Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.03.Art019

Assessment of Risk Factors Associated with Common Fungal Pathogens Causing Otomycosis at a Tertiary Care Hospital in South India

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Abstract

Otomycosis is one of the most prevalent fungal diseases of the external auditory canal in the tropical and sub-tropical regions of the world. Numerous environmental and host factors can put a person at risk for otomycosis. This cross-sectional study was undertaken over a one-year period at a tertiary care hospital, in South India after approval from Institutional Human Ethics Committee (IHEC), as there is limited data available on the clinical-mycological profile and its association with various risk factors in our region in the recent past. 90 ear swabs from clinically diagnosed otomycosis patients were sent to the laboratory for mycological examination from the Ear, Nose, and Throat (ENT) out-patient Department. Their clinical data was evaluated using a self-administered questionnaire. Mycological examination yielded 63 fungal isolates with Aspergillus niger as the predominant organism followed by other fungi; common risk factors identified were diabetes mellitus (92%), CSOM patients on steroid drops (91.6%), swimming (80%), CSOM patients on antibiotic drops (75%) with a significant association of $p<0.05$ between the above-mentioned risk factors and otomycosis. The current study highlights that proper identification of the causative agents is mandatory to prevent complications and recurrences, even when the patients' otoscopic findings and clinical presentations strongly suggest a fungal infection. Comparable with other superficial fungal infections, otomycosis does not represent a threat to life. However, prompt microbiological identification are essential for timely and effective therapy to avert major issues from otomycosis.

Keywords: *Aspergillus Niger, Candida Albicans, Chronic Suppurative Otitis Media (CSOM), Otomycosis.*

Introduction

Medical professionals are increasingly concerned about otomycosis, a frequent external ear canal fungal infection. This disorder, which is marked by swelling, itching, and sometimes discharge, can have a serious negative effect on a person's quality of life. New insights into the epidemiology, diagnosis, and therapy of otomycosis have given medical practitioners a more thorough approach to treating this illness. The epidemiology of otomycosis varies geographically; studies in resource-poor nations have found greater prevalence rates [1]. Worldwide, there is a prevalence of the infection ranging from 9% to 30% in individuals exhibiting external auditory canal (EAC) infection [2, 3]. Numerous host factors, such as immunocompromised status (diabetes, malignancies), long-term use of steroids or prolonged antibiotic usage, underlying dermatological diseases, relatively high humidity in EAC, dirt and epithelial debris accumulation, have been implicated in

Received: 22.07.2024 ***Accepted: 30.08.2024*** ***Published on: 30.09.2024***
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predisposing to otomycosis. Otalgia, pruritus, tinnitus, hearing loss, blocked feeling, and ear discharge are frequent symptoms seen in patients with otomycosis [4]. It has been demonstrated that an individual's susceptibility to this illness is influenced by underlying medical disorders like diabetes or immunosuppression, as well as environmental factors like climate and cleanliness [5].

Numerous distinct fungal species, each with its own specific traits and clinical manifestations, can be implicated in the aetiology of otomycosis. Molds of the genus *Aspergillus*, especially *Aspergillus niger* complex, and yeasts of the genus *Candida*, particularly *Candida albicans*, are the most frequent causes of otomycosis [6]. The genus *Aspergillus*, which includes *A. fumigatus, A. flavus*, and *A. niger*, is one of the most frequent causes of otomycosis [7]. These ubiquitous fungi can easily colonise the external auditory canal, especially in people who have immune system disorders, diabetes, or prolonged exposure to water [8]. These conditions can foster an environment that is favourable for the fungi's growth and thrive. Other fungus, like *Candida* spp., have also been linked to the aetiology of otomycosis in addition to *Aspergillus* species [9]. *Candida* infections, which are frequently linked to mucosal involvement, can also appear in the external auditory canal and cause symptoms that resemble otomycosis caused by *Aspergillus*. Additionally, several tropical and subtropical areas have documented cases of otomycosis brought on by uncommon fungal species such *Penicillium* spp. and *Fusarium* spp. [10]. Even though they are less common, these fungi can still be rather dangerous, especially in those who already have immunosuppression or live in environments where they can thrive.

