symptoms in each case. The record of mycologic appearance and symptoms were noted and the results of mycological examinations were compiled to obtain a gross pathogenic appearance of diseases of external ear canal. However, in the present study only the positive cases of otomycosis were compared.

Clinical record of the patients examined includes symptoms and gross pathogenic appearance and the particular type of fungus isolated.

(2) MYCOLOGICAL EXAMINATION:

Swabs from each patient were examined by two different methods. The details of these are as follows:

(A) Direct Microscopic Examination:

A small fragment of the plug or debris was placed on one end of clean sterilized glass slide, taking care to spread the material out flat and evenly in single
layer, possibly free from folds the material was than treated with 10% KOH solution, added drop by drop to make the specimen free from wax and other kerotinic substances. The remaining protein of the specimen was than stained using aqueous cotton blue. Than a coverslip was applied and so prepared slides were than left at room temperature for 24 hours in moist chamber. The specimen was viewed by microscope to verify the presence of fungal propagules in them.

(B) Culture Method:

For the isolation of infecting microorganisms from the sample swabs method of Yassin et al., (1964) was followed. For this sample swabs were immersed in test tubes containing 10 ml sterile distilled water and shaken well for 15 minutes. So prepared suspension was further diluted and a series of dilution up to \(10^{-3}\) were prepared to insure isolation of all infecting microorganisms present in them. 0.1 ml of diluted suspension from each dilutions was then plated in Petridishes containing 20 ml sabouraud's dextrose agar (40 gm. Dextrose, 10 gm Peptone, 17 gm Agar Agar, 1000 ml Distilled water). The plates were incubated at 28°C \(\pm 1^\circ\)C to facilitate the growth of microbial propagules. Any fungal growth appearing on the agar plates was examined and collected in
purified form for further studies.

IDENTIFICATION:

The isolates were identified with the help of available literature in the laboratory of The Applied Microbiology and Biotechnology, Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) and secured through the courtesy of various workers of allied fields. Living cultures are also stored in the culture collections of the department.
PLATE - 3:

A. A swab with a tight cotton plug used for obtaining the debris from the infected ear

B. Test organisms -
   1. *Aspergillus niger*
   2 & 6. *Aspergillus flavus*
   3. *Absidia corymbifera*
   4. *Panicillium nigricans*
   5. *Candida albicans*
RESULTS

DISTRIBUTION OF FUNGAL SPECIES IN NORMAL AND INFECTED EAR CANAL

In all 480 samples including a total of 350 normal ear and 130 samples from infected ears, (Table - 1) respectively, were collected during present investigations. Mycological examination of these samples indicated fungal occurrence in 10.57 percent samples of normal ears, while a 43.84 percent samples belong to infected ears were found positive for fungal occurrence.

A total of 16 fungal species belonging to 14 genera were isolated from all the samples examined during present investigation. Amongst these 15 fungal species belonging to 13 genera were isolated from samples of the normal ear, while only 9 fungal species belonging to 7 genera were collected from samples of infected ear canal.

Three species belonging to genus Aspergillus i.e., A. niger, A. flavus and A. fumigatus were isolated from samples of both normal and infected ear canals. Besides these Penicillium nigricans, Absidia corymbifera and unidentified species belonging to genus Rhizopus, Mucor and one sterile fungus were also found to be present in
<table>
<thead>
<tr>
<th>Organisms</th>
<th>Normal Ear</th>
<th>Infected Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>07</td>
<td>18</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>02</td>
<td>09</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td><em>Penicillium nigricans</em></td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td><em>Absidia corymbifera</em></td>
<td>03</td>
<td>08</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>–</td>
<td>03</td>
</tr>
<tr>
<td><em>Rhizopus sp.</em></td>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td><em>Mucor sp.</em></td>
<td>02</td>
<td>02</td>
</tr>
<tr>
<td><em>Fusarium sp.</em></td>
<td>03</td>
<td>00</td>
</tr>
<tr>
<td><em>Curvularia sp.</em></td>
<td>03</td>
<td>00</td>
</tr>
<tr>
<td><em>Sterile mycelia</em></td>
<td>04</td>
<td>02</td>
</tr>
<tr>
<td><em>Alternaria sp.</em></td>
<td>02</td>
<td>00</td>
</tr>
<tr>
<td><em>Epidermatothyton flaccosum</em></td>
<td>01</td>
<td>00</td>
</tr>
<tr>
<td><em>Helminthosporium sp.</em></td>
<td>02</td>
<td>00</td>
</tr>
<tr>
<td><em>Mirospora sp.</em></td>
<td>02</td>
<td>00</td>
</tr>
<tr>
<td><em>Trichoderma sp.</em></td>
<td>02</td>
<td>00</td>
</tr>
</tbody>
</table>

