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Isolation, Characterization, and Antifungal Sensitivity Pattern of Fungal Species with Potential Resistance to Antifungal Drugs in Patients with Otomycosis

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Abstract

Background: Otomycosis is defined as a superficial fungal infection, accounting for about 10% of infectious otitis externa cases.
Objectives: This study investigated patients with suspicious symptoms through the examination of their demographic information, isolate etiological agents, and in vitro antifungal susceptibility patterns.
Methods: The samples of 170 patients with otitis externa symptoms were collected and confirmed for otomycosis by mycological examination (e.g., potassium hydroxide, methylene blue staining, and fungal culture) and molecular sequencing. In vitro antifungal susceptibility tests against miconazole, fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, and caspofungin were performed according to the Clinical and Laboratory Standards Institute (M27-A3/S4 and M38-A2).
Results: Out of 170 patients, 145 subjects (85.29%) showed positive mycological findings. In this study, 55.8% of the patients were male, and the most common age group affected was 50 - 59 years (26.2%). Hearing loss and pruritus were the most common clinical manifestations. The most common occupation was being a housewife (47.5%), and most cases occurred during the winter (40%). *Aspergillus niger* was the most common species, followed by *Aspergillus fumigatus*, *Candida albicans*, and *Candida glabrata*. Caspofungin showed the highest activity against *Aspergillus* and *Candida* isolates; nevertheless, itraconazole demonstrated the lowest activity against *Aspergillus* isolates. Fluconazole showed the weakest power against *Candida* species.
Conclusions: Due to climatic conditions, humidity, and dust, otomycosis has a high occurrence in Iran. Although otomycosis needs long-term antifungal therapy and recurrence is high in some cases, it is rarely life-threatening, and eardrop antifungals are usually enough to eradicate the infection. Local information about the antifungal pattern is useful for the control, prevention, and treatment of otomycosis.

Keywords: Otomycosis, Antifungal Agents, *Aspergillus* Species

1. Background

Otitis externa is a frequent disorder causing pruritus, pain, edema, and erythema in the auditory canal, auricle, tympanic membrane, and middle ear (1). This condition might be infectious, inflammatory (psoriasis and eczema), or both (2). Otomycosis is defined as a superficial fungal infection, accounting for about 10% of infectious otitis externa cases (2), which might be acute, subacute, or chronic (3). This condition is more common in tropical and subtropical areas due to the hot, dusty, and humid climatic conditions (4). The prevalence of this condition in Iran ranges from 5.7% to 81% (2, 5).

Otomycosis symptoms are typically unilateral and include otalgia, hearing loss, pruritus, tinnitus, erythema, and aural discharge with debris that looks like a wet newspaper (6, 7). Sometimes fungal hyphae and spores could be observed during a clinical examination (8). The various predisposing factors to otomycosis include heat, humidity, bacterial infections, topical use of antibiotics or steroids, immunodeficiency, poor hygiene, ear surgery, fre-

quent swimming, trauma and foreign objects, diabetes mellitus, tympanic membrane perforation, self-cleaning with cotton swabs, and seborrheic dermatitis (5, 9-12). Otomycosis tends to be caused by the genera *Aspergillus* (60 - 90%) and *Candida* (10 - 40%), with the predominance of *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans* (13).

Otomycosis accounts for about 10% of ear, nose, and throat outpatients (14). Although otomycosis mortality is very rare, the course of the disease can be exhausting due to frustrating treatment, regular follow-up, and a high recurrence rate (13). The similarity of clinical findings of otomycosis to other ear infections might result in the administration of wrong antifungals (15). Moreover, different responses to empirical antifungal therapy can lead to therapeutic failure (16).

2. Objectives

The present study aimed to investigate patients with suspicious symptoms through the examination of their demographic information, isolate etiological agents, and in vitro antifungal susceptibility patterns.

3. Methods

3.1. Collection and Identification of Samples

The samples were obtained from 170 patients with symptoms of fungal otitis externa in hospitals affiliated with Shahid Beheshti University of Medical Sciences and Tehran University of Medical Sciences, Tehran, Iran, within 2017 - 2022. The samples were collected using sterile forceps and two cotton swabs. The first swab was used for direct examination by potassium hydroxide and methylene blue. The other swab was rolled on Sabouraud Dextrose Agar (SDA, Merck, Germany) for 2 - 7 days at 30°C. The deoxyribonucleic acid of the fresh colonies was extracted using the previously described method (17, 18). The ITS1-5.8SrDNA-ITS2 of the yeast and beta-tubulin regions of the molds were amplified using the ITS1-ITS4 primers and beta-tubulin primers, respectively (19, 20). Polymerase chain reaction products were subjected to the sequence.

