Original Article

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Fungal Profile and Its Characteristics in Patients of Otomycosis-A Prospective Study

Microbiology Section

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ABSTRACT

Introduction: Fungal ear infection is one of the most common infections, which is termed as otomycosis. Common causative agents are *Aspergillus* and *Candida* species.

Aim: To find the prevalence, identification and susceptibility testing of fungal species in samples collected from patients with ear infection.

Materials and Methods: Samples were collected from 105 patients of otomycosis and were inoculated on SDA and BA tubes. KOH preparations were made to see the presence of fungal elements. Morphology of filamentous fungi was identified by using Lacto Phenol Cotton Blue (LPCB) and yeast-like fungi by Gram stain and CHROM agar medium. Susceptibility testing was done to find out the sensitivity and resistivity against Voriconazole, Itraconazole, Fluconazole and Amphotericin-B by disc diffusion method.

Results: Otomycosis was common in the age group between 20-40 years, and males were more affected as compared to females. Common isolates found were moulds such as *Aspergillus* niger (14.89%), *A. flavus* (21.2%), *A. fumigatus* (21.2%), *Rhizopus oryzae* (2.1%) and *Scedosporium* (2.1%); yeast-like fungi were *Candida tropicalis* (34%) and *C. glabrata* (8.5%).

Conclusion: Microscopic examination and fungal culture is essential for confirmation of the diagnosis. Proper identification of fungus followed by antifungal treatment according to susceptibility testing is important for treating such patients.

Keywords: Antifungal susceptibility testing, *Aspergillus*, *Candida*

INTRODUCTION

Ear is continuously exposed to biotic elements of the atmosphere, as a consequence easily accessible to various microorganisms, including fungi which leads to inflammation and fluid development inside the ear. Local lesions observed in otitis externa, such as congestion, increases the vascular permeability and temperature to create favourable conditions for the growth of fungi and development of mycoses both in the external and middle ear [1]. Fungi are abundant in soil or sand, which contains decomposing vegetable matter. These are dehydrated rapidly in tropical area and blown in wind as small dust particles. The air borne fungal spores are carried by water vapours, which correlates with higher rate of infection, when relative humidity rises to 80% [2]. Andrall and Gaverret were the first to describe fungal ear infection in 1843 and by Mayer in 1844 [3].

Otomycosis has been described as superficial fungal infection of the external auditory canal while infections in middle ear occur due to perforated tympanic membrane. Permanent damage of the middle ear and inner ear is caused by chronic fungal infection of ear [4]. It is characterised by the presence of otalgia, ear discharge, pruritus, ear fullness, hearing loss. Common predisposing factors are heat and humidity, change in pH, temperature, qualitative and quantitative change in cerumen, immuno-compromised patients and systemic diseases, trauma, Chronic Suppurative Otitis Media (CSOM), use of antibiotics and non-sterile oils [4-6].

Common fungal isolates are *Aspergillus* and *Candida* species. In recent years, there has been an increase in the incidence of otomycosis due to increase in prevalence of co-morbid conditions like diabetes mellitus, malignancies and use of ear buds [4,5,7]. Although there has been controversy with respect to whether the fungi are the true infective agents versus mere colonisation of the species as a result of compromised local host immunity secondary to bacterial infection, most clinical and laboratory evidence show that otomycosis is a true pathological entity [2]. Otomycosis is sporadic and is caused by a wide variety of fungi, most of which are saprophytes growing in diverse types of environmental conditions [3]. The fungus is usually a secondary contaminant in cases of otitis externa and hence can be found mixed with bacterial infections also [8]. Though the disease can be diagnosed clinically, microscopic examination and fungal culture is required for confirmation of the diagnosis. Antifungal susceptibility testing methods help to detect the antifungal resistance. Diagnosis and management of otomycosis can be challenging in immuno-compromised patients; also recurrences are more common among them.