- **No. of Positive Samples: Occurrence (%):**

- **Total Number of Normal Ear Examined = 350**
- **Total Number of Infected Ear Examined = 130**
OCCURRENCE OF FUNGAL SPECIES IN NORMAL AND INFECTED EXTERNAL EAR CANAL

FIG. 2

1- A. aiger, 2- A. flavus, 3- A. fumigatus, 4- F. nigricans, 5- A. corynebacteria, 6- C. albicans, 7- Rhizopus sp.
8- Mucor sp., 9- Fusarium sp., 10- Curvularia sp., 11- Sterile mycelia, 12- Alternaria sp.,
TABLE NO. 2

Samples showing more than one fungi from the infected ear

<table>
<thead>
<tr>
<th>Fungal combination</th>
<th>No. of samples</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger + A. flavus</td>
<td>3</td>
<td>2.30</td>
</tr>
<tr>
<td>A. niger + A. fumigatus</td>
<td>2</td>
<td>1.52</td>
</tr>
<tr>
<td>A. niger + P. nigricans</td>
<td>1</td>
<td>0.76</td>
</tr>
<tr>
<td>C. albicans + A. niger</td>
<td>2</td>
<td>1.52</td>
</tr>
<tr>
<td>C. albicans + A. flavus</td>
<td>1</td>
<td>0.76</td>
</tr>
<tr>
<td>Absidia corymbefera +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>4</td>
<td>3.04</td>
</tr>
<tr>
<td>Absidia corymbefera +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>3</td>
<td>2.30</td>
</tr>
</tbody>
</table>

Total 16 12.30

Total number of infected ear examined = 130
both normal and infected ears. Candida albicans was also isolated from some samples of infected ears. Fungi belonging to genus Fusarium, Curvularia, Alternaria, Epidermatophyton, Helminthosporium, Nigrospora and Trichoderma were isolated from samples of normal ear only. A. niger was found most frequent in its occurrence in both normal and infected ears. It was isolated from 13,846 and 2,000 percent samples of infected ears and normal ears, respectively. Absidia corymbefera a mucoraceous fungus was found next to A. niger and was isolated from 6,153 percent samples of infected ears and 0.857 percent samples of normal ears. However, A. flavus showed little more percentage of its occurrence in infected ear than A. corymbefera.

During mycological examination a number of samples yielded more than one type of fungal species (Table-2). Only 16 samples yielded more than one fungal species on their mycological examination, in such samples A. niger was found most frequent as its was isolated in combination to A. flavus, A. fumigatus, Penicillium nigricans and Absidia corymbefera. Other two fungi i.e., Candida albicans and Absidia corymbefera were also found in combination with A. niger and A. flavus. Three percent samples yielded Absidia corymbefera in combination to A.
niger, while only 2.3 percent samples yielded either *A. niger* in combination with *A. flavus* or *A. corymbifera* with *A. flavus*.