3.2. In vitro Antifungal Susceptibility Test

For the evaluation of the antifungal susceptibility pattern, Clinical and Laboratory Standards Institute guideline was used for yeasts (M27-A3/S4) and filamentous fungi (M38-A2) (21-23). The minimum inhibitory concentration (MIC) of miconazole, fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, and caspofungin was determined. All antifungal agents were obtained from Sigma-Aldrich, USA. The medium used for these experiments was RPMI 1640 (Sigma-Aldrich, USA) with MOPS (Sigma-Aldrich, USA).

The final ranges of drug concentrations tested were 0.064 - 64 μg/mL for fluconazole, 0.016 - 16 μg/mL for posaconazole, voriconazole, itraconazole, and amphotericin B, and 0.008 - 8 μg/mL for caspofungin. *Candida* suspensions were obtained from colonies grown on SDA at 35°C and were adjusted to give a final inoculum concentration of about 0.5 - 2.5 $\times 10^3$ CFU/mL (24). *Aspergillus* suspensions were obtained after 7 days of growth on potato dextrose agar (Merck, Germany), and the cell density was adjusted to 0.4 - 2.5 $\times 10^4$ CFU/mL (25).

For all drugs except amphotericin B and caspofungin, the lowest concentration of drug that caused 50% growth inhibition was regarded as MIC. Moreover, 100% inhibition of growth was MIC for amphotericin B and caspofungin.

Candida parapsilosis (ATCC 22019) was used as a quality control strain. All the tests were performed in duplicate. Since fluconazole is not commonly effective for the *Aspergillus* genus, this agent was not used for this species.

3.3. Statistical Analysis

Statistical analysis was performed using SPSS software (version 16.0). The chi-square test was used to test associations, and the p-value was calculated. A P-value of 0.05 or less was considered statically significant.

4. Results

The samples of 170 patients with otitis externa symptoms were collected during a 5-year period. After initial evaluations, 145 patients (85.29%) showed positive mycological findings. Table 1 shows a summary of the demographic information (e.g., gender, age, affected ear, risk factors, and clinical manifestations).

In the present study, otomycosis was observed in various occupations, with most cases observed in housewives (n = 69; 47.5%), followed by farmers (n = 24; 16.5%), employees (n = 13; 8.9%), and other occupations (n = 39; 26.8%). The seasonal distribution in patients was reported as 58 cases in winter (40%), followed by autumn (n = 45; 31%), spring (n = 22; 15%), and summer (n = 20; 13.7%). The results after sequencing showed that *A. niger* was the predominant species (n = 75; 51.72%), followed by *A. fumigatus* (n = 33; 22.75%), *C. albicans* (n = 24; 16.55%), and *C. glabrata* (n = 13; 8.96%). Table 2 summarizes the identification and antifungal susceptibility of the isolates.

According to the obtained results, all tested drugs were effective against *Aspergillus* isolates. Caspofungin showed the highest activity against *Aspergillus* isolates; however,

Table 1. Distribution of Patients with Otomycosis Based on Different Characteristics

Characteristics	No. (%) (Total = 145)
Gender	
Male	81 (55.8)
Female	64 (44.1)
Age groups (y)	
< 20	2 (1.4)
20 - 29	14 (9.7)
30 - 39	36 (24.8)
40 - 49	30 (20.7)
50 - 59	38 (26.2)
60 - 69	15 (10.3)
≥ 70	10 (6.8)
Affected ear	
Right	81 (55.9)
Left	60 (41.4)
Both	4 (2.7)
Risk factor	
Ear manipulation	112 (77.2)
Antibiotics	82 (56.5)
Hearing aid	15 (10.3)
Swimming	12 (8.2)
Clinical manifestations	
Hearing loss	134 (92.4)
Pruritus	118 (81.3)
Otorrhea	96 (66.2)
Edema	89 (61.4)
Otalgia	85 (58.6)

Table 2. Antifungal Susceptibility of *Aspergillus* and *Candida* Strains Isolated from Otomycosis Patients

