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Cinicomycological study of otomycosis with antifungal drug susceptibility testing of Candida isolates using disk diffusion method in Kota region, Rajasthan, India

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A B S T R A C T

Otomycosis or fungal otitis externa is a superficial, sub-acute or chronic infection of the external auditory canal. Its prevalence has been quoted to range from 9% to 27%. The aim of the study is to find the prevalence of otomycosis along with its clinicomycological profile and to detect antifungal drug susceptibility of Candida isolates by disk diffusion method. A total of 100 samples were collected using swabs from ENT OPD based on symptoms and otoscopic finding suggestive of otomycosis and were processed in mycology lab. Otomycosis was diagnosed in 94 % of the cases with highest prevalence in 11-30 years of age group. Male to female ratio was 1.12:1. *Aspergillus niger* (58%) was the predominant isolate followed by *Aspergillus flavus* (23%), *Candida* spp (12%), *Aspergillus fumigatus* (4%), *Penicillium* (1%), *Geotrichum* (1%) and *scopulariopsis* (1%). Among *Candida* species, *Candida albicans* (50%), *C. tropicalis* (25%), *Candida glabrata* (16.66%) and *Candida kefyr* (8.33%) were isolated. Antifungal drug susceptibility testing results shows 100% sensitivity to Amphotericin B for all candida isolates. Resistance against fluconazole was present in 16 % of *C.albicans* isolates, 25% of *C. tropicalis* isolates. Nystatin was resistance among 16% of *C.albicans* & 25% of *C. tropicalis*, whereas clotrimazole resistance was present in 23% of *C.albicans*, 25% of *C. tropicalis* & 50% of *C.glabrata* isolates. Ketoconazole was resistant among 16% of *C. albicans*. Our study showed a high prevalence of otomycosis in the Kota region, thus proper diagnosis and treatment by aseptic techniques for this disease is required.

Introduction

Otomycosis or fungal otitis externa is a superficial, sub-acute or chronic infection of the external auditory canal, usually unilateral, which is characterized by inflammation, pruritis, scaling and otalgia1.The fungal agents responsible for this clinical entity are found as saprotrophic in the environment and true fungal pathogens are rarely recovered from these patients. The fungi are usually secondary invaders of tissue already rendered susceptible by bacterial infections, physical injury or excessive accumulation or lack of cerumen in the external auditory canal. As such no age group is immune to this disease but it is commonly seen between 2nd and 3rd decades of life (2). Otomycosis is one of the common conditions encountered in a general otolaryngology clinic setting and its prevalence has been quoted to range from 9% 3to 27.2% (4, 5) among patients who present with signs and symptoms of otitis externa and

up to 30% (6, 7) in patients with discharging ears. The fungal agents responsible for this clinical entity are *A. niger, A. flavus, A. fumigatus, A. terreus, A. sydowii, C. albicans, Penicillium, scopulariopsis* and dermatophytes like *Epidermophyton floccosum, Trichophyton mentagrophytes* and *Trichophyton violaceum* (8). Other organisms like *Malassezia sympodialis* (9) and *Pseudallescheria boydii* (10) have also been reported.

Most patients suffering from early otomycosis complains of severe itching which often progresses to pain, hearing loss, and may lead to tympanic membrane perforations (11-13). Manifestations are usually unilateral2. Various factors have been proposed as predisposing factors for otomycosis, including a humid climate, excessive presence or absence of cerumen, swimming, evidence of fungal infection somewhere else in the body, instrumentation of the ear, immune compromised host and more recently increased use of topical antibiotic/steroid preparations14. It is more common in individuals with lower socioeconomical status with poor hygienic conditions. The infection has also been observed in people who do not clean their ears after taking bath or swimming. The wetness predisposes to fungal infection.

The present study was carried out to ascertain the etiologic agents, epidemiologic patterns of otomycosis in Kota region and to detect antifungal drug susceptibility of the Candida isolates using disk diffusion method.

Materials and Methods

The study involved 100 patients who presented with symptoms of otomycosis at ENT OPD of MBS hospital, Kota from 2015 to 2016. Clinical details such as chief complaint, name, age, gender, suspected risk factors, occupation, history of infection, address, and other relevant information were recorded. Informed written consent was obtained from all subjects. After establishing a clinical diagnosis, specimen and clinical materials from the external auditory canal were collected from all patients by means of sterile cotton swabs. Materials were divided into two samples for mycological processing. With one sample slide KOH(10%) was perfo rmed. The morphology(yeast and mold), and other relevant characteristics (spores, arthroconidia, septate and non-septate hyphae, etc.) were identified. The second sample was inoculated on SDA and SCCA(Himedia).The media were incubated at room temperature (25°C) and observed for 3 weeks. Cultures were examined every day to determine the probable growth of fungi colonies and their identification.