**CLINICAL INVESTIGATIONS AND THEIR CO-RELATION WITH FUNGAL OCCURRENCE:**

The symptoms correlated by the patients at the time of collection of samples, were noted (Table - 3) and a resume of main symptoms is as follows:

**IRRITATION:**

This is the symptom seems to be appeared just after the onset of infection in the ear. The irritation and sensation of discomfort was noted in the ear canal of the patients. However, a very few patients have reported sensation of irritation in deeper part of the ear canal.

**PAIN:**

This is the major symptom reported by many patients. Few cases have also complained headache accompanying with severe pain in the ear, while others were having only pain in the ear canal.
**TABLE NO. 3**

Fungal isolates from infected ear showing
different symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>irritation</td>
<td><em>A. niger, P. nigricans</em></td>
</tr>
</tbody>
</table>
| Pain        | *A. niger, A. flavus, C. albicans, P. nigricans*  
              | Mixed fungi, Aspergilli and Candida. |
| Tinnitus    | *P. nigricans*                                 |
| Deafness    | *A. niger*                                     |
| Itching     | *A. flavus, C. albicans, Mixed fungi, Mixed Aspergilli and Candida.* |
| Discharge   | *A. niger, C. albicans, A. corvmefera, P. nigricans, Mixed fungi mixed Aspergilli and Candida.* |
| Toxaemia    | Mixed fungi, Mixed - Aspergilli and Candida.    |
TINNITUS:

This symptom was recorded in some of the patients only. In addition to this an immediate relief was recorded once the plug was removed.

DEAFNESS:

This is the symptom reported by those patients which were bearing a spongy plug of almost black colour. Inflammation in the ear canal was found associated with this symptom in many cases.

ITCHING:

Many of the patients complained severe itching in their ear canal. No actual plug was detected in many of these patients, but only a small amount of debris was present in the deep meatus. Other symptoms found associated with itching were the pain and irritation.

DISCHARGE:

Discharge of mucous was noted in some cases.

TOXAEMIA:

Some of the patients have reported fever and other complaints.
Besides these symptoms four typical mycologic appearance have been recorded (Table - 4) in the infected ear canal and are as follows:

(1) Dry mycelial mat
(2) Soft debris
(3) Wet mycelial mat
(4) Mycologic plug

In course of present study the symptoms which were found associated with fungal occurrence are given in Table-3 and a grass pathogenic appearance of the symptoms, mycologic appearance and the causal agents of otomycosis are given in Table-4.

The data recorded indicated that A. niger and P. nigricans infection mostly cause irritation in the external ear canal with some exceptions in the exterior of canal near tympanic membrane. The cause of deafness was noted mostly due to A. niger which is occasionally found associated with pain, discharge and irritation. Itching is caused by almost all fungi causing infection in the ear. However, cases of toxaemia were only few in these cases debris from ear canal was found to possess mixed infection of some fungi mostly Aspergillus or/and Candida.
<table>
<thead>
<tr>
<th>Organisms</th>
<th>Mycologic Appearance</th>
<th>Particular Symptoms Noted</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Mycologic plug common, wet mat and fungal appear in early stage, dry mat rare.</td>
<td>Irritation marked with mild to severe pain, discharge least.</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>Mycologic plug common, dry mat rare.</td>
<td>Severe pain with frequent itching.</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Mycologic plug.</td>
<td>Severe pain with frequent itching.</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Mycologic plug formation very rare, wet debris found some times.</td>
<td>Severe itching and pain are common symptoms, discharge rare.</td>
</tr>
<tr>
<td><em>Absidia corymbifera</em></td>
<td>Soft debris common, wet mycelium appear stage, mycologic plug common.</td>
<td>Discharge is a major symptom.</td>
</tr>
<tr>
<td><em>Rhizopus</em></td>
<td>Wet mycelium in early stage, and dry myceli rare.</td>
<td>Pain, irritation and tinnitus are main symptoms.</td>
</tr>
<tr>
<td><em>Penicillium nigricans</em></td>
<td>Mycologic plug very common.</td>
<td>Severe pain and itching abundant discharge toxaemia rare.</td>
</tr>
<tr>
<td>Mixed <em>Aspergillus</em> and <em>Candida</em></td>
<td>Mycologic plug.</td>
<td>Severe pain with abundant, discharge, toxaemia rare.</td>
</tr>
</tbody>
</table>
DISCUSSION