This research on otomycosis described clinical presentation, fungal profile and its characteristics along with antifungal susceptibility testing of various commonly involved species and whether the fungal species found in the study were similar to other studies mentioned in literature. We also studied in detail about the regional differences in fungal species causing otomycosis.

MATERIALS AND METHODS

Study Group

Mycological analysis was carried out on debris or exudate samples from the ear auditory canals of 105 patients clinically diagnosed to have otomycosis infection, collected from Outpatient Department (OPD) of Otorhinolaryngology of Sir Sunder Lal Hospital, Banaras Hindu University, Varanasi, Uttar Pradesh, India, from January 2018 to December 2018. All patients were informed about the study and consent was taken before taking the ear swab. The collection of ear swab and to send it for culture and sensitivity, is a routine procedure at the study institute. However, all the ethical considerations were taken care of.

Ethical Considerations

All patients were informed about the study and proper consent from all the patients was taken before taking the ear swab. Hence, no ethical obligations were involved in our study.

Collection of Samples

The samples were collected under aseptic conditions using sterile cotton swab from the external auditory canal. To diagnose otomycosis, detailed history, clinical examination, otoscopic findings and laboratory identification of fungus were considered [9].

Culture

Two samples were taken, one was used for inoculation on SDA and BA tubes and other was used for KOH mount. The inoculated SDA tubes were incubated on 25°C and examined after 2-3 days of incubation. BA tubes were incubated on 37°C for 24 hours.

Identification

Direct microscopy: For detection of fungal elements, KOH (10%) preparation was used. Gram stain was done for identification for yeast-like fungi and LPCB was used to identify the filamentous fungi. CHROM Agar was used to identify the *Candida* species.

Antifungal Susceptibility Testing

***Candida* species:** The disc diffusion assay was performed according to CLSI guideline (M44-A2) to determine the susceptibility of obtained isolates. In brief, Mueller-Hinton agar was supplemented with 2% glucose, provided a suitable growth for most of the yeasts, and 0.5 mg/L methylene blue was used. The pH of the medium was maintained between 7.2 and 7.4 after gelling and the agar was 4 cm high. Inoculum was taken with a sterile swab stick from sub-cultured SDA tubes, mixed with normal saline (1 mL) and prepared in tubes, inoculum was standardised to 0.5 McFarland, it was swabbed on MHA plates supplemented with 2% glucose and 0.5% methylene blue, antifungal drugs (Voriconazole, Itraconazole, Fluconazole, Amphotericin-B) were applied and were incubated at 35°C for 24 hours; some strains showed insufficient growth and these strains required 48 hrs of incubation to grow.

Filamentous Fungi

CLSI guideline (M-51A) protocol was followed. PDA sub-cultured tubes were taken, 1 drop of Tween 80 was added in each of the tubes, 1 mL normal saline was added and mixed with the spores. Then, 1-2 drops of prepared solution was taken in tubes of prepared normal saline, which was standardised to 0.1 McFarland. The prepared mixture was then swabbed on plain MHA plates with the swab stick, and antifungal drugs (VIFA) were applied and incubated at 25°C for 48 hours; some strains showed insufficient growth and would require 72 hours of incubation.

RESULTS

A total of 105 patients with suspected otomycosis were included in the study; 114 samples were obtained (as some patients had otomycosis in both the ears).

The most prevalent age-group was 20-40 years (44 patients) followed by age-group of 1-20 years [Table/Fig-1].

Age in years	Male	Female	Total
1-20	17	16	33
20-40	26	18	44
40-60	11	06	17
60-80	08	02	10
80-100	01	00	01
Total	63	42	105

[Table/Fig-1]: Age and sex distribution of the otomycosis patients.

The common presenting symptoms solely or in combination encountered in the study group have been summarised in [Table/Fig-2]. Ear discharge (72.38%) was the most common complaint.

Otomycosis was unilateral in 105 (92%) cases and 9 (7.8%) cases were bilateral. Among the unilateral cases, the right side (48.2%) showed predominance [Table/Fig-3].

Majority (15.2%) of the patients gave history of CSOM [Table/Fig-4].