Within the *A. niger* complex, 28 distinct species of black mould have been identified by recent molecular technologies and studies. *A. tubingensis, A. foetidus, A. awamori, A. welwitschiae* are the species that have also been implicated in this illness, despite their rarity [11]. Accurate genetic identification of these black fungi is necessary to develop rapid diagnostic methods for Aspergillus otomycosis and to determine the optimal treatment plan for different species within this complex. *A. flavus* are also frequently isolated. In addition to the previously listed moulds, *A. fumigatus, A. terreus, A. luchuensis, A. versicolor* are less frequently found to be the cause of infections [12]. *A. sydowii* has been recognised as a possible human infection in individuals with impaired immune systems. Two cases have been documented in which the fungi were discovered using molecular analysis using EAC material extracted during tympanomastoidectomy [13].

Occasionally, reports of otomycosis have also been made about yeasts belonging to the genera *Geotrichum candidum, Rhodotorulla* spp., and *Cryptococcus*. Moreover, dermatophytosis can easily lead to EAC skin infection through autoinfection with fungi belonging to the dermatophyte group (*Trichophyton mentagrophytes*). Fungal species belonging to the genera *Penicillium, Fusarium, Mucorales, Paecilomyces, Scopulariopsis, Chrysosporium, Alternaria, Acremonium* spp. have been identified as the source of this infection in the category of non-dermatophyte moulds [14].

Patients with otomycosis may have a variety of symptoms, such as ear pain, itching, and discharge, depending on the particular fungal agent causing the condition. If ignored, these symptoms may result in hearing impairment. Primary care physicians must diagnose and treat otomycosis as soon as possible due to the variety of fungus species involved and the possibility of severe consequences [15].

The goal of our study is to identify the clinico-mycological profile of clinically diagnosed otomycosis prevalent in our region and their associations with several risk factors.

Materials and Methods

Study Design

The current study was a cross-sectional hospital-based study carried out in the Department of Microbiology at a tertiary care hospital in Chennai, South India, between June 2023 and May 2024, after receiving approval from the Institutional Human Ethics Committee (IHEC). The study included 90 clinically diagnosed patients with otomycosis who were attendees at the outpatient Department of Ear, Nose, and Throat.

Inclusion Criteria: The study included ninety clinically diagnosed otomycosis patients of different age groups. The symptoms such as itching, soreness, feeling of a blocked ear, difficulty in hearing, ear discharge, and positive otoscopic findings were used as criteria to establish the diagnosis of otomycosis. The study also included patients with CSOM on antibiotic/steroid drops.

Exclusion Criteria: Patients who were recently treated for otomycosis and chronic suppurative otitis media. Also, the samples which had bacterial growth were excluded from the study.

The clinical history, demographic status, risk factors of patients with a clinical diagnosis were assessed and analysed using a predesigned proforma. The patients' age, sex, occupation, and socioeconomic position were noted. Any prior use of oil, topical steroids, antibiotic ear drops, or wooden sticks to remove wax was documented. Clinical manifestations of patients, including discomfort, itchiness, sense of fullness in the ears, discharge from the ears, and tinnitus, were also noted.

Microbiological analysis

Two ear swabs were aseptically taken after a thorough inspection of the ear using an otoscope. The organisms were identified mycologically by following standard operating procedures [16]. The first swab was used for direct microscopy (Gram staining for identification of yeast-like fungi, a 10% KOH wet mount and Lactophenol-Cotton Blue (LPCB) were employed for identification of filamentous fungi). The other swab was reserved for mycological cultures. Sabouraud's Dextrose Agar (SDA) slant (mixed with 50 ug/mL of the antibiotic gentamicin) and incubated for two to three weeks at 37°C and 25°C. Slide culture was also performed from the growth on SDA. The colony morphology on SDA agar and lactophenol Cotton Blue (LPCB) mount from the growth and slide culture were used for the identification of moulds. For *Candida* isolates, colony morphology, Gram stain, Germ tube test and HiCrome Candida Differential agar were used to identify the species [16]. SDA slant and HiCrome Candida Differential agar was procured from HiMedia, Mumbai, India.

Statistical Analysis

For data entry, Microsoft Excel was used. The data were analyzed using IBM Corp.'s Armonk, NY SPSS, Version 25.0. A P-value of less than 0.05 was considered statistically significant. Tables showed the frequency and percentage representations of the descriptive statistics.

Results

Table 1 depicts the demographic profile of the patients under study. The ratio of males to females was 1.4:1 (53:37) [Table 1].