The identification process of the isolated fungi was done based on macroscopic and microscopic morphology. Lactophenol cotton blue preparations were made from the cultures and then examined microscopically. The slide culture technique was also used where morphological details of various fungi was necessary for exact identification. The isolated yeast species were identified using various test- germ tube production, Corn meal agar morphology, assimilation and fermentation of carbohydrates and Chrome agar morphology. Antifungal sensitivity of various fungal isolates was performed by the disc diffusion method on Mueller Hinton Agar supplemented with 2% glucose and 0.5mg/ L Methylene Blue (HiMedia make) and tested for Fluconazole 10µg,Nystatin 100U, Amphotericin 100U, Ketoconazole 10µg, Clotrimazole 10µg disk (HiMedia). After the measurement of zone of inhibition, the results of antifungal sensitivity were interpreted according to criteria given with HiMedia antifungal discs. All mycological investigation was carried out in the mycology section of the Microbiology department.

Results and Discussion

Otomycosis was diagnosed in 94 % of the cases with highest prevalence in 11-30 years of age group. Male to female ratio was 1.12:1(Male 53%, Female47%). 98 % cases were unilateral (Right ear57%, Left ear 41%) and rest 2 % were bilateral in presentation. Out of 94 culture positive cases 90 has single type of growth whereas rest 4 has mixed growth. Total fungal isolates were 100 as 2 cases were of bilateral otomycosis (both were culture positive) and 4 cases has mixed growth. Among various isolates, *Aspergillus niger* (58%) was the predominant isolate followed by *Aspergillus flavus* (23%), Candida spp (12%), *Aspergillus fumigatus* (4%), *Penicillium* (1%), *Geotrichum* (1%) and scopulariopsis (1%) [Table 1]. Among *Candida* spp. isolated most common species was *Candida abicans* (50%), followed by *Candida tropicalis* (25%), Candida glabrata (16.66%) and *Candida kefyr* (8.33%). Maximum incidence of cases were recorded in the rainy season from July to September with peak number of cases in the August month. As per the occupation field workers (40%) were the most commonly affected group, followed by Housewives (24%), Office workers (15%) and rest 23 % in students and retired personnel. Among various predisposing factor, use of oils like mustard oil, coconut oil and instillation of other form of ear drops like antibiotic drops, wax dissolving drops was present in 56 % of the cases. Following it, use of wooden, metallic and paper roll was present in 35% of the cases as predisposing factor. Cerumen was absent in 70 % of the patients [Table2]. History of swimming was present in 4% cases, 7% of the cases were diabetic and 5 % cases had history of covering their heads.

Whereas in 10 % no such predisposing factors and any chronic illness was present. In our study itching (86%) was the most common symptom followed by ear pain (40%), sensation of ear blockage (42%), tinnitus (22%), decreased hearing (15%) and discharge (12%) [Table3]. Antifungal drug susceptibility testing results shows sensitivity to Amphotericin B by all candida isolates. Resistance against fluconazole was present in 16 % of *C. albicans* isolates, 25% of *C. tropicalis* isolates. Nystatin was resistance among 16% of *C. albicans* & 25% of *C. tropicalis*, whereas clotrimazole resistance was present in 23% of *C. albicans*, 25% of *C. tropicalis* & 50% of *C. glabrata* isolates. Ketoconazole was resistant among 16% of *C. albicans*

Otomycosis, a fungal infection of the ear, is found throughout the world. It is worldwide in distribution with a higher prevalence in the hot, humid, and dusty areas of the tropics and subtropics (4) Itching and pain in the ear are the most common presenting symptoms of otomycosis 14, 15. This usually progresses to discomfort, irritation, sensation of sound in the ear, sense of blocked ear, hearing loss and aural discharge. Tympanic membrane perforation can occur, but is rare. In our study, the prevalence of otomycosis was 94%, which is higher than the results found in other studies, including work by Kumar16 who found otomycosis in 75.9% of patients; Pardhan *et al.,* (7), who found otomycosis in 79.4% of patients, Kaur *et al.,* 8, who found the disease in 74.7% of patients. An analysis of the age group suggested that otomycosis can occur at any age. In our study highest incidence of cases were found in 11-30yrs (48%) of age group, and lowest among extreme of ages <10 years(4%) and > than 60 years(4%). The same observation was made by Paulose *et al.,* (17), HS Satish *et al.,* 18 and RP Rao *et al.,* 19 study.