[Table/Fig-5] shows the list of associated medical conditions in the study, out of which a majority of 7.6% had common cold and cough.

Also, it was found that 6 patients with immuno-compromised state and 35 patients with immuno-competent state were positive for otomycosis. Three patients were bilaterally positive in immuno-competent patients while rest of the patients were unilaterally positive.

Symptoms	No. of patients	Percentage (%)
Pruritus	28	26.6
Ear discharge	76	72.38
Tinnitus	50	47.6
History of hearing loss	43	40.9
Ear pain	18	17.1
Ear fullness	10	9.5

[Table/Fig-2]: Symptomatology of otomycosis patients.

Ear	No. of patients	Percentage (%)
Right	55	48.2
Left	50	43.8
Bilateral	9	7.89

[Table/Fig-3]: Laterality distribution of otomycosis patients.

Factors	No. of patients	Percentage (%)
Use of oils, antibiotics and self-cleaning	6	5.71
Trauma	4	3.8
Swimming	1	0.95
CSOM	16	15.2
Presence of ear wax	2	1.9

[Table/Fig-4]: Predisposing factors for otomycosis.

Medical history	No. of patients	Percentage (%)
Diabetes	03	2.85
Blood pressure	02	1.9
Cold and cough	08	7.6
Heart problem	01	0.95
Herpes	01	0.95
Immuno-compromised	06	5.7
Immuno-competent	35	33.3

[Table/Fig-5]: Associated medical history.

Only 45 samples were positive for the presence of fungal elements. Out of 114 samples, 64 were negative both by microscopy and culture, two were positive with KOH but no growth on culture and 3 were negative with KOH but no growth [Table/Fig-6].

Positivity	Culture positive	Culture negative	No growth	Total
KOH positive	22	2	02	26
KOH negative	21	62	03	88
Total	45	64	05	114

[Table/Fig-6]: Specificity of KOH preparation compared to culture (Total samples-114).

A total of 45 out of 114 samples, were positive for fungal growth by culture. The most common fungal isolates belonged to the species of *Aspergillus*. *Aspergillus flavus* and *fumigatus* (20%) were the most common isolate followed by *Aspergillus niger* (15.5%) and *Aspergillus terreus* (4.4%). Two samples were moulds and two samples having yeast like species (which could not be identified) [Table/Fig-7].

Aspergillus niger was found in 7 samples, all were sensitive to Voriconazole showing a clear zone inhibition. Yeast-like fungi included 10 samples of *Candida* tropicalis, all were sensitive to Voriconazole showing a clear zone of inhibition. All 10 were sensitive to Fluconazole and Amphotericin-B. *Candida glabrata* was grown in 2 samples; all were sensitive to Voriconazole, Itraconazole, Fluconazole and Amphotericin-B [Table/Fig-8].

Fungal species	No. of patients	Percentage (%)
Aspergillus niger	07	15.5
Aspergillus *flavus*	9	20
Aspergillus *fumigatus*	9	20
Aspergillus terreus	02	4.4
Rhizopus oryzae	01	2.2
Scedosporium	01	2.2
Candida tropicalis	10	22.2
Candida glabrata	02	4.4
Species unidentified (molds)	02	4.4
Species unidentified (yeast-like)	02	4.4
Total	45	100

[Table/Fig-7]: Spectrum of fungal isolates from otomycosis patients (Total samples-45).

Fungal species	Voriconazole			Itraconazole			Fluconazole			Amphotericin-B		
	S	I	R	S	I	R	S	I	R	S	I	R
A.niger	07	00	00	03	02	02	01	01	05	07	00	00
A.flavus	08	00	01	06	03	00	01	01	07	02	01	06
A.terreus	02	00	00	00	02	00	00	00	02	00	00	02
C.tropicalis	10	00	00	08	00	02	10	00	00	10	00	00
C.glabrata	02	00	00	02	00	00	02	00	00	02	00	00

[Table/Fig-8]: Antifungal susceptibility testing.