Table 1. Demographic Profile of the Study Population

Age Group (Years)	Male n (%)	Female n (%)	Total n (%)
0-20	5	3	8
21-40	25	18	43
41-60	21	14	35

>60	2	2	4
TOTAL	**53 (59)**	**37 (41)**	**90 (100)**

According to complaints, itching (78%), clogged ears (73%), and ear pain (68%) were the most common symptoms in clinically diagnosed otomycosis patients, whereas hearing loss (43%) and ear discharge (22%) were the least common complaints [Figure 1]. Table 2 depicts the unilateral and bilateral distribution of clinically diagnosed otomycosis.

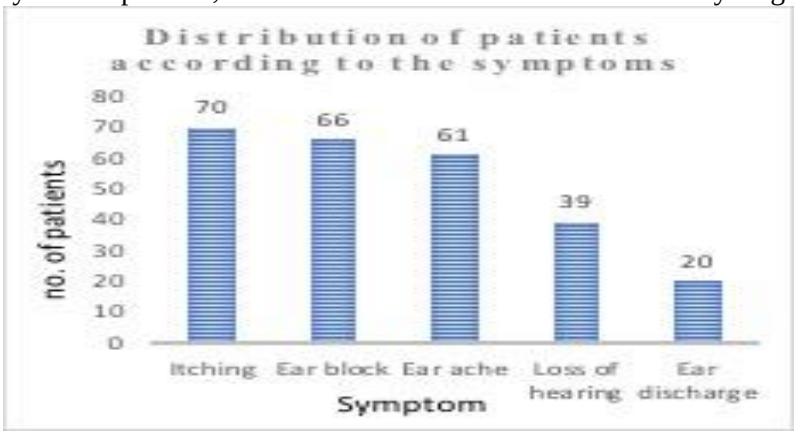

Figure 1. Distribution of Clinically Diagnosed Otomycosis Patients with Respect to the Symptoms

Table 2. Laterality of Otomycosis

Gender	Right	Left	Bilateral	Total
Male	28	23	2	53 (59%))
Female	18	17	2	37 (41%)

Out of 90 clinically diagnosed otomycosis samples sent to the laboratory, 63 samples showed fungal growth. The maximum mycology culture positive cases were found in the age group of 41-60 years i.e., 49% while minimum cases were detected in the age group of >60 years i.e., 2% [Figure 2].

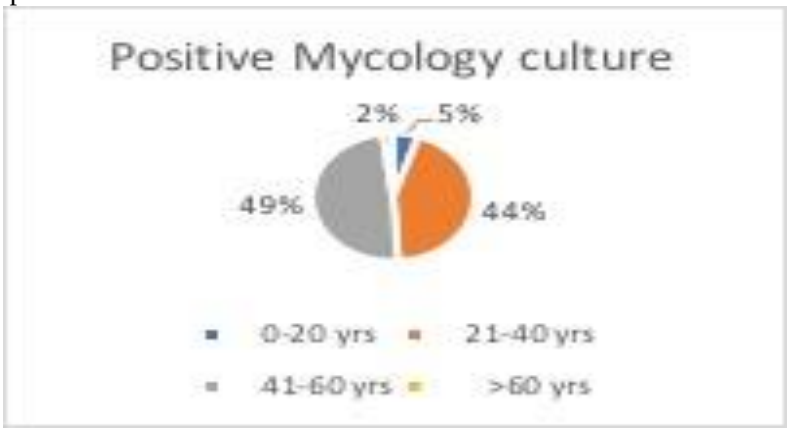

Figure 2. Prevalence of Mycology Culture Positive Among Various Age Groups

Table 3 depicts association between various risk factors and mycology culture positive otomycosis. There was statistically significant relationship ($P<0.05$) between positive mycology culture results and all the risk factors except fungal nail infection, instillation of oil, earphone usage. Diabetes mellitus, CSOM patients on steroid drops, swimming, CSOM

patients on antibiotic drops were most common predisposing factors leading to otomycosis i.e., 92%, 91.6%, 80%, 75%, respectively [Table 3].

Table 3. Association Between Risk Factors and Otomycosis

Risk factor	**Total**	**Positive Mycology Culture n (%)**	**P value**
CSOM patients on antibiotic drops	20	15 (75)	0.0087
CSOM patients on steroid drops	24	22 (91.6)	0.0001
Diabetes mellitus	38	35 (92)	0.0001
Swimming	20	16 (80)	0.0002
Fungal nail infection	10	3 (30)	0.181
Instillation of oil	25	6 (24)	0.064
Earphone usage	44	7 (15.9)	0.479

In the present study, *A. niger* comprised 48% of the fungal isolates, *A. flavus* (35%), *C. albicans* (6%), *A. fumigatus* (5%) followed by other fungi as mentioned in Table 4.