The people in age group from 11-30 years usually spend more time in the outdoors and are more exposed to the fungal spores due to occupational exposure, travelling etc. making them more vulnerable to otomycosis. In our

study, otomycosis cases were found to be more common in males (53%) than females(47%).These findings were relatively close to Kaur *et al.*,8, HS Satish *et al.*,18, SC Prasad *et al.*, 14 and A. Kazemi *et al.*, 15 study. As males usually spend more time outside, so are more exposed to dust, fungal spores. These result could also be attributed to the difference in surface lipids between males and females, as surface lipids are under the control of sex hormones20. So males are supposed to have more lipids contents in the skin of the external auditory canal, thus making it more favorable for the growth of fungus. The percentage of females was 47% in our study, which may be due to the household work like dusting, cleaning or gardening thus exposing them to the fungal spores. Although in RP Rao *et al.*, 19 study females percentage was higher than males. Whereas, in Chander J *et al.*, 21 study male to female ratio was 1:1.This may be due to more number of females attending the OPD as compared to males.

Most of the studies revealed otomycosis to be unilateral disease. In our study 98% of the cases were unilateral. Out of which right ear was involved in 57% and left ear in 42 %. These results correlates with Paulose *et al.*,(17), Kaur *et al.*, 8 and H S Satish *et al.*,(18) study. The unilateral nature of the disease may be attributed to the habits like self-manipulation of ear canal with wicks or inserting fingers and as majority of the population in our study were coincidentally right handed, so more chances of manipulating right ear may be present. In our study bilateral otomycosis was present in patients who had history of swimming. Our study revealed higher rate of occurrence of otomycosis cases from july to September. Rainy season in our study area also commences at july with peak rainfall during July and August. In August 2016, Kota city witnessed a whopping 193 mm of rainfall, against the previous all-time highest rainfall of 122.1 mm for the month. Also 100% humidity was reported in the month of August 2016. The air borne fungal spores are carried by water vapors, a fact which correlates the higher rates of infection in monsoon when relative humidity rises. SC Prasad *et al.*, 14 noticed similar results. Whereas, in Than KM *et al.*, 27, Barati22 and Ahmad *et al.*, 23 study they had more occurrences of cases in the dusty dry season or in autumn. This difference may be due to the fact that the symptoms may have started in the rainy season; but patients did not present on time to the clinic until the dry season.

In our study field workers (40%) were found to be the highest affected occupation followed by housewives (24%), Office workers (13%). Whereas, the rest 23% of cases were students and retired personnels. This finding correlates with the Jaiswal *et al.*, 24study. As the field worker are more exposed to the environmental fungal spores, so are the highest affected group. In our study, history of instillation of oil (mustard & coconut) and ear drops like antibiotic drops, steroid drops, wax removal drops was present in 56% of total cases. Similar finding was found in HS Satish *et al.*, 18, M. abdelazeem *et al.*, 20 and RP Rao *et al.*, 19 study. History of using wicks was present in 35%, followed by association with diabetes in 7% and swimming history in 4%. This findings correlates with HS Satish *et al.*, 18 study. Whereas no predisposing factor was present in 10 % of the cases which is similar to Lakshmipathi *et al.*, 25, HS Satish *et al.*, 18 study. This may be due to improper history given by the patient. Oils have fatty acids that provides a suitable medium for the growth of fungus, which explains the higher incidence of otomycosis in people who instill oils regularly. Recurrent use of antibiotic drops, steroids, antiseptics or wax solvent ear drops applications alters the local environment of

the external ear canal and allows super infection by fungus. Use of metallic /wooden/paper roll commonly used for cleaning ear canal, often leads to trauma of the canal skin into which the fungal spores may seed in.

