

Otomycosis in Adolescent Patients Referred to the Therapeutic Centers in Babol City, Iran

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Background: Otomycosis is an external ear canal infection caused by various fungi. This disease is prevalent in some tropical and subtropical regions or countries.

Objectives: Given the crucial role of fungal agents in the treatment of the disease, the aim of the present study was to identify the fungi in ear canal of patients with otomycosis admitted to the hospitals in Babol City, Iran.

Patients and Methods: This study included 56 patients with otomycosis. After removal of ear infectious samples, some of them were placed on the slides for direct examination and also a portion of them was plated on the Sabouraud dextrose agar with chloramphenicol for fungal growth. The slides were studied for the presence of fungal elements. Conventional methods were performed to determine fungal colonies.

Results: Thirty-three patients (55.36%) were female and the rest were male. Fungal elements were observed in 11 cases (19.64%) in the direct examination, alone, and 45 specimens (80.36%) had fungi and bacteria combined. Septate mycelia, with 43 cases, had the most frequent fungal elements in direct examination. *Aspergillus* and *Candida* genera were the prevalent fungal colonies in culture media.

Conclusions: According to the role of different genera of fungi in the process of otomycosis, much attention on the macroscopic and microscopic examination of the samples leads to special treatment decisions of a physician.

Keywords: Otomycosis; *Aspergillus*; *Candida*

1. Background

The anatomy of ear canal which acts as a route for hearing can lead to infectious diseases via some microorganisms if the natural defense is lost or decreased through some activities. Otomycosis produced by different genus of fungi is not a very uncommon disease in almost all countries, worldwide especially the tropical and subtropical regions (1-4). Otomycosis is usually an inflammatory and infectious disease of the ear canal without any destruction of tympanic membrane (2, 5). This disease has a local inflammation process, but it can diffuse to other tissues if suitable treatment is not started especially in immunosuppressed patients (6, 7). Some factors such as moisture, humidity, swimming in the pool, trauma and use of hearing aids can lead people to otomycosis, although there are no exact predisposing factors to be detected after the accurate history and physical examination in some cases (8-10). Otomycosis exists in all ages, especially in middle ages, and also in both genders (5, 11), but it is prevalent in swimmers and also with mixed bacterial otitis (12-14).

The main signs and symptoms are itchiness, hearing loss, otorrhea and or pain, which can help in identification of the

original infection (6, 9, 15). Identification of otomycosis is based on direct microscopic examination; and also macroscopic and microscopic morphology of fungal colonies (16). Saprophytic fungi such as *Aspergillus* and *Candida* genera are the most etiologic agent for otomycosis, although corporation with bacteria is seen (6, 17-19). Dermatophytes are the third group of fungi in otomycosis (20-23). *Aspergillus niger* is the prevalent fungi as a cause of otomycosis; also, *Candida albicans* is the most commonly isolated species of genus of *Candida*. Other species of *A. flavus*, *A. fumigatus*, *C. tropicalis* and *C. parapsilosis* are the less frequent fungi in this illness in almost all studies (22, 24). Naturally these latest mentioned fungi are the dominant agents in otomycosis in some other studies (2, 11, 23, 25). In a rare study, other saprophytic fungi such as *A. terreus* are predominant (4); and some genus or species of fungi such as *Fusarium* spp., *Penicillium* spp., *Scedosporium* spp., *Geotrichum* spp. etc are the fungi isolated from otitis discharges (23, 25-27).

2. Objectives

According to the type of climate in Mazandaran Prov-

ince, which is moist temperate, the present study aimed to evaluate fungal agents and use these results for the prevention of the disease in patients with otitis externa (otomycosis) referred to the treatment center in Babol City, Iran, for laboratory examinations.