DISCUSSION

In the present study, analysis of the age-group revealed that otomycosis can affect any age from 1 to 100 years. However, the incidence was highest in the age-group of 20-40 years, similar to the findings mentioned by Fasunla J et al., and Ologe FE et al., [10,11]. The higher incidence in these age-groups was due to the fact that these people are more exposed to the mycelia due to occupational exposure, travelling etc., whereas the older and younger age-groups are less exposed to these pathogens.

In the study, males were more commonly affected than females, as males spend more time outdoors leading to more exposure to fungal spores. It is well known that the outdoor air is an important vector for locally prevalent fungal flora. Our study correlates well with the study of Than KM et al., Kaur R et al., and Ho T et al., which showed 58%, 60% and 56% incidence in males, respectively [12-14]. Unilateral involvement was seen in 92% in our study, which correlated with the study done by Paulose KO et al., in which unilateral involvement was 87% [15]. Ho T et al., observed a bilateral involvement in 7% of the patients, which corresponds to the present study where we found bilateral otomycosis in 7.89% patients [14].

Common symptoms of otomycosis as mentioned in literature are itching, ear discharge, ear pain, blocking sensation, decreased hearing and tinnitus [16,17]. In our study, ear discharge (34%) was the more common symptom followed by tinnitus (22%).

In the present study, immunocompromised patients having otomycosis were commonly associated with co-morbid illnesses like common cold & cough, diabetes, high blood pressure, heart problems and herpes. Also, 3.8% of the patients had trauma with stick, feather, metal picker, pin etc., The habit of cleaning the ear with feathers, match-stick or contaminated finger tips is known to encourage the inoculation and growth of the spores of fungus on the moist external auditory canal especially in patients applying oil to the area. Excessive cerumen in some patients with poor personal hygiene favours the germination of spores and conidia of the prevalent fungi.

The moisture, warmth, and acidic pH of the external auditory canal provide ideal growth medium for the fungi. Swimming was also a predisposing factor in our study, similar to the study by Ozcan KM et al., Fasunla J et al., and Paulose KO et al., [5,10,15].

Lack of formal education in people in many parts of India has let them to believe in myths that coconut oil application in ears is beneficial for a variety of ear ailments. Coconut oil has been reported to be sporostatic and therefore may help preserve the viability of fungal conidia deposited in the external ear for long and indirectly contribute to occurrence of otomycosis [18]. Similarly the use of mustard oil is associated with high incidence of otomycosis [17].

Overview of literature shows that among the fungal isolates, *Aspergillus niger* and *Candida* were the most common species causing otomycosis worldwide but more than 50 causative fungi species have been isolated in their studies by various authors which belong to genera *Penicillium*, *Fusarium*, *Mucoraceae*, *Scopulariopsis*, *Alternaria*, *Malassezia* and as well various dermatophytes [16,19]. In the present study, we recorded 60% of *Aspergillus* species, which correlated with the study of Satish HS et al., (54%) and Paulose KO et al., (54.4%) [3,15]. However, present study differed from the study conducted by Aneja KR et al., as the most prevalent microorganism in their study was *Aspergillus niger* (43.31%), whereas most prevalent species in present study was *Candida tropicalis* (22.2%) [7]. In some other studies mentioned in the literature, yeast fungi such as *C. albicans* had more frequency than *A. niger*, similar to this study [19-22].

Aspergillus flavus species found in this study was 20%, which correlated well with the study of Satish HS et al., and Yahiya MM et al., [3,20]. Infection with *Candida* can be more difficult to detect clinically because of its lack of a characteristic appearance like *Aspergillus* and can present as otorrhea not responding to aural antimicrobials. Otomycosis attributed to *Candida* is often identified by culture data [13,15]. The colour of discharge from the ear and also the laboratory identification may help towards the probable identification of the disease agent [22]. Usually the black discharge may be due to *Aspergillus* genus, especially *A. niger*, and creamy or white discharge may be due to *Candida* genus [23].