The most significant feature of this investigation has been the frequency with which fungi have been isolated from normal ear and infected ear. In the present survey the fungal occurrence was noted in 10.57 and 43.84 percent of the total samples collected from normal and infected ear, respectively. It is interesting that the normal ear which were found positive for fungal occurrence yielded more number of fungal species than the number of fungal species recorded from samples of infected ears. Fungal species which were isolated from infected ear includes species belonging to genus \textit{Aspergillus}, \textit{Penicillium}, \textit{Absidia}, \textit{Mucor}, \textit{Rhizopus} and \textit{Candida}. Few isolates of unknown taxonomic position, which failed to produce spores were also recorded from infected ear (Table-1). Other species belonging to genus \textit{Alternaria}, \textit{Curvularia}, \textit{Helminthosporium}, \textit{Nigrospora} and \textit{Trichoderma} along with few \textit{Sterile mycelial} forms were recorded from normal ear only. This is somewhat surprising that none of the above fungi has been found associated with infections of the otomycosis.

How and when the fungal propagules start infecting the ear and what are the suitable condition met in the external ear canal for the establishment of
infection is a subject of great enquiry. Some reports have also indicated the occurrence of fungal species in normal ear (Singer et al., 1952; Sood et al., 1967). Singer et al. (1952) collected samples from military personnel at the MacDill Air Force Base, Tampa and the U.S. Naval Air Technical Training Center Jacksonville, Florida and found 38.3% and 42.7% percent positive samples for fungal occurrence belonging to debris from normal ear and infected ear, respectively. They have also reported a low number of fungal specimen in samples of infected ears than normal ear. In addition to these various surveys have been undertaken to characterize the percentage frequency of otomycosis in the reported cases of ear infections. Smyth (1962) examined 282 patients and found 109 cavities positive for fungal occurrence (19.32%). He reported 5 species of Aspergillus, Penicillium sp., Scopulariopsis sp. and Candida albicans from these cases. Maximum cavities yielded A. niger (5.3%), A. terreus (4.4%) and others with their lesser frequencies. Youssef and Abdou (1966) formed Aspergillus and Candida as being the major cause of otomycosis in U.A.R., Wolf (1947) found that species belonging to genus Aspergillus as a major cause of otomycosis. Haley (1950) isolated a large number of fungi (39 fungal species) from 367 cases of otomycosis and reported Aspergillus and Penicillium as major cause of this
disease, while Paulose et al. (1989) reported only four species from the cases of otomycosis from Bahrain and found maximum frequency of *A. niger* (54.4%) in the reported cases. From India reports on otomycosis were made by Sood et al. (1967) and Jaiswal (1990) from Delhi and Bihar, respectively. Jaiswal reported maximum incidence of this disease in farmers and labourers of Bihar. Sood et al. (1967) conducted studies to produce otomycosis in human volunteers, on transfer of fungal infection from infected to healthy ear canals. He found 36.67 percent cases in which otomycotic infection produced successfully.

