



Otomycosis in the South of Iran with a High Prevalence of Tympanic Membrane Perforation: A Hospital-Based Study

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Abstract

Introduction Otomycosis is a superficial infection of the external ear caused by fungal pathogens. The genera *Aspergillus* and *Candida* are considered the main fungal causative agents, with the predominance of *Aspergillus* section *Nigri*. The present study aimed to evaluate the clinical symptoms of patients with otomycosis and predisposing factors and to identify fungal etiological agents using molecular approaches. We also present an overview of published papers on

tympanic membrane perforation (TMP) secondary to otomycosis.

Materials and Methods An otorhinolaryngologist collected specimens from external ear canals of patients with suspected otomycosis based on the patient's history and clinical examinations. The specimens were collected using sterile swabs. Fungal isolates were confirmed in clinical specimens by direct microscopy and culture methods. Fungal isolates were identified based on molecular approaches.

Results In total, specimens from 211 patients with suspected otomycosis were examined. The presence of

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fungi was confirmed in about 51% of patients based on fungal elements in direct microscopy and culture-positive fungi. *Aspergillus tubingensis* was the most commonly isolated species (52.77%), followed by *Aspergillus niger* (25.92%). Otomycosis due to infection with *Candida* species was observed in 16% of cases. Of note, in 36.11% of cases, otomycosis was associated with TMP.

Conclusion A mycological examination is indispensable for a correct diagnosis in patients with otitis extern. TMP should be considered in patients with otomycosis, as it appears to be relatively common in this population.

Keywords Otomycosis · Tympanic membrane perforation · Epidemiology · *Aspergillus tubingensis*

Introduction

Otomycosis is a superficial fungal infection of the external ear canal and a common disorder in tropical and subtropical countries [1, 2]. The infection may sometimes affect the middle ear, especially in patients with perforated eardrums and those undergoing a mastoidectomy [3]. The symptoms of otomycosis include pain, itching, aural discharge or fullness, tinnitus, and hearing impairment [3–5]. The predisposing factors for otomycosis include heat, humidity, a history of aural surgery, bacterial infections, oil installation in the ear, frequent swimming, and poor personal hygiene. Other predisposing factors for otomycosis include the use of hearing aids, common disorders, such as diabetes, topical applications of broad-spectrum antibiotics, trauma due to the insertion of contaminated foreign objects into the ear, and perforation of the tympanic membrane [1, 6–8].

Many fungal species, such as saprophytic filamentous fungi and yeasts, have been identified as causative agents of otomycosis, with dermatophytes a rare cause [9]. Among these species, the genera *Aspergillus* and *Candida* are considered the main causative agents, with the predominance of the *Aspergillus* section *Nigri*, including *Aspergillus niger* and *Aspergillus tubingensis* [6, 10–12]. High phenotypic similarity among these closely related species complicates their identification and may lead to misidentification [13]. New molecular methods have enabled the

identification of many novel species, and the application of these methods may revise the current understanding of the etiological agents of otomycosis [6].

The clinical symptoms of otomycosis are similar to those of other ear infections, leading to an improper administration of antifungals [10]. Due to various responses of some etiological causative fungi to antifungal drugs, empirical treatment can result in therapeutic failure. Furthermore, recurrence of otomycosis post-treatment poses a challenge because some of the main causative agents of otomycosis, such as *Aspergillus* and *Candida* species, including the multidrug-resistant *Candida auris*, have an inherent resistance to some antifungal drugs [13–15]. It is important to identify the causal etiological agent of otomycosis to ensure that the most appropriate treatment is administered.

The present study aimed to evaluate the clinical symptoms of patients with otomycosis and the fungal causative agents using molecular approaches. We also present an overview of published papers on tympanic membrane perforation (TMP) secondary to otomycosis.

Materials and Methods

Patients' Selection

This was a descriptive cross-sectional study. Patients with a clinical suspicion of otomycosis who visited the otorhinolaryngology outpatient clinic of Peymaniyeh Hospital in Jahrom, Iran over 2 years (April 2018 to April 2020) were included in the study. The patients had different complaints, including otalgia, pruritus, and otorrhea, with or without hearing loss.

