CHAPTER I

GENERAL INTRODUCTION
Fungi form a large polyphyletical group. It has been estimated that there are between 250,000 to 300,000 fungal species widely distributed in all the ecosystem. It is estimated that the weight of all the fungi living on our planet largely exceeds that of all the human beings (Muller and Loeffter, 1976). Generally, fungi develop a complex ecological relations with other living organisms and can cause an incidence of new morphological and taxonomic entities. In a survey, Howard (1983) listed 80 pathogenic genera for human and lower animals which according to Ainsworth (1968), would include more than 200 species.

Human and other mammals are exposed to fungal contamination from birth. This can happen:

A - When human and other mammals penetrate in an ecosystem that is not theirs (deep mycosis called "exotic" occurring through a respiratory route and mycetome, chromoblastomycosis and sporotrichosis infesting via the taguments).

B - When they weakened or more susceptible because of intercurrent pathologies or in iatrogenic factors (opportunistic mycosis).
C - When they are exposed to the infesting stage of pathogenic fungi very well adapted to parasitism and quite able to invade the host (dermatophytes).

These circumstances allow us to characterise three groups of pathogenic fungi for humans and lower animals according to their degree of parasitic adaptation (Biguet, 1979).

- Exosaprophytic fungi rather common in the outside medium whose conidia, either inhaled or reaching the organism through other routes, are almost systematically infectious (Blastomyces, Histoplasma, Coccidioides).

- Endo or exosaprophytic fungi which have a pathogenic parasitic effect only on hosts made susceptible, this corresponds to the definition of opportunistic micro-organisms most commonly admitted (Dei-Cas et al., 1984) are the species of Candida, Aspergillus, Mucor, Rhizopus, Absidia and Penicillium.

- Genuine and obligatory parasitic dermatophytes.

Regarding the opportunistic moulds it is said that in the early stages of aspergillosis, Aspergillus fumigatus develops in the lungs of the host in the form of
short and wide hyphal branches (5-10 \( \mu m \) wide), which only later on will look more like the saprophytic mycelium. The morphology of the mycelium becomes "normal" during the final phases of experimental infections. This fact might show that the resistance of the host has been overcome (Austwick, 1968). Some times it has been observed that during a case of chronic otomycosis due to *Aspergillus fumigatus*, the fungus developed in vivo its typical asexual-fruited bodies. This phenomenon is more frequent in the aerial sacs of birds. Finally, the development of *Aspergillus* reproductive sexual form (cleistothecia) and hulle cells were observed in vivo in one case of human sinus al aspergillosis (Doby and Favry, 1978). However, this phenomenon seems to be very rare.

The occurrence of opportunistic mycosis is rising rapidly, for an organism to cause disease it must enter the host, multiply or grow in host tissues, resist or not stimulate host defence mechanisms, and finally, damage the host. However, the disease process is an interaction between an organism and the host and this process can not be fully understood if one studies only the host of an organism as separate entities. There are several unique features of the opportunistic infections. Aspergillosis, Mucormycosis, Penicilliosis and Candidiasis, unlike the
traditional pathogenic fungi, which make their study particularly interesting. The causative organisms are saprophytic or commensal organisms and usually infect only patients predisposed by some underlying diseases or treatment. Because the hosts are predisposed often times distinct defence mechanisms are defective and it is possible to utilise this to determine which aspects of host defence are of importance in controlling the infection. The etiologic agents of Aspergillosis, Penciliosis, and Mucormycosis are not dimorphic. like the true pathogenic dimorphic fungi as they grow both in the environment and within the host as hyphal forms. However, the host must contend with several forms of the organism to successfully eliminate them.

A resting metabolically inactive spore or conidium enters the host, swells and germinates in the susceptible host and invades tissues as a hyphal organism. Each form displays different antigenic and surface features and elicit different host responses.