The results of the present investigation confirms the findings of above surveys of otomycosis. In present investigation *Aspergillus niger* was found associated with 31.58 percent positive cases of otomycosis. *A. flavus* and *A. corymbiferum* were recorded only from 15.79 and 14.03 percent cases. In the surveyed area the cause of otomycosis by *C. albicans* was found with lesser frequency. Data given in Table-2 indicate some of the cases of otomycosis which possess mixed fungal infection. Our results correlates the findings of Youssef and Abdou (1966) they also reported some cases of mixed infection in the patients of otomycosis examined by them. Various factors were also studied by Yassin et al. (1964); Youssef and
Abdou (1966). According to them fungi causing otomycosis were found to grow optimally at about 35°C. However, the growth rate of these fungi was found optimum at about pH 6.0 but were found to grow at a range of pH 4.0 to 9.0 (Youssef and Abdou, 1966). The temperature requirement of these pathogenic fungi confirms the high incidence in tropical and subtropical countries. Relative humidity of the atmosphere and occasional entry of water in the ear canal also seems to play an important role in the incidence of this disease (Yassin et al., 1964). Nature of source of infection and the number of fungal spores and hyphae in the atmosphere also determines the incidence of otomycosis. Besides external atmospheric conditions and the presence of pathogenic fungal spores in the air resistance of the host is equally important. Some workers attach greater importance to the presence of secretions of the apocrine and sebaceous glands which provides a protective covering to the skin of mastoid cavity (Smyth, 1962). According to him entry of water in the cavity during swimming and excessive sweating are likely to dissolve some of the water miscible waxes, and cholesterol esters which provides easy establishment to the propagules of fungi in the mastoid cavity.
PART -B

TAXONOMY OF CAUSAL ORGANISMS
TAXONOMIC DESCRIPTION OF THE
FUNGAL ISOLATES

INTRODUCTION

During the present study a number of fungal species were isolated from normal and infected external ear canal (Table - 5). All the isolates were identified and kept in stock culture. A total of five fungal species were isolated and described.

Taxonomic description of all the five fungal species is given here (Plate - 3), they were also compared with their type descriptions.
TABLE NO. 5

Fungi isolated from the external ear canal of the patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Absidia corymbifera</em> Lichth</td>
</tr>
<tr>
<td>2.</td>
<td><em>Aspergillus flavus</em> Link</td>
</tr>
<tr>
<td>3.</td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td>4.</td>
<td><em>A. niger</em> Van Tiegham</td>
</tr>
<tr>
<td>5.</td>
<td><em>Alternaria</em> sp.</td>
</tr>
<tr>
<td>6.</td>
<td><em>Candida albicans</em> Robin Berkhout</td>
</tr>
<tr>
<td>7.</td>
<td><em>Curvularia</em> sp.</td>
</tr>
<tr>
<td>8.</td>
<td><em>Epidermatophyton fluccosum</em></td>
</tr>
<tr>
<td>9.</td>
<td><em>Fusarium</em> sp.</td>
</tr>
<tr>
<td>10.</td>
<td><em>Helminthosporium</em> sp.</td>
</tr>
<tr>
<td>11.</td>
<td><em>Mucor</em> sp.</td>
</tr>
<tr>
<td>12.</td>
<td><em>Nigrospora</em> sp.</td>
</tr>
<tr>
<td>13.</td>
<td><em>Penicillium nigricans</em> (Bainear) Thom</td>
</tr>
<tr>
<td>15.</td>
<td><em>Sterile mycelia</em></td>
</tr>
</tbody>
</table>
Aspergillus niger Van Tiegham

(Plate 4 Fig. 3 ) Isolate No. 4

Synonyms:

- Sterigmatocystis nigra Van Tiegham

Colonies rapidly growing with abundant submargined mycelium, colourless or less yellowish in colour, aerial hyphae usually scanty. Conidial heads blackish brown, globose 210 to 280 μ in diameter, conidiophore arising from the substratum smooth wall 2.8 to 4.5 μ thick, almost colourless near the foot, brownish towards the vesicle, 1 to 1.5 mm in height by 14 to 21 μ in diameter. Vesicle globose, brownish, fertile all over the surface 25 to 48 μ in diameter, sterigmata in two series, brownish, closely packed.

The fungus tallies with the description given by Thom and Raper (1945). Fungus grow fast on sabouraud's dextrose agar medium attaining a diameter of 53 mm in four days at 28°C ± 1°C temperature.