Informed consent was gained from all the participants before enrolment. Ethical approval for this project was granted by the ethics committee of Jahrom University of Medical Sciences under IR.JUMS.-REC.1397.062 approval code.

Specimen Processing and Culture-Based Identification

An otorhinolaryngologist collected samples from the external ear canal of each patient with suspected otomycosis based on the patient's history and clinical examinations, while TMP was identified by otoscopy

and tympanometry tests. The samples were collected using sterile swabs. Clinical symptoms, demographic variables, and predisposing factors were recorded using a questionnaire.

Following the clinical diagnosis, the external ear canal specimens were sent to a specialized medical mycology laboratory for analysis in Jahrom.

Each specimen was subjected to direct microscopy analysis after treatment with 10% potassium hydroxide. The presence of fungal elements, including septate/aseptate mycelium, yeast/pseudohyphae, or *Aspergillus* conidia, was considered a definite diagnosis of otomycosis. Specimens were also cultured on two Sabouraud dextrose agar (SDA) plates (Merck, Germany), supplemented with chloramphenicol, incubated at 25 °C and 35 °C, and monitored for fungal growth for at least 4 weeks. Moreover, CHROMagar™ *Candida* (CHROMagar, France) was used for primary isolation and presumptive identification of the yeast isolates. To provide a primary identification at the genus or species level, phenotypic characteristics of mold isolates were explored using the slide culture method with SDA after staining with lactophenol aniline blue.

Molecular Identification

Total DNA was extracted from the yeast and mold isolates using the simple boiling method and phenol–chloroform method, respectively [14, 15]. Subsequently, the internal transcribed spacer (ITS) polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used to identify the yeast strains at the species level using the *MspI* restriction enzyme [16]. For isolates of the *Candida parapsilosis* complex and other yeast isolates that could not be identified by PCR–RFLP, the ITS regions of their rDNA were amplified and sequenced using ITS1 and ITS4 primers under PCR conditions described previously [14]. The identification of *Aspergillus* isolates was performed by amplification and then sequencing of a part of the beta-tubulin gene via beta-2A and beta -2B primers as described previously [17].

The definitive identification of the fungal isolates was accomplished by comparison of the sequences with relevant reference sequences in GenBank utilizing the nucleotide BLAST system (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), with a maximum identity of $\geq 98\%$ and query coverage of $\geq 98\%$.

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Results

Of 211 patients who underwent primary evaluations and had a clinically suspected otomycosis, 108 (51.1%) patients were culture positive, and samples from these patients revealed fungal elements in direct microscopic examinations.

Table 1 summarizes the demographic information of the patients, including sex, age, residence status, occupation, affected ear, predisposing factors, and clinical manifestations, and provides information on the seasonal distribution of otomycosis. Of the 108 patients, the female: male ratio was 54.63:45.37%. The patients ranged in age from 14 to 80 years (mean: 45), with otomycosis most frequent in patients in their 30 s (25.93%). As shown by our results, 76.85% of the confirmed cases resided in urban areas, and 23.15% resided in rural areas. In terms of occupation, the majority of the patients (55.56%) were housewives. Fifty patients (46.29%) had right-sided otomycosis, and the remaining patients had left (39.81%) or bilateral (13.89%) ear infections. Canazole (clotrimazole) and tolnaftate were used for treatment for 7 to 10 days. There was no difference in treatment between *A. niger* and *A. tubingensis*. In terms of the possible predisposing factors, ear manipulation (76.90%) was the most common factor, followed by antibiotics usage (51.85%), diabetes (13.89%), swimming (12.96%), hypertension (6.48%), and hearing aid usage (3.70%). The most frequent symptoms were otalgia and pruritus, which were found in 80.56% and 75.93% of the cases, respectively. TMP was present in 36.11% of patients. In terms of the seasonal distribution of otomycosis, most cases (54.63%) occurred during the fall, followed by summer (24.07%), spring (14.81%), and winter (6.49%).