Fig.1 *A.niger* a) macroscopy (SDA growth), b) microscopy (40x, LCB)

Fig.2 *A.flavus* a) macroscopy (SDA growth), b) microscopy (40x, LCB)

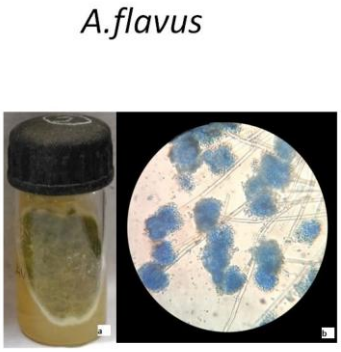


Fig.3 *A.fumigatus* a) macroscopy (SDA growth), b) microscopy (40x, LCB)

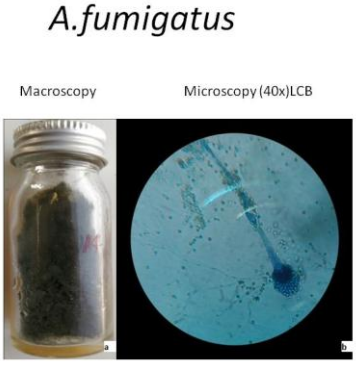


Fig.4 *Penicillium* a) macroscopy (SDA growth), b) microscopy (40x, LCB)

Penicillium spp.

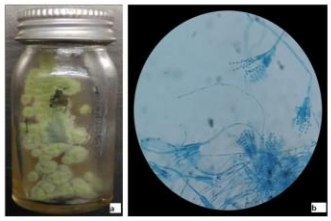


Fig.5 *Scopulariopsis* a) macroscopy (SDA growth), b) microscopy (40x, LCB)

Scopulariopis spp.

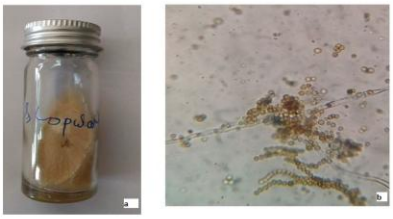


Fig.6 *Geotrichum* a) macroscopy (SDA growth)

Geotrichum spp.

Fig.7 *Candida spp* a) macroscopy (SDA growth), b) microscopy (100x, gram)

Candida spp.

Table.1 Showing various fungal isolates

Fungal Isolated (N=100)	No. of cases
A.niger	**58**
A.flavus	**23**
A.fumigatus	**4**
Candida spp	**12**
Penicillium	**1**
Scopulariopsis	**1**
Geotrichum	**1**

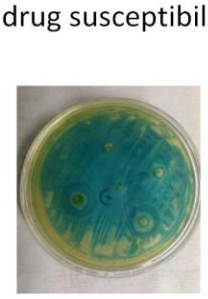
Table.2 Showing the distribution of cases as per the Predisposing Factors present Among the Study Population

Predisposing Factors	Percentage of cases (N=100)
Use of Oils/ antibiotic drops	**56%**
Use of wooden/metallic wick/paper roll	**35%**
Covering of head as per customs	**5%**
Diabetes	**7%**
Swimming	**4%**
No Cerumen	**70%**
No predisposing factor	**10%**

Table.3 Showing distribution of case as per the presenting Symptoms Among the Study Population

Symptoms	Percentage of the cases (N=100)
Itching	86%
Ear pain	40%
Tinnitus	22%
Discharge	12%
Sensation of Blockage	42%
Decreased hearing	15%

Fig.8 Antifungal drug susceptibility for candida isolates using disk diffusion method



In our study 7% cases had diabetes. Similar results were in seen in HS Satish *et al.,* 18 where 16% cases had diabetes. History of swimming in local ponds and swimming pools was present in 4% of cases. This findings was similar to HS Satish *et al.,* 18 study where 8% of the total cases had history of swimming. The lipid mantle layer formed by the cerumen in the external canal is considered as the key factor for the protection of the canal wall, and its removal by frequent irrigation of the external canal while swimming, frequent bathing is incriminated as a cause of recurrent otomycosis. In our study, history of covering of head was present in 5 % of cases. Mostly these patients were Muslim females who used to wear burka. Head covers increases moisture, heat and humidity around the ears thus predisposing to fungal infection. In Aneja KR study 26 the major predisposing factors responsible for the otomycosis have been found as the wearing of traditional customary clothes. In 70 % of patients, cerumen was lacking. This is in correlation with M. abdelazeem *et al.,* 20, Pontes *et al.,* 4 and SC Prasad *et al.,* 14 study. Absence of cerumen may lead to infection, as cerumen serves an antimicrobial role by physically protecting the external auditory canal skin, establishing a low pH, making inhospitable environment for pathogens by producing antimicrobial compounds such as lysozyme.