3. Patients and Methods

This experimental study was performed on patients with fungal otitis externa referred to therapeutic centers in Babol City, Iran, during 2011 - 2012. The exclusion criteria were otitis media, ruptured tympanic membrane and history of surgery on ear canal. According to the positive results in direct examination, the total sample size was 56 cases. The patients' samples of pus or otorrea were picked up by an ENT specialist, after completing the questionnaire. The samples were removed using an ear speculum or with sterile loop, separately for direct examination and culture, respectively. For the removal of pus, no material was used to softening.

A portion of the sample was spread on a clean slide glass for direct examination and another sample inoculated in the Sabouraud dextrose agar (Biolife, Italia) supplemented with chloramphenicol (Merck, Germany) medium for fungal growth. The plates were incubated at room temperature for two weeks. The slides were stained with Gram staining method, and observed microscopically for the existence of any microorganisms. Mycelium, pseudomycelium and also fruiting bodies (for *Aspergillus* spp.) in microscopic examination may be seen using a light microscope.

Fungal colonies were identified according to macroscopic and microscopic appearances, such as mycelia and fruiting bodies, for filamentous fungi; and budding cells or pseudomycelia for yeast fungi. Slide cultures were performed for some filamentous fungi if identifying them was impossible in wet mount examination. Sterile mycelia may report for fungus that does not produce any propagule for identification. Germ tubes on human sera and production of vesicles on corn meal agar (HiMedia, India) supplemented with tween 80 (Sigma-Aldrich, Germany) were done for the identification of yeast. The identification of otomycosis was based on the positive direct examination results for the presence of fungi and also the positive result for the growth of fungal colonies.

3.1. Data Analysis

The data were entered into SPSS software version 18 and statistical analyses were done to compare the qualitative or quantitative variables using chi-square and t-test, respectively. In all of them, a p-value less than 0.05 was considered as statistically significant.

3.2. Ethical Considerations

Patients were aware and agreed to the research. All information was saved in a private place.

4. Results

The mean age of the patients was 43.21 ± 17.1 years. Based on the 6 age groups, middle-aged patients (35 - 44 years) with 29 persons (32.14%) had the highest frequency of otomycosis, followed by young patients (25 - 34 years) with 16.07%; elderly patients (> 64 years) with 6 cases had the lowest frequency (Table 1).

Table 1. The Age Groups of Patients Suffered From Otomycosis Who Were Referred to Therapeutic Centers in Babol City, Iran ^a

Age groups, y	Values
15 - 24	8 (14.29)
25 - 34	9 (16.07)
35 - 44	18 (32.14)
45 - 54	8 (14.29)
55 - 64	7 (12.5)
> 64	6 (10.71)
Total	56 (100)

^a Data are presented as No. (%).

Among these patients, 25 cases (44.64%) were male and 31 (55.36%) were female; there was no statistically significant difference between the two groups. The color of discharge ranged from white to black. Most color of ear discharge, which may help otologists and mycologists in the identification of organism was black (23 cases, 41.07%) followed by white (30.36%) and yellow (21.43%). The rest were brown and yellow brown with 5.36% and 1.78%, respectively (Table 1).

Out of the 56 cases of otomycosis, the most prevalent element was related to the coexistence of fungal and bacterial elements in 45 cases (80.36%). Fungal elements were seen in 11 samples (19.64%). Septate mycelia accompanied with bacteria were the most common elements (35 samples, 62.5%) in direct microscopic examination of slide samples, followed by pseudomycelia accompanied with bacteria with 10 cases (17.86%). The rest were pseudomycelia (5.36%) and septated mycelia (14.28). Fungal colonies grew in 54 samples (96.43%) and in 2 cases (3.57%), the growth of fungi was not seen in cultures. The most prevalent fungal strains related to *Aspergillus* genus (36 colonies, 64.28%); and *A. niger*, were more than the other species of *Aspergillus*. *Candida* genus was the other most prevalent fungi in culture, with 11 cases (19.64%) (Table 2).