The molecular identification of the fungal isolates is shown in Table 2. The resulting nucleotide sequences were deposited into GenBank with accession numbers from MT348163 to MT348208 and from MT303056 to MT303061. In this study, *A. tubingensis* (52.77%) was the most commonly isolated pathogen, followed by *A. niger* (25.92%), *C. parapsilosis* (6.48%), *Candida glabrata* (5.60%), *Aspergillus flavus*

Table 1 Demographic information of the 108 patients diagnosed with otomycosis

Characteristics	No. of patients	Percentage
<i>Gender</i>		
Male	49	45.37
Female	59	54.63
<i>Age groups</i>		
< 20	2	1.85
20–29	16	14.81
30–39	28	25.93
40–49	21	19.44
50–59	16	14.81
60–69	16	14.81
≥ 70	9	8.33
<i>Residence status</i>		
Urban areas	83	76.85
Rural areas	25	23.15
<i>Occupation</i>		
Housewife	60	55.56
Self-employment	17	15.74
Employee	10	9.26
Retired	10	9.26
Farmer	9	8.33
Student	2	1.85
<i>Affected ear</i>		
Right	50	46.29
Left	43	39.81
Right and left	15	13.89
<i>Predisposing factors</i>		
Ear manipulation	83	76.90
Antibiotics usage	56	51.85
Diabetes	15	13.89
Swimming	14	12.96
Hypertension	7	6.48
Hearing aid usage	4	3.70
<i>Clinical manifestations</i>		
Pain	87	80.56
Itching	82	75.93
Hearing loss	73	67.59
Suppuration	69	63.88
Inflammation	55	50.93
Tympanic membrane perforation	39	36.11
Tinnitus	30	27.77
<i>Seasonal distribution</i>		
Autumn	59	54.63
Summer	26	24.07
Spring	16	14.81
Winter	7	6.48

Table 2 Molecular identification of the fungal species isolated from 108 cases of otomycosis

Fungal genus no. (%)	Species	Frequency no. (%)	GenBank accession no
<i>Candida</i> species 17 (15.74%)	<i>C. parapsilosis</i>	7(6.48)	MT303057- MT303060
	<i>C. glabrata</i>	6(5.60)	–
	<i>C. albicans</i>	3(2.77)	MT303056
	<i>C. orthopsilosis</i>	1(0.92)	MT303061
<i>Aspergillus</i> species 91 (84.26%)	<i>A. tubingensis</i>	57(52.77)	MT348180- MT348208
	<i>A. niger</i>	28(25.92)	MT348167- MT348179
	<i>A. flavus</i>	5(4.62)	MT348163- MT348165
	<i>A. awamori</i>	1(0.92)	MT348166
Total		108(100)	

(4.62%), *Candida albicans* (2.77%), *Candida orthopsilosis* (0.92%), and *Aspergillus awamori* (0.92%).

Of the 108 patients, 39 subjects had TMP as a complication of otomycosis. The demographic information of these patients is shown in Table 3. The 39 patients had TMP ranged in age from 20 to 86 years. 79.48% of the confirmed cases resided in urban areas, and 20.52% resided in rural areas. In terms of occupation, the majority of the patients with TMP were housewives and the possible predisposing factors, ear manipulation (94.8%) was the most common factor. In terms of the seasonal distribution of otomycosis with TMP, most cases (71.8%) occurred during the autumn, followed by spring (10.25%), winter (10.25%), and summer (7.7%).

Discussion

The incidence of mycotic infections due to opportunistic fungi has been increasing recently worldwide [18, 19]. Our study demonstrated that fungi were the etiological agents of otomycosis in 108 cases (51.1%). Similar results were observed in previous studies conducted in various regions in Iran [11, 20, 21], although one study conducted in Iran found a lower frequency of otomycosis (10.23%) [22]. The rate of otomycosis varies, depending on the geographic area, with a high incidence in tropical and subtropical areas [23]. The present study was conducted in Jahrom in Iran, a region with a hot semi-arid climate and average annual precipitation of about 285 mm. Such a climate may influence the distribution of this infection.