Table 4. Prevalence of Fungi Isolated form Clinically Diagnosed Otomycosis

Type of fungi	**Frequency (n)**	**%**
Aspergillus niger	30	48
Aspergillus flavus	22	35
Candida albicans	4	6
Aspergillus fumigatus	3	5
Penicillium spp.	2	3
Candida tropicalis	1	1.5
Candida krusei	1	1.5
TOTAL	**63**	**100**

The macroscopic appearance of the fungal colonies on SDA agar are shown in Figures 3-5.

Figure 3. Macroscopic Appearance of *Candida* spp. on SDA

Figure 4. Macroscopic Appearance of *A. niger* on SDA

Figure 5. Macroscopic Appearance of *Penicillium* spp. on SDA

The microscopic appearance of the fungi are shown in Figures 6-8.

Figure 6. Microscopic Picture of *Candida* spp. Under Gram Stain

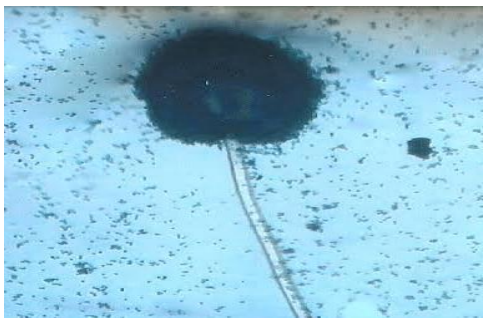

Figure 7. Microscopic Picture of *A. niger* Under LPCB Mount

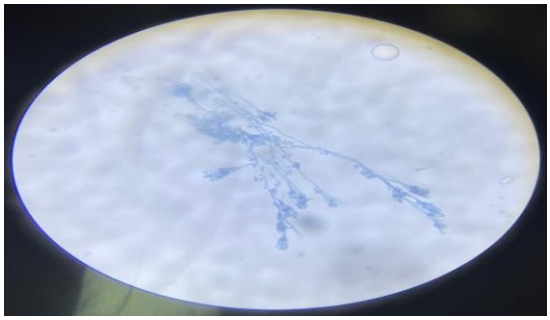

Figure 8. Microscopic Picture of *Penicillium* spp. Under LPCB Mount

Discussion

This study aimed to identify the mycological profile and risk factors associated with otomycosis. Out of 90 clinically diagnosed cases of otomycosis, 53 (59%) were males and 37 (41%) were females. Males between the ages of 21 and 40 had the highest frequency. Otomycosis is a worldwide illness that is more prevalent in tropical areas and affects people of all ages. It is often diagnosed based on clinical criteria [17] and treated experimentally. If left untreated, chronic ear infections can cause learning problems in children, hearing loss, and decreased productivity in adults [18]. The patients in our study had itching (78%), blocked sensations (73%), earache (68%), loss of hearing (43%), discharge from the ears (22%) as the most prevalent signs of otomycosis. Bilateral otomycosis is most likely caused by infection spreading from one ear to the other [19], potentially as a result of cleaning both ears with the same cotton bud. However in our study, the prevalence of bilateral otomycosis was very low.

In the current study, *A. niger* was isolated from 48% of the fungal isolates, followed by *A. flavus* (35%), *C. albicans* (6%), *A. fumigatus* (5%), *Penicillium* spp. (3%), *C. tropicalis* (1.5%), *C. krusei* (1.5%). In the study by Prasad SC et al., *Aspergillus* species were found in 80% of cases, followed by *Penicillium* spp. (8%), *C. albicans* (4%) and others [20]. *Aspergillus* was the most frequent cause of otomycosis in the majority of investigations. Nonetheless, the cause for the reported species variation across different places is the global spread of fungi. Among the *Aspergillus* species, *Aspergillus flavus* was the most frequently isolated fungus in the majority of the investigations. Our study revealed that *Aspergillus* spp. was the most predominant fungal infections in cases with otomycosis. These findings are in line with studies conducted by Hagiwara S et al., Aneja KR et al. and Agarwal P et al. [21, 12, 2]. As a result, etiological proof is required before beginning local antifungal treatment.