In some dermatophytes soil is an important constituents of the natural focus. The soil creates the main link in the extra human circulation of the agent. However, the question arises why some species of fungi can
live and survive in soil while others cannot. Emmons (1950), evaluated that there was a sound basis for the hypothesis that pathogenic fungi causing human and animal diseases are more commonly present in soil and vegetation than mycologists generally realised. During recent years there have been many reports on the isolation of dermatophytes and related keratinophilic fungi from Indian soils (Padhye, 1961; Randhawa and Sandhu, 1964; Mohapatra and Gugnani, 1969; Jain and Agrawal, 1977; Deshmukh et al., 1981; Deshmukh and Agrawal, 1983; Kushwaha et al., 1985; Rathore, 1987). The occurrence of these moulds is essentially related to the human-beings and animals. Studies on soil inhabiting keratinophilic fungi from various habitats are receiving considerable attention in recent years.

For the last 100 years surveys (Berzon, 1983) have described the pattern of ear disease in patients consulting general practice or hospital clinics in different areas of the U. K. (Williamson, 1856; Duncanson et al., 1981; Knopp, 1982). In United Kingdom in the year 1971 and 1972 the National Morbidity Surveys were conducted on a larger scale, involving over 200,000 patient/years in randomly selected practice. This survey reported consultations and episodes of ear disease
(H.M.S.O., 1974, 1979). However, a high incidence of fungal external otitis in India and other tropical countries are apparent (Maipani, 1962; Proctor, 1965; Jain, 1967).

Andral and Gavarret (1843), were the first to describe fungus infection of the ear, it has long been recognized to be due to Aspergillus (Capps, 1938). Up to the past decade otitis externa was called "Otomycosis". The earlier literature (Singer et al., 1952) on this condition reported that mycotic infection was the cause. The term otomycosis, should be redefined to include fungal infection of the external ear, middle ear, and open mastoid cavity as against the present definition of otitis externa of fungal etiology (Paulose et al., 1969).

Otomycosis has a worldwide distribution with a higher prevalence in the hot, humid and dusty climate of the tropical and subtropical regions. Conant et al. (1954) exposed the opinion that more than 15-20 percent of ear infections are true otomycosis. Jones (1965) reported that patients who had recurrent attacks of otitis externa had primary fungal infections with a superadded bacterial pathogen. In the past there were controversies regarding the prevalence and even existence of otomycosis. It is now
considered to be a definite clinical entity and a continuing problem (Sood et al., 1967; Mugliston and O'Donoghae, 1985). Various synonyms such as a swimming pool ear, Singapore ear, fungus ear, swimmers ear, hot weather ear, mildew ear, Hongkong ear and itching ear all are caused by micro-organisms as previously suspected (Alonso, 1951; Singer et al., 1952; Hardey et al., 1954; Sanders, 1959; Cassini et al., 1977; Lawerence et al., 1978, Pauloose et al., 1989). Since 1950s, attention has been drawn to the increasing problem of otomycosis and its prevalence.

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 Aspergillus is responsible for about 90 percent of otomycosis cases. The genus Candida was also reported by Daggett (1942) and Ward (1948) as a causative of otomycosis. Conley (1948) examined 200 patients having external otitis with special emphasis on its relation to ear wax. Haley (1950), isolated 39 species of fungi from 367 cases of otomycosis and reported that Aspergillus and Penicillium were the most common genera of fungi responsible for the disease. Yassin et al. (1964) isolated 37 Aspergillus cultures, the four
different species found are *A. niger*, *A. flavus*, *A. nidulans* and *A. terreus*.

Micro-organisms exist parasitically on skin and on its appendages in the external auditory meatus. The external auditory meatus has a lining of skin similar to that of other parts of the body. However, it differs in the following respects:

I - It is only exposed to the atmosphere by the small meatal inlet and therefore represents a skin lined culture tube which provides optimum conditions for the growth of micro-organisms.

II - The skin has a very rigid support as it is closely applied to the periconidium and periosteum forming its margins, so there is little room for lateral expansion and oedema is accompanied by intensive pain.