Species and Antifungal agent	MIC Parameter (μg/mL)	
	G-Mean	Range
Aspergillus niger **(n = 75)**		
ITC	0.229	0.016 - 0.5
VRC	0.223	0.032 - 8
POS	0.162	0.016 - 2
AMB	0.216	0.016 - 4
CAS	0.062	0.016 - 0.5
Aspergillus fumigatus **(n = 33)**		
ITC	0.234	0.063 - 0.5
VRC	0.217	0.125 - 8
POS	0.134	0.063 - 0.5
AMB	0.122	0.032 - 0.5
CAS	0.046	0.016 - 0.25
Candida albicans **(n = 24)**		
FLC	1.915	0.5 - > 64
ITC	0.339	0.25 - 0.5
VRC	0.115	0.016 - 0.5
POS	0.229	0.063 - 0.5
AMB	0.569	0.25 - 1
CAS	0.016	0.008 - 0.032
Candida glabrata **(n = 13)**		
FLC	3.775	0.5 - > 64
ITC	0.297	0.125 - 0.5
VRC	0.334	0.032 - 0.5
POS	0.354	0.125 - 1
AMB	0.297	0.125 - 1
CAS	0.025	0.008 - 8

Abbreviations: MIC, minimum inhibitory concentration; FLC, fluconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; AMP, amphotericin B; CAS, caspofungin.

itraconazole demonstrated the lowest activity. In this study, five *A. niger* isolates were resistant to itraconazole (MIC: 2 μg/mL). Among *A. fumigatus* isolates, three isolates were resistant to amphotericin B (MIC: 8 μg/mL), and five isolates were resistant to voriconazole (MIC: 16 μg/mL).

Fluconazole showed the weakest power with a high Geometric Mean (G-Mean) against *C. albicans* (GM: 1.915) and *C. glabrata* (GM: 3.775). Caspofungin showed the most activity against *C. albicans* isolates (GM: 0.016) and *C. glabrata* isolates (GM: 0.025), respectively. Furthermore, eight *C. albicans* isolates were resistant to fluconazole with a MIC of 32 μg/mL. Two isolates of *C. glabrata* were resistant to caspofungin with a MIC of 8 μg/mL, and four isolates were resistant to itraconazole with a MIC of 2 μg/mL.

5. Discussion

Otomycosis has a global distribution with a prevalence of 4 per 1,000 individuals (26). The samples of 170 patients with otitis externa symptoms were evaluated in this study. Similar results reported by Kazemi et al. revealed that the frequency of otomycosis in a 2-year period was 92% (129 out of 140) in northwest Iran (27). However, several studies in different regions of Iran demonstrated lower frequencies of otomycosis, including Jahrom (n = 108/211; 51.1%) in the south of Iran (28), Semnan (8/70; 11.4%) in the north of Iran (1), Lorestan (15/79; 18.98%) in the west of Iran (29), Khouzes-

tan (293/881; 32.25%) in the south of Iran (26), Yasuj (144/275; 52%) in the south of Iran (5), Rasht (43/100; 43%) in the north of Iran (30), and Isfahan (118/171; 69%) in the center of Iran (12). Based on the evidence, the prevalence of otomycosis differs in different geographical regions due to various climatic conditions (29). Therefore, the incongruity between the findings of the present study and others in Iran could be attributed to diverse geographical regions, duration of sampling, and different inclusion and exclusion criteria for patients.

Among the studied patients in this study, the prevalence was higher among those in the age range of 50 - 59 years (26.2%) but rare among adolescents ($>$ 20 years) and older patients ($\geq$ 70 years). Javidnia et al. and Prasad et al. reported that otomycosis was uncommon among teenagers and older patients (28, 31). However, the results of the present study do not support those obtained in previous studies, which reported the highest prevalence of otomycosis among working groups (5, 28, 31).

Based on the present study's results, otomycosis is more prevalent among female patients (44.1%), which is consistent with earlier reports (5, 28-30, 32, 33). However, some other studies reported higher frequency in males than in females (27, 34). The higher prevalence of otomycosis in the current study can be explained by factors, such as wearing a scarf, women's higher tendency to visit physicians than men, and daily housework, which expose housewives to fungal spores in the dust (24, 27, 32, 35). However, wearing a head scarf was not a possible risk factor for developing otomycosis (12).

Based on previous reports, otomycosis is mostly unilateral (32). In this study, 2.7% of patients presented with the bilateral involvement of the ears, which is in line with previous studies reporting that 9%, 7%, 13.8%, and 5% of patients suffered from the simultaneous affliction of both ears, respectively (5, 11, 28, 31). A few studies reported higher rates (25% and 19.23%) of the bilateral involvement of ears (35, 36). These discrepancies might be attributed to different conditions of patients' immune systems. Viswanatha et al. showed that bilateral otomycosis is more prevalent among immunocompromised patients than in immunocompetent patients (37).

