



Prevalence and Etiology of Otomycosis in West Libya

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ABSTRACT

Fungal otitis media is a frequently case facing otolaryngologists due to its recurrence and long-Fungal otitis media is a frequent condition faced by otolaryngologists due to its recurrence and long-term treatment period. The infection starts in the outer ear canal and may reach the inner ear. The aim of this study was to isolate and identify the fungal species causing inflammation of the auditory canal, identify the main causes and contributing factors to the spread and increased incidence of ear fungal infection, and determine the relationship between otomycosis occurrence and age, gender, and diabetes. Additionally, the study aimed to compare the incidence of otomycosis among different areas. A total of 205 ear swabs were collected from patients visiting national clinics and hospitals. The samples were cultured on Sabouraud dextrose agar and Czapek-Dox agar and incubated at 30°C for a week. Nine fungal species belonging to four genera were isolated: *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp., and *Candida* spp. The current study showed a predominance of *Aspergillus* spp. isolates, with a rate of 82.67%, making it the main cause of fungal ear infection; however, *Penicillium* spp. was the least prevalent. Among the fungal species, *A. niger* was dominant, with a prevalence rate of 41.16%. The prevalence of ear infection was higher in females (62.82%) than in males (37.17%), and the infection was most common in the age group from one to twenty years (39.35%), while it was less frequent in older age groups. In conclusion, The study concluded that otomycosis is widely prevalent in western Libya, with *Aspergillus niger* as the predominant etiological agent.

انتشار ومسببات التهاب الأذن الفطري في غرب ليبيا

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الكلمات المفتاحية:

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الملخص

التهاب الأذن الوسطى الفطري هو حالة متكررة تواجه أطباء الأنف والأذن والحنجرة بسبب تكرارها وفترة العلاج الطويلة، تتركز العدوى الأولية في القناة السمعية للأذن الخارجية، وتمتد أحياناً إلى الأذن الداخلية عبر طبلة الأذن، هدفت هذه الدراسة إلى عزل وتحديد الأنواع الفطرية المسببة لالتهاب القناة السمعية، والتعرف على أكثر الأسباب والعوامل المساهمة في انتشار وزيادة الإصابة بفطريات الأذن، وتحديد العلاقة بين حدوث فطار الأذن والعمر، والجنس ومرضى السكر، وكذلك مقارنة نسبة انتشار فطار الأذن بين المناطق المختلفة، جمعت 205 مسحة أذن من المرضى الذين قاموا بزيارة العيادات والمستشفيات الوطنية. تمت زراعة العينات في أجار سابورود دكستروز وأجار، و تشابيك دوكسي، وحضنت عند درجة حرارة 30°م لمدة أسبوع، عزلت تسعة أنواع فطرية تنتمي إلى أربعة أجناس: *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp., و *Candida* spp.، أظهرت

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الدراسة الحالية وجود عزلة سائدة لفطر *Aspergillus* spp. بنسبة (82.67%) تعتبر السبب الرئيسي للإصابة بفطريات الأذن، إلا أن جنس *Penicillium* spp. كان الأقل انتشاراً. أما بالنسبة للنوع الفطري فكان *A. niger* هو السائد بنسبة انتشار (41.16%)، وكانت نسبة الإصابة بالتهاب الأذن أعلى عند الإناث (62.82%) عنها عند الذكور (37.17%)، بينما كانت الإصابة الفطرية أكثر انتشاراً في الفئة العمرية من سنة إلى عشرين سنة بنسبة (39.35%). وأقل انتشاراً في الفئة العمرية الأكبر. خلصت الدراسة إلى أن داء الأذن الفطري احد أهم الأسباب لالتهابات الإذن وهو منتشر بشكل واسع في غرب ليبيا، في حين كان فطر *Aspergillus niger* هو المسبب الرئيس للمرض وهو الأكثر انتشاراً من بين الفطريات الأخرى.

1. Introduction

Fungi are among the many organisms that can cause damage to the human ear by secreting toxins, making it susceptible to damage, even affecting the prominent part of the ear [1, 2, 3]. They also affect the middle ear, with *Aspergillus* spp. and *Candida* spp. being among the most important fungi that cause this infection. Otomycosis is common in tropical and subtropical regions, particularly in areas with hot and humid climates [4, 5, 6, 7]. In addition, wind plays a major role in the transmission of fungal spores. Weakness or loss of hearing, a feeling of blockage in the ear, secretions (white, gray, yellow, or green) from the ear canal, and redness or inflammation of the ear tip or scales around it [8, 9, 10, 11] are among the most important manifestations of ear fungal infection. Earwax is crucial in protecting the lining of the auditory canal from fungi and other microbes. Therefore, anything that reduces the amount of wax or changes the pH of the auditory canal, such as the use of ear cleaning sticks, earphones, oils in the ear, or water sports like swimming and surfing, can affect the amount of wax in the ear, which is the first line of defense for the auditory canal, and thus facilitates the occurrence of fungal infection [8, 12, 13, 14, 15]. Studies have found that fungal infections of the ear are more prevalent in females than in males, as head coverings are considered one of the factors that predispose individuals to infection [2, 5]. It is also known that some chronic diseases increase the risk of fungal infection, including diabetes, eczema, and immune deficiency disorders [16, 17]. Fungal infections are more prevalent in individuals with diabetes, particularly those caused by *Candida* spp. [18]. Furthermore, living in tropical and subtropical climates with high temperatures and humidity are common factors that increase the risk of otomycosis, as the infection is more frequent in the summer than in the winter [5, 20, 15]. Otomycosis is a subacute or acute superficial fungal infection of the external auditory canal, primarily caused by opportunistic fungi. This infection is typically unilateral and presents with symptoms such as inflammation, itching, scaling, and significant discomfort, including suppuration and pain. In one study, 15 patients (11 females and 4 males) with symptomatic otomycosis were diagnosed using direct microscopy and culture methods. The most frequently identified fungal pathogen was *Aspergillus niger* (8 cases), followed by *A. flavus* (2 cases), and one case each of *A. fumigatus*, *Penicillium* species, *Candida albicans*, *C. parapsilosis*, and *Rhizopus* species [19]. Otomycosis has been observed in many countries, including northern Iraq [21], as an endemic disease in João Pessoa, Brazil [13], in the Indian Subcontinent [20], and southern Serbia [22]. In northern Iraq, *Aspergillus* species were the predominant fungi isolated, accounting for 92.1% of cases [21]. In Nigeria, *Candida albicans* was the most common fungal isolate, occurring in 28.3% of patients. Other isolates, listed in order of frequency, included *Aspergillus fumigatus* (5.7%), *A. niger* (1.9%), *Penicillium* spp. (1.9%), and *Mucor* spp. (1.9%) [23]. Due to the lack of information about the prevalence of fungal diseases and the characteristics of otomycosis in patients clinically suspected of having external otitis in western Libya, the purpose of the present investigation was to estimate the prevalence of otomycosis and the distribution of causative species in these infections.

2. Materials and Methods

Samples were collected from prospective patients of all age groups referred to Al-Taqwa Sanatorium in Al-Zintan, Al-Firdous Sanatorium in Tripoli, Yafran Hospital, and Al-Rayina Hospital

during the period from March to September 2019. A total of 205 specimens were included in this study: 9 cases involved patients with diabetes, 81 cases were clinically diagnosed with fungal infection, 159 samples were from healthy participants, and 37 samples were from patients with some symptoms but no confirmed infection. Sterile cotton swab samples were taken from the secretions of the auditory canal. The samples were then transferred to the laboratory and divided into two parts.

The first part was used for direct microscopic examination