Table 3 The distribution of the 39 otomycosis cases associated with tympanic membrane perforation

Characteristics	
Gender (no.)	Male (13), Female (26)
Age [years (no.)]	44 (4), 22 (3), 68 (2), 48 (2), 45 (2), 36 (2), and [20, 23, 26, 27, 28, 31, 32, 33, 38, 39, 40, 41, 42, 43, 50, 52, 54, 56, 60, 62, 65, 67, 71, 86] each one (1)
Residence status (no.)	Rural (31), Urban (8)
Occupation (no.)	Housewife (25), Farmer (7), Retired (5), Teacher (1), Employee (1)
Affected ear (no.)	Left (25), right (9), right & left (5)
Predisposing factors (no.)	Ear manipulation (37), antibiotic usage (32), ear washing (12)
Clinical manifestations (no.)	Pain (35), itching (34), suppuration (25), tinnitus (5)
Seasonal distribution (no.)	Autumn (28), spring (4), winter (4), summer (3)
Fungal species (no.)	<i>A. tubingensis</i> (22), <i>A. niger</i> (12), <i>C. parapsilosis</i> (2), <i>A. flavus</i> (1), <i>C. albicans</i> (1), <i>C. glabrata</i> (1)

In our patient population, otomycosis was common in those aged 30–39 years but uncommon in teenagers and elderly individuals (≥ 70 years). Hence, otomycosis may be associated with occupational activity as it is more frequent in working-age groups. In the present study, the prevalence of otomycosis was higher among women, consistent with the findings of previous studies [24, 25]. The finding can be attributed to the social and cultural behaviors of Iranian women, such as wearing the traditional headscarf and the higher tendency of females as compared with that of males to seek medical help. In terms of occupation, otomycosis was more frequent among housewives. It was also more frequent among those living in urban areas. These findings are consistent with those of previous reports, which showed that housewives were more prone to otomycosis, as their daily housework activities exposed them to fungal spores in dust [26, 27].

As reported previously, otomycosis is usually unilateral [26]. In our study, 13.8% of the patients had simultaneous involvement of both ears. An earlier study reported bilateral involvement in 7% of otomycosis patients [8].

Several possible predisposing factors were identified in this study. In addition to antibiotic usage, diabetes, swimming, hypertension, and hearing aid usage, ear manipulation by patients was a common predisposing factor. Previous studies showed that ear manipulation appeared to be the main factor in otomycosis progression in patients [28]. A study in Mangalore, India indicated that swimming is an important predisposing factor for otomycosis [29]. In addition, a recent study linked extensive use of antibacterial eardrops to an increase in the prevalence of otomycosis [30].

In the present study, the main complaints of the patients with otomycosis were pain, itching, hearing loss, suppuration, and inflammation. Sabz et al. reported similar results, with pain and itching ear the most common symptoms in patients with otomycosis [24]. Despite these clinical symptoms being common in otomycosis, they are not pathognomonic [21]. Accordingly, mycological examinations should be performed for a definitive diagnosis.

An important finding of our study was that 36.11% of patients with otomycosis had TMP. Table 4 provides an overview of literature reports in chronological order on TMP secondary to otomycosis [8, 10, 31–37]. TMP is an infrequently reported

complication of otomycosis [38]. As reported previously, TMP is one of the most misleading complications of otomycosis and can result in an inaccurate diagnosis [38]. The diagnosis of TMP is based on a clinical examination by a specialist physician and can be difficult. Nevertheless, we cannot conclusively affirm whether perforation is caused by a fungus or ear manipulation, or other factors in the otitis media [39].

Another important finding of our study was the observed linkage between the occurrence of otomycosis and season. More than half the patients (54.6%) were diagnosed during the fall. Thus, it appears that this season provides conditions conducive to the occurrence of otomycosis (6).