Some rarest species had also been found in the index study, with 2 isolates of *A. terreus*, 1 isolate of *Rhizopus oryzae* and 1 isolate of *Scedosporium*. The *Scedosporium* genus encompasses a group of filamentous fungi isolated from water, soil, stalled or polluted water all over the world. Two species cause human infection: *S. apiospermum* (asexual anamorphous of *Pseudoallescheria boydii*) and *S. prolificans* (*S. inflatum*). These are considered rare human pathogens, especially in immunocompromised patients [24,25]. Next common group in the study was *Candida* species, accounting for (26.6%) of total isolates. This correlated with the study by Satish HS et al., (53.4%) and Viswanatha B et al., [3,26]. There was a difference in isolation of fungi in the present study when compared to other studies which may be due to geographical variation [Table/Fig-9] [7,10,13,17-19,22,26,27].

There is no consensus on the most effective agent. Application of appropriate topical antifungal agents coupled with frequent mechanical debridements usually results in prompt resolution of symptoms, though recurrence or residual disease can occur [28]. Administration of mildly acidic drops, such as boric acid and alcohol, or modified Burow's solution can be a cost-effective therapy for primary treatment of otomycosis [29]. Clotrimazole and Fluconazole are very effective drugs in the treatment of otomycosis [30].

In the index study, susceptibility test disc diffusion method was performed against Voriconazole, Itraconazole, Fluconazole and Amphotericin-B. It was found that patients suffering from *Aspergillus niger* infection were sensitive to Voriconazole and Amphotericin- B, showing a clear zone of inhibition. *Aspergillus flavus* and *terreus* were also sensitive to Voriconazole. Both of them were resistant to Fluconazole and Amphotericin-B. Yeast-like fungi included *Candida tropicalis* were sensitive to Voriconazole, Itraconazole, Fluconazole and Amphotericin-B. *Candida glabrata was* sensitive

Authors	Year	*A. niger/A. fumigatus/A. flavus*	*Candida* sp.	*Mucor*	*Rhizopus*	*Scedosporium*	Species unidentified
Kaur R et al., [13]	2000	36.9/41.1/1.4	13.7	1.4	2.7	-	1.4
Pradhan B et al., [17]	2003	25.5/6.6/37.7	10.4	-	-	-	-
Vishwanatha B et al., [26]	2012	56/18/-	16	-	-	-	-
Pontis ZBVDS et al., [19] (Brazil)	2009	20/5/10	55	-	-	-	10
Aneja KR et al., [7]	2010	39.8/12.9/16.6	10.2	-	-	-	-
Fasunla J et al., [10] (Nigeria)	2008	48.35/33.96/5.43	12.26	-	-	-	-
Barati B et al., [27] (Iran)	2011	41.6/5.5/49	7.6	-	-	-	0.9
Jain SK and Agarwal SC [18]	1992	56.3/15.6/4.7	6.3	6.3	-	-	3
Jaiswal SK [22]	1990	34/-/-	46	-	12	-	8
Present study	2019	15.5/20/20	22.2	-	2.2	2.2	

[Table/Fig-9]: Comparison of percentage of various fungi in otomycosis as reported by different authors [7,10,13,17-19,22,26,27].

to Voriconazole, Itraconazole, Fluconazole and Amphotericin-B. Overall, it was concluded that all the fungal species were sensitive to Voriconazole and Amphotericin-B, though some were resistant to Fluconazole and Itraconazole.

LIMITATION

1. Ear swab method was used for sample collection, which is not a good method for diagnosis of species in otomycosis.
2. A control group should have been taken, in which sample from patients with purulent ear discharge should have been examined for diagnosis of species, so that the various pathogens involved in ear discharge could have been compared.

CONCLUSION

The most common clinical feature was ear discharge and the common age-group was 20-40 years. CSOM was the most common predisposing factor. The most common fungal species found was *C. tropicalis*. All species were sensitive to both Voriconazole and Amphotericin-B.

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