In our study itching (86%) was the most common presenting symptom which correlates with Than KM, Naing KS and Min M27, SC Prasad *et al.,* 14, Abdolhassan Kazemi *et al.,* 15 and M. abdelazeem *et al.,* 20 study. Sense of ear blockage was present in 42% of cases, similar to Than KM, Naing KS and Min M27, SC Prasad *et al.,* 14, Abdolhassan Kazemi *et al.,* 15studies. Otalgia was present in 40% of cases and tinnitus in 22% of the cases. The aforementioned symptoms in similar percentage were found in HS Satish *et al.,* 18 study. Discharge was present in 12 % of the cases. In Abdolhassan Kazemi *et al.,* 15 study similar results were found. Decreased in hearing was present in 15 % of the cases which correlates with M. abdelazeem *et al.,* 20 study. Discharge was present in 12 % of the cases. In Abdolhassan Kazemi *et al.,* 15 study similar results were found. Decreased in hearing was present in 15 % of the cases which correlates with M. abdelazeem *et al.,* 20 study. The mycosis of external ear canal results in superficial epithelial exfoliation, inflammation of the ear canal skin, formation of masses of debris containing hyphae and suppuration. Inflammation of the ear canal skin results in itching and pain..In addition, symptoms like tinnitus, aural fullness and decreased hearing are as a result of accumulation of fungal debris in the ear canal thus obstructing the ear canal. Discharge is usually a more common symptom in bacterial origin otitis externa. In our study discharge was present maximally in candida origin otomycosis.

In our study out of 100 samples, 94 were culture positive. The negative cultures might have been the result of previous treatment before these patients entered our study. Single fungal isolate was present in 90 cases whereas mixed growth was present in 4 cases and two cases had bilateral otomycosis, making a total isolates to 100. In our study *Aspergillus niger* was the most common isolate accounting for 58% which simulates to results of Yassin *et al.,* 28, Chander J *et al.,* 21, Kaur *et al.,* 8, HS Satish *et al.,* 18, Abdolhassan Kazemi *et al.,* 15 and RP Rao *et al.,*19 studies. Next to *Aspergillus niger,* the most common isolates were *Aspergillus flavus* (23%) and *Aspergillus fumigatus* (4%). These findings correlates with that of Abdolhassan Kazemi *et al.,* 15 and RP Rao *et al.,* 19 study. In our study Candida was isolated in 12 % of the cases which is similar to Kaur *et al.,* 8 and RP Rao *et al.,* 19 study. *Penicillium* isolated was

1%. In Kaur *et al.*, 8, RP Rao *et al.*, 19 and HS Satish *et al.*, 18 study also similar results were obtained. *Geotrichum* (1%) 29 and Scopulariopsis 30(1%) were also isolated.Scopulariopsis is a rare isolate found to be associated with otomycosis. Among 12 % of the Candida spp isolated Candida albicans (50%) was the major isolate followed by Candida tropicalis (25%), Candida glabrata (16.66%) and Candida kefyr (8.3%).

Aspergillus is abundant in soil or sand which contains decomposing vegetable matter. Whereas, *Aspergillus niger* is a common food contaminant, a black mold which often grows on a variety of fruits and vegetables. There conidia being aerodynamic in nature are dessicated rapidly in tropical sun and blown in wind as small dust particles and are carried by water vapors, a fact which correlates the higher rates of infection, in monsoon when relative humidity rises to 80%. And also the human external auditory canal is an ideal environment for this fungus to grow and abundance of proteins and carbohydrates and favorable humidity and temperature explains this finding. Also Aspergillus are found to be more common in hot and humid countries whereas Candida spp. has more preponderance of infections in temperate regions13.Our study area comes under subtropical zone, so Candidal isolates were less in our study. The secretion of aspartic proteinases (Sap1p to Sap10p) is an important virulence determinant of *C. albicans*. Saps facilitate invasion and colonization of host tissue by disrupting host mucosal membranes and degrading important immunological and structural defense proteins.

Antifungal drug susceptibility testing results shows sensitivity to Amphotericin B by all candida isolates. Resistance against fluconazole was present in 16 % of *C. albicans* isolates, 25% of *C. tropicalis* isolates. Nystatin was resistance among 16% of C.albicans & 25% of *C. tropicalis*, whereas clotrimazole resistance was present in 23% of C.albicans, 25% of *C. tropicalis* & 50% of C.glabrata isolates. Ketoconazole was resistant among 16% of *C. albicans*. Clotrimazole which is the most commonly prescribed drug for the treatment of otomycosis was found to be resistant in 23 % of the candida isolates. This clearly reflects that all cases of otomycosis should not be treated on just OPD basis, but rather should be sent for fungal culture and antifungal drug susceptibility testing and then should be treated accordingly.

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