Note:

The fungus was isolated from the external ear canal. It was earlier reported by Andral and Gavarret, 1843; Tigham, 1867; Capps, 1938; Wolf, 1947; Gregson and
FIG. - 3

Aspergillus niger Van Tiegham

A. Septated mycelium
B. Vesicle with sterigmata and chains of conidia
C. Conidia
PLATE - 4:

A. *Aspergillus niger* colony on Sabouraud's dextrose media

B. Fungal colony - as above enlarged view

The living culture of this fungus is kept at the culture collection of Applied Microbiology and Biotechnology lab., Deptt. of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar.
*Aspergillus flavus* Link

(Plate 5 Fig. 4 ) Isolate No. 2

Synonyms:

- Monilla flava Persoon
- Eurotium Aspergillus flavus De Barm and Woronin

Colonies growing rapidly, floccose, limited to scanty growth of sterile hyphae in older and dryer areas among crowded conidiophores, conidial areas range in colour from velvety to yellowish green, colours are persistent in old colonies, reverse yellowish to orange. Conidial heads vary from small with a few chains of conidia to large radiate, conidial heads, green, globose to loosely radiate, occasionally splitting into 3 to 4 columns of conidia, 285 to 417 μ in diameter, conidiophores pitted, colourless, walls strongly rough (0.7 to 1 μ thick), 0.7 to 1.2 mm in length by 8 to 12 μ in diameter, arising from submerged hyphae, vesicle globose to flask shaped, 40 to 54 μ in diameter. Sterigmata usually in two series, primary in 7 to 10 μ by 3.5 to 4.0 μ secondary 7.5 to 8.4 μ by 2.5 to 3.0 μ in single series on the same vesicle measuring 7 to 7.5 μ by 2.8 to 3.0 μ. Conidia rough 2.8 to 3.5 μ in diameter, sclerotia not formed.

The fungus tallies with the type description given
Aspergillus flavus Link

A. Septated mycelium

B. Vesicle with sterigmat and chains of conidia

C. Conidia
PLATE - 5:

A. Ampyrgillus flavum colony
   on Sabouraud's dextrose media

B. Fungal colony - as above
   enlarged view
by Thom and Raper (1945). Fungus grow fast on sabouraud's dextrose agar medium attaining a diameter of 62 mm within four days at $28^\circ C \pm 1^\circ C$ temperature.

NOTE:

This species is very common in nature and among the most abundant of all the Aspergilli. During the course of the present studies it was collected from an external ear canal. The fungus has been earlier reported by Andral and Gavarret 1843; Tigham, 1867; Capps, 1938; Wolf, 1947; Ismail, 1962; Bickley et al., 1988; Paulose, 1989; Jaiswal, 1990.

The living culture of the fungus is deposited at culture collection of Applied Microbiology and Biotechnology lab., Deptt. of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar.
Absidia corynefera Lichth

(Plate 6 Fig. 5) Isolate No. 1

Synonyms:
- Absidia lichtheimii (Lucet and Costantin) Lender

Sporangiophores prostrate, branched in corymbs, forming white felt, woolly. They terminate in corymbeform branching by carrying the sporangia on longer or shorter pedicels. A little below the terminal corymb frequently occur in groups of branches carrying smaller sporangia. Sporangia erect, hyaline, pear-shaped, with an infundibuliform apophysis, becoming attenuate gradually to the sporangiophore having an average diameter of 45 to 60 μ and the least 10 to 20 μ, wall of sporangium colourless, transparent, smooth, different leaving a basal collarette. Columella large hemispherical or globular, 10 to 20 μ, smooth smoky gray. The apophysis and pedical also similarly coloured, spores spheric or subspheric, colourless, small, usually 2 μ in diameter × 3 μ long.

The fungus tallies closely with the description given by Gilman (1959). Fungus grow fast on Sabouraud's dextrose agar medium.