Table 2. Fungal Colonies Were Isolated From Patients With Otomycosis ^a

Fungi	Values
<i>A. niger</i>	31 (55.36)
<i>A. fumigates</i>	3 (5.35)
<i>A. flavus</i>	2 (3.57)
<i>C. albicans</i>	8 (14.29)
Non- albicans <i>Candida</i>	3 (5.35)
<i>Penicillium</i> sp	1 (1.79)
<i>Rhizopus</i> sp	2 (3.57)
<i>Cladosporium</i> sp	2 (3.57)
Sterile mycelia	1 (1.79)
<i>Geotrichum</i> sp	1 (1.79)
None grown	2 (3.57)
Total	57 (100)

^a Data are presented as No. (%).

5. Discussion

The results of the present study show that *Aspergillus* genus, especially *A. niger* and also *C. albicans* were the predominant fungi isolated in otomycosis. Fungal diseases are not related to gender, but some studies had different results around otomycosis. In our study, infection of otomycosis was seen in women more than men. Women were more infected with otomycosis than men in other studies (5, 13, 26, 28, 29), in agreement with the present study. In a study, otomycosis was more prevalent in females; the rate of the disease in females to males was 2/1 (30). Of course, other researchers (22, 23, 25, 31) reported that men are more sensitive and infected than women. These differences may be due to headress of women, the use of a hairdryer, or the differences in health management (13).

The color of discharge from the ear may help and lead ENT and also the laboratory 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 (1). The more frequency of the black and white color in the present study is the corporation of fungal elements in direct examination and also culture. Direct examination showed a coinfection between fungi and bacteria. Of course, culture in appropriate media can be important for the identification of fungal agents in otomycosis (22). We found 94.74% coordination between positive results in direct examination and positive culture results. Accordingly, the direct result is more important than the culture result; thus, this harmony showed a high validity of our experiments. Success in positive culture was less in some articles (12, 23).

In the present study, *A. niger* and *C. albicans* were the two fungal species in otomycosis. Our results are comparable and in agreement with more results reported by some previous studies, as almost all of these studies reported that the *Aspergillus* and *Candida* were the most prevalent fungi in otomycosis. Similar to our results, *A. niger* and *C. albicans* were the most prevalent fungi in their studies as infectious agents in otomycosis (7, 13, 19, 22, 30, 32). In some few studies, yeast fungi such as *C. albicans* had more frequency than *A. niger* (21, 28). Araiza et al. (33) reported that *A. fumigatus*, *C. albicans* and *A. niger* were the predominant fungi from 97 patients with proven otomycosis. Nwabuisi et al. found *A. fumigatus* was more prevalent than other fungi in otomycosis (11). In Yavo et al.'s (34) report, we can see that *A. flavus*, *C. albicans*, *C. parapsilosis* and *A. niger* were the most fungal colonies from this illness. Few studies mentioned that non-*albicans Candida* such as *C. parapsilosis* was more common than other *Candida* species in otomycosis (31). Paulose et al. (35) reported that *A. niger* and *fumigatus* have been the most common fungal pathogens in ear canal infections. According to the quick growth of these fungi, as well as the moist and acidity of the ear canal, these organisms can be the causal agents of otomycosis (13).

We found only 2 positive growth results of *Rhizopus* sp. and also *Cladosporium* sp. Some articles mentioned that *Rhizopus* spp. can produce infection in ear canal (22, 23). The *Penicillium* spp. was isolated in one case as an etiologic agent of otomycosis in rare (5, 36, 37). We did not find any Dermatophytes in the present study; this may be due to the decrease of dermatophytosis, change of climate and seasons (13, 22, 38). The results of the present study show a corporation and mixed presence of fungi and bacteria in direct examination of otomycosis; thus, we propose that it is better to perform these microorganisms in treatment protocols.

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Authors' Contributions

Kiakojuri arranged ear sample collections and also primary design, Rajabnia and Mahdavi Omran designed the study, wrote and edited the manuscript, Jalili carried out the examinations and Khafri performed statistical analysis.

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