The most common predisposing factors among the patients of the current study included ear manipulation, followed by topical antibiotic therapy, hearing aid usage, and swimming, similar to previous studies by Sabz et el. and Loh et el. in which the manipulation and self-cleaning of ears were highlighted as the most common risk factors for otomycosis (5, 38). However, the aforementioned results differ from those of other studies, which reported swimming as a major risk factor (31, 39). Furthermore, the presence of cerumen, diabetes, humid climate, hypertension, immunodeficiency, and configuration of the ear canal has been suggested as the predisposing factors of otomycosis (5, 28, 40).

In the present study, the most common symptom was hearing loss, followed by pruritus. This result is inconsistent with the results of other studies in which otalgia and pruritus were reported as the most frequent symptoms (5, 12, 15, 27, 28, 34, 41, 42). Furthermore, in two other studies, blockage of the ear (43) and otorrhea (44) were reported as the most common symptoms of otomycosis.

Based on the literature, the etiology of otomycosis is greatly divergent and has different antifungal susceptibility patterns (2, 39). In this study, out of the total 145 ears diagnosed with otomycosis, 108 and 37 ears were infected with filamentous fungi and yeast agents, respectively. *A. niger* was the predominant species, followed by *A. fumigatus*, *C. albicans*, and *C. glabrata*. Barati et al. reported that *A. flavus* is the most frequent etiology in otomycosis patients in central Iran (12). In opposition to the preset study's results, Javidnia et al. reported *A. tubingensis* (52.7%) and *A. niger* (25.9%) as the most frequent isolates (28). In numerous studies, *A. niger* was considered to be the most prevalent etiology of otomycosis (27, 32, 33, 41, 44, 45). However, in a few studies, *C. albicans* was reported as the leading cause of otomycosis (29). An earlier project by García-Martos et al. showed that *C. parapsilosis* was the more frequent etiology of otomycosis than *C. albicans* (46). Some studies reported rare cases of otomycosis caused by *Penicillium* spp. (29) and *Alternaria* spp. (2, 29).

There is adequate evidence to show that azoles are the most effective agents against otomycosis without any ototoxicity (30). The results of the current study demonstrated that fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, and caspofungin were active against *Aspergillus* isolates, among which caspofungin and itraconazole displayed the most and the least activity against these strains, respectively. In this study, five *A. niger* isolates were resistant to itraconazole. Moreover, three and five *A. fumigatus* isolates were resistant to amphotericin B and voriconazole, respectively. In addition, caspofungin presented the highest activity against *C. albicans* and *C. glabrata* isolates; nevertheless, fluconazole showed the weakest potency. Moreover, five *C. albicans* isolates were considered fluconazole-resistant, and two and three *C. glabrata* isolates were resistant to caspofungin and itraconazole, respectively.

Szigeti et al. reported that all strains of *Aspergillus* showed moderate sensitivity to amphotericin B, ketoconazole, and fluconazole (47). Nemati et al. demonstrated that all *A. niger* isolates were sensitive to fluconazole, clotrimazole, and ketoconazole. In contrast with the results of the present study, Nemati et al. demonstrated that *C. albi-*

cans isolates had the most susceptibility against fluconazole (30). Nong et al. in China reported that *Aspergillus* species were susceptible to itraconazole and ketoconazole, but not to fluconazole (48). The results of the aforementioned study showed that *C. albicans* isolates were susceptible to itraconazole, ketoconazole, fluconazole, and amphotericin B (48). Based on the evidence, the antifungal susceptibility patterns of several *Aspergillus* species, such as *A. niger*, have demonstrated variable sensitivities depending on geographical regions and various sources (49, 50).

5.1. Conclusions

Due to climatic conditions, humidity and dust, otomycosis has a high occurrence in Iran. The manipulation and self-cleaning of the ear canal with unhygienic tools were suggested as the main risk factors. Education in this regard is important to prevent this disease. To sum up, although otomycosis needs long-term antifungal therapy and recurrence is high in some cases, it is rarely life-threatening, and eardrop antifungals are usually enough to eradicate the infection.

Footnotes

Authors' Contribution: All authors contributed to the study concept and design. EL, RGH, ZR, FYH, and MF, material preparation, clinical collection, and statistical analysis; EL, NM, DM, and SS, searching in databases; EL, RGH, and MF, drafting the manuscript; EL and MF, critical review of the manuscript. All authors read and approved the final manuscript.

Conflict of Interests: All the authors declare that they have no conflict of interest.

Data Reproducibility: The data presented in this study are uploaded during submission as a supplementary file and are openly available for readers upon request.

Ethical Approval: The project received approval from the Ethics Committee of Shahid Beheshti University of Medical Sciences (code: IR.SBMU.MSP.REC.1400.559; Link: ethics.research.ac.ir/EthicsProposalView.php?id=232232).

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Informed Consent: Informed consent was obtained from the patients upon admission.

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