NOTE:
The fungus was isolated from the external ear canal. The fungus was also reported by Chisom and Sutton, 1925; Lawrence et al., 1978 as human pathogen.
FIG. - 5

*Absidia corymbifera* Lichth

A. Coenocytic mycelium

B. Sporangiophores with sporangia filled with spores

C. Spores
PLATE - 6:

A. *Absidia corymbifera* colony
   on Sabouraud's dextrose media

B. Fungal colony - as above
   enlarged view
Penicillium nigricans (Bainier) Thom

(Plate 7 Fig. 6) Isolate No. 13

Synonyms:
- Penicillium echinatum Bainier

Colonies dark gray at first becoming mouse gray, reverse yellow to deep orange shades, mycelium branched, septate, conidia bearing hyphae variously short branches of aerial hyphae, penicilli terminal on short branches about 50 μ long, consisting of variously diverging branchlets bearing few to many sterigmata and chains of conidia occasionally parallel but usually divergent with individual chains commonly up to 52 μ to 80 μ in length, metulae strongly divergent, variable about 8 to 14 μ by 2.2 to 4.2 μ. Sterigmata usually born in clusters of 6 to 12, more or less divergent about 7 to 8 μ by 2.0 μ, conidia 3.0 to 4.0 μ in diameter, globose and spiny.

The fungus tallies with the description given by Raper and Thom (1949). Fungus grow fast on sabouraud’s dextrose agar medium attaining a diameter of 30 mm in diameter at 28°C ± 1°C temperature within four days.

NOTE:

The fungus was isolated from the external ear canal. The fungus was also reported by 1867; Wolf, 1947; Gregson and La Touche, 1961; Sood et al., 1967 as human pathogen.
FIG. - 6

*Penicillium nigricans* (Bainier) Thom

A. Septated mycelium

B. Sterigmeta with chains of conidia

C. Conidia
PLATE - 7:

A. *Penicillium nigricans* colony on Sabouraud's dextrose media

B. Fungal colony - as above
   enlarged view
**Candida albicans.** Robin Berkhout

(Plate 8 Fig. 7) Isolate No. 6

**Synonyms:**

- *Oidium albicans* Rabin
- *Saccharomyces albicans* Robin (Rees)
- *Monilia albicans* Robin (Lucrent)

The young colonies were short yellowish white, easily detachable crusts throughout its entire length, reverse of the colonies whitish. *Candida albicans* is often referred to as a diamorphic fungus. Blastoconidia (Yeast cells), which reproduce by budding, are ovoid to nearly spherical cells varying in size from 2.7 x 2.0 to 10 x 6 μm. Hyphae which elongate continuosly and lay down septa at intervals behind the growing tip, pseudohyphae are essentially blastoconidial cells, elongated to a variable extent, which arise by an acropetal budding process and remain unseparated, some times giving an appearance of a filamentous cell, chain that is often narrow enough to be confused with true hyphae chlamydosores are thick-walled, highly refractile, spheres approximately 10 μm in diameter.

Fungus grew fast on sabouraud’s dextrose agar medium attaining a diameter of 40 mm within four days. at 28°C ± 1°C temperature.
FIG. - 7

*Candida albicans* Robin Berkhout

A. Blastocoenidia attached on mycelium
B. Chlamydospores
C. Blastocoenidia (*Yeast cells*)
PLATE - 8:

A. *Candida albicans* colony
   on Sabouraud's dextrose media

B. Fungal colony - as above
   enlarged view
NOTE:

The fungus was isolated from the external ear canal. It is a well known pathogen (Taschdjian, 1957; Conant et al., 1971; Howard, 1983; Barzon, 1983; Benjak and Chondy, 1989).

The fungus is characterized by liquid serous discharge in the external acoustic meatus (Palchoun and Voznesensky, 1988).

The living culture is being deposited at the culture collection of the Applied Microbiology and Biotechnology Lab. of the Botany Department, Dr. H. S. Gour Vishwavidyalaya, Sagar.