In the current study, otomycosis was associated with *Aspergillus* and *Candida* species, with a frequency of 84.3% and 15.7%, respectively. *Aspergillus* species are ubiquitous environmental filamentous fungi that exist in soil and plant debris, where they thrive as saprophytes and can be harmful to humans under certain conditions [24]. As shown in Table 2, *A. tubingenensis* (52.7%) and *A. niger* (25.9%) were the most common pathogens isolated from the patients. *A. tubingenensis* is a black fungus belonging to the *Aspergillus* section *Nigri* that has a broad distribution worldwide [44]. Our findings are congruent with those of previous studies in Iran that reported black *Aspergillus* was the most frequent agent of otomycosis [6, 24, 27, 40, 41]. Species of *Aspergillus*, particularly *A. niger*, have been considered the most common filamentous cause of otomycosis in various studies based on morphological methods [42, 43]. However, more recent molecular studies identified non-*niger* black *Aspergillus* species as the most common causative agents of otomycosis [44]. Antifungal susceptibility profiles of members of the *Aspergillus* section *Nigri* have shown variable susceptibilities that are dependent on geographical regions and different sources [45, 46]. *A. tubingenensis* was less susceptible to some azole drugs compared with *A. niger* [47, 48]. But resistant species of *A. tubingenensis* have also been reported in other few studies [49]. While some studies have indicated no significant differences in the susceptibilities among these members [37, 49]. Azole resistance mechanisms in the section *Nigri* have been described mainly in *A. tubingenensis* [45], they are commonly attributed to point mutations in the *cyp51A* gene encoding the azole target protein (14 α -sterol demethylase [CYP51A]) [50, 51]. However, the molecular mechanisms of azole

Table 4 Overview of published reports on tympanic membrane perforation secondary to otomycosis

Author. year (Ref.)	Country	No. of patients	No. of cases with TMP (%)	Fungal species
Kurnatowski et al. 2001 [31]	Poland	96	12 (12.5)	ND
Pradhan et al. 2003 [32]	Nepal	107	17 (15.8)	ND
Ozcan et al. 2003 [33]	Turkey	87	3 (3.4)	ND
Ho et al. 2006 [8]	USA	132	18 (14)	ND
Visvanatha et al. 2012 [34]	India	100	3 (3)	ND
Jia et al. 2012 [10]	China	108	18 (16.67)	ND
Anwar et al. 2014 [35]	Pakistan	180	28 (15.5)	ND
Aboutalebian et al. 2019 [36]	Iran	97	19 (19.6)	ND
Kiakojuri et al. 2021 [37]	Iran	237	16 (6.75)	<i>A. flavus</i> , <i>A. tubingensis</i> , and <i>C. albicans</i>
Current study	Iran	108	39 (36.11)	<i>A. tubingensis</i> , <i>A. niger</i> , <i>C. parapsilosis</i> , <i>C. glabrata</i> , and <i>C. albicans</i>

TMP Tympanic Membrane Perforation

ND Not determined

resistance in these species are still controversial and not well defined [45].

A previous study in central Iran reported that *A. flavus* was the most common fungus in patients with otomycosis [20]. In our study, *A. flavus* was isolated from 5 (4.62%) patients, and *Candida* species were responsible for 15.74% of cases of otomycosis (Table 2). *C. parapsilosis* and *C. glabrata* were more common than other *Candida* species (Table 2). A previous study showed that non-*albicans Candida*, such as *C. parapsilosis*, was a more frequent causative agent of otomycosis than *C. albicans* [52]. Due to their endogenous nature, otomycosis caused by *Candida* species differs markedly from otomycosis caused by *Aspergillus* species. This fact needs to be considered in the treatment, control, and prevention of infection. Molecular tests may aid the diagnosis of otomycosis in suspected clinical cases.

Conclusion

In our patient population, *A. tubingensis* and *A. niger* were the predominant causative agents of otomycosis. Moreover, manipulation of the ear was an important predisposing factor in the progression of otomycosis in patients. Successful treatment of otomycosis and recurrence prevention depends on the accurate identification of predisposing factors and the causative fungal agents.

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Declarations

Conflict of interest The authors declare they have no conflicts of interest to disclose related to this study.

Ethical Approval This research was approved by the Ethics Committee of the Jahrom University of Medical Sciences, Jahrom, Iran (Approval Code: IR.JUMS.REC.1397.062).

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