Age groups 41–60 years (49%), 21–40 years (44%) showed the highest prevalence. Furthermore, Onotai and Osuji observed that their peak prevalence occurred between the ages 1 and 10 years (30.7%) and between 21 and 30 years (26.2%) [22]. The age group of 20–40 years old had the highest frequency in multiple additional studies [23]. In our study the age groups under 20 and over 60 had the lowest prevalence which was similar to a research by Prasad et al. [20]. In our study, the prevalence was more in men than in women which was in contrast to a research by Gupta and Mahajan [24] who found that the prevalence of women was greater in India. Equal incidence and a preponderance of men have also been reported [25]. In light of the varied findings seen globally, otomycosis may not be associated with any particular gender. 70% of cases were reported by patients who worked in agriculture, according to research by Prasad SC et al. [20]. The biggest number of cases, however, were reported by Agarwal P to be outdoor farmers and labourers who were

exposed to dust and fungal spores as a result of their work conditions [2].

Of the 63 fungal isolates, the most frequent predisposing factor was found to be diabetes mellitus, CSOM patients on steroid drops, swimming, CSOM patients on antibiotic drops. Among the CSOM patients on steroid drops, 91.6% and among CSOM patients with antibiotic drops usage, 75% were mycology culture positive cases. The prevalence of fungi was high among diabetics (92%); there was 80% prevalence in swimmers. Overall, our data showed a higher prevalence when compared to studies from urban areas [26, 27]. However, patients from rural areas are more likely to have unhygienic mopping of the ear or instillation of hot oil or other substances into the ear, which can spread infection from the external environment to the middle ear [28]. Antibiotic use in the past may also have inhibited the proliferation of microorganisms. This result was consistent with research by Mawson S. et al. [29] and Kunelskaya et al. [30], which found that topical treatment with antibiotics and steroids was the primary cause of the development of the fungal process in the middle ear in the majority of instances. Good glycaemic management is essential for diabetic patients to prevent consequences such as tissue necrosis in *Aspergillus*-caused otomycosis [31]. Otomycosis is multifactorial in patients who have had prior ENT surgery. Frequent drainage, the use of antibiotics, anatomical changes that lead to cerumen production—all play a crucial part [32].

A study by Pontes ZB et al. showed the incidence of otomycosis was high (81.33%) among individuals who had a history of habitual ear picking [33]. Cleaning an ear with contaminated objects, like a key, hairpin, etc., causes the inoculation of fungal debris in the EAC. However, injecting coconut oil into the ear was the most popular method, accounting for 42% of the causative agents [20]. There was a very low prevalence in other dermatophytic nail infection, instillation of oil, earphone usage. In our study otomycosis following autoinfection by dermatophytic nail infection was low which was similar to a study by Asoegwu CN et al. [19]. The possibility of low prevalence following instillation of oil could be due to awareness among the urban population about the consequences of ear injury following insertion of sharp objects, hot oil into the ear for removal of wax [34]. A study done by Suresh, S et al. showed insignificant correlation between earphone usage and otomycosis and added that using earphones for a long duration could be a contributing factor in patients with pre-existing ear conditions and not a sole risk factor for otomycosis which was in line with our study [35].

Limitations

Although we isolated and characterised fungal agents down to the species level using conventional techniques, there may be opportunities to use molecular technologies to distinguish between different species of fungal isolates. Antifungal susceptibility testing could have been employed. Also, other predisposing factors such as prior ear surgery, relationship between occupation and its prevalence were not included in the study.

Conclusion

Our study emphasises how much more common otomycosis is in men than in women. Otomycosis is usually a benign illness that poses no threat to life, much like other superficial fungal infections. Prompt identification of the fungi are the need of the hour for providing timely and effective therapy due to its high prevalence among patients with co-morbid conditions, chronicity, decline in the quality of life experienced by afflicted patients, and the infrequent complications such as perforation of the tympanic membrane or very rarely disseminated infection involving the central nervous system and the bones. Laboratory investigations are advised when there is persistent inflammation of the external

auditory canal. Mycological analysis in addition to bacteriological study, should be a part of microbiological examination which includes techniques for isolating both yeasts and moulds. Specific antifungal agents should be taken into consideration when choosing a treatment plan, including the application technique. Certain risk factors, such as swimming, the application of alternate treatments, are advised to be avoided or reduced. These factors have the potential to harm the skin's mechanical barrier or microbiota in the EAC, which could reduce the production of cerumen. In the meantime, a history of otologic surgeries, immunosuppressive drugs, diabetes, prolonged systemic antibiotic therapy and other pre-existing diseases may be predictive of the onset of EAC fungal infections. Accurately identifying the fungi and host variables that cause otomycosis would also help these patients recover.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors want to acknowledge the Central Diagnostic Laboratory, SBMCH for providing the laboratory facilities.

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