III - The hair follicles, sweat, ciruminous and sebaceous glands are confined entirely to the outer third of the ear canal. Ceruminous glands are modified sweat glands. They differ from the characteristic apocrine type in that the cells are filled with fat droplets and brown pigment granules which form ear wax. Sweat has normally an acid reaction due to the presence of acid sodium phosphate, which maintains a pH level of 4.7 to 7.5 (West and Todd, 1961), but this becomes
alkaline in the presence of excessive sweating (Macleod and Muende, 1946). For this reason tropical ear-wax fails to provide the protective sleeve normally attributed to it in temperate conditions. In addition, the more dilute oliferous form of wax renders it more readily removed by irrigation, which is associated with swimming and diving.

Some micro-organisms have developed enzymatic systems that enable them to attack the substrates furnished by hosts. The pathogenic capability of micro-organisms can also depend on its capacity to produce toxins or potentially destructive enzymes. Thermotolerant isolates of certain species produce an alkaline proteinase with proteolytic activity within the normal physiological pH range (Fromentin et al., 1970). These enzymes might also play a part in inhibiting the defensive responses of the host (Biguet, 1979). Aspergillus fumigatus is reported to produce endotoxins (Tilden et al., 1961), C substance (Longbottom and Pepys, 1964), tremorgenic (Yamazaki et al., 1971) and mycotoxins (Choudhary and Singh, 1982).

A. fumigatus produces gliotoxin, a compound containing an epidithiadioxopiperazine ring, which has immunosuppressive and antiphagocytic activity in vitro (Mullbacher and Eichner, 1984). Acid proteinases are
secreted by most strains of *Candida* sp., reflecting the order of virulence of *Candida* sp. for man (Ruchel et al., 1963; Ruchel, 1964). Toxins and enzymes may augment the pathogenic potential of fungal agent and the variations of toxigenic potential of fungi may be responsible for the differences in the pathogenic effects of various strains. Further studies are needed to verify the role of enzymes and toxins in virulence of these organisms.

To understand the mode of infection of pathogenic moulds, host and parasite relation and nature of parasitism, it is essential to study the various aspects of the physiology of different parasites which can be of some help for a scientific approach towards the eradication of the diseases.

*A. terreus* and *A. fumigatus* are reported from aural cavities. English and Dalton (1962) occasionally found *A. niger* and *A. flavus* as a common cause of otomycosis of the intact ear. All these fungi are of very common occurrence outside the human body, are primarily saprophytic and of world-wide distribution. They occur in soil (Gilman, 1959), on all sorts of decaying vegetable matter (Ainsworth and Austwick, 1959; Emmons, 1960; Smith, 1960) and in the air of residential districts (Veries, 1960). All except *A. terreus* were found in house dusts by
Davies (1960). It is, therefore, not difficult to suggest possible sources for aural infections.

It is essential to study various aspects of the physiology of parasites, which can suggest better therapeutic measures and improved diagnostic approaches. This would help in the study of the epidemiology of otomycosis (fungal infection of the external ear canal). It is because of this, the present study was planned to acquire a better understanding in this line, for which five test fungi i.e., *Aspergillus niger*, *A. flavus*, *Absidia corymbifera*, *Penicillium nigricans* and *Candida albicans* were selected for their detailed study. Wolf (1947) found that *Aspergillus* is responsible for about 90 percent of otomycosis cases. Gregson and La Touche (1961) stated that fungal organisms were predominantly species of *Aspergillus* or *Candida*, and isolated six different species of *Aspergillus* namely, *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus* and *A. herbariorum*. According to Yassin et al. (1964) infection cases are recorded more in the summer than in winter time; this may be due to relatively high temperature in summer, besides relatively high humidity which stimulates better growth of the fungus. Pollution of water and swimming pools is another factor which increases the incidence of the disease in summer.
Concerning sources of infection, fungi infecting the ear are mainly airborne pathogens. Fungal spores and hyphae contaminate the atmosphere are able to grow in the external auditory meatus and are able to include infection owing to the presence of favourable conditions such as (i) relatively high percentage of humidity in the external ear canal or external auditory meatus (ii) high temperature which closely approximates "body temperature" (iii) epithelial debris and serous exudate in various stages i.e., light/dark and pH of chemical break-down.

Otomyososis infection caused by various species of fungi are widely distributed and constitute in some areas of public health a problem of great magnitude. The use of plant parts and their components against pathogenic fungi have been suggested by several workers (Boas, 1939; Meruzzella and Liguori, 1958; Nene et al., 1968; Khanna and Chandra, 1972; Grover and Rao, 1976; Jain and Agrawal, 1976; Deshmukh and Jain, 1981; Shrivastava et al., 1984). Reports of mycotic infection (Tinea capitis) from our country indicate an uncommon condition in India. The causes of this rarity are not clear, though use of mustard oil has been considered as a probable preventive factor (Ajello, 1960; Kaira et al., 1964). Hajni et al. (1970) demonstrated that the
incorporation of vegetable oils and fatty acids in Sabouraud’s dextrose agar medium inhibit the growth of most of the dermatophytes. The fungitoxicity of some vegetable oils, herbal seeds mixed with boiled oil and different types of milk against causal organisms have also been evaluated in the present study. An emphasis was paid for a search of some antifungal agents from perfumes obtained from medicinal plants and animals.

The in vitro study of some antibiotics, phenolic compounds and sulfa drugs against the otomycotic pathogens can suggest their use to cure ear diseases. There are some fragmentary reports on the sensitivity of some antibiotics against fungi causing otomycosis (Singer et al., 1952; Stride, 1962; Youssef and Abdou, 1967; Lawerence et al., 1978; Schneider, 1981; Paulose et al., 1989). Phenols are known to be most frequently involved in the disease resistance mechanisms of plants especially against fungal pathogens (Greathouse and Rigler, 1940; Walker and Stahmann, 1956; Friend, 1981, 1982). Generally, the orthodihydroxy phenols are more fungitoxic to microorganisms (Le Tourneau et al., 1957) and are quite useful for combating skin infections. The use of thymol in alcohol as an antifungal agent had been described by some workers (Gill, 1932; Mac Bunery and Seurcey,
1936; Reeh, 1942; Johnston, 1944) they also demonstrated their clinical effectiveness in the treatment of otomycosis. Senturia (1957) reported that the Aspergilli, he treated were inhibited to a large extent by sulfanilamide, while sulfathiazole, sulfadiazine, sulfaguanidine and sulfamazine were completely ineffective. No data are available on the fungitoxic effect of the combinations of antibiotics and sulfadrgugs against otomycotic pathogens and due to this the present study has been taken.

A number of fungi are known to cause otomycosis in man. The causal agents of such diseases may survive saprophytically in nature. It is now well established that fungi in practically all habitats, rich in an atmosphere containing various organic volatile substances, which may be the exudates from the decomposing materials like forest litter, domestic and industrial waste. Many of these substances are inhibitory rather than stimulatory. Cholodny (1943) demonstrated the existence of volatile organic compounds acting upon the soil microbes. Some workers have evaluated the inhibitory or stimulatory effect of volatile organic compounds against keratinophillic fungi and relatd dermatophytes (Jain and Agrawal, 1978b; Thind and Agrawal, 1978; Singh and
Agrawal, 1981; Deshmukh and Agrawal, 1982; Rathore and Agrawal, 1986; Singh and Agrawal, 1987). Fungal infection of ear particularly in children is posing a great threat to public health due to their severe effect on hearing and ear drum perforation. To investigate the mode of survival of these pathogenic fungi in natural habitats, the in vitro studies were planned and performed. These also include the antagonistic effect of some common saprophytic fungi on the growth of otomycotic pathogens.

Otomycosis are common in central India and particularly in the rural areas where persons remain in close contact with the soil and also come in close association with the diseased domesticated animals. The present study was undertaken to investigate the distribution of pathogenic fungi in the normal and in infected ear canal. These type of studies will be of immense help for proper understanding of the role of soil, various physiological and biochemical conditions for the survival of the pathogens causing otomycosis of man. The data of the present work will definitely provide information regarding the etiology of the otomycosis and can help in getting to get efficient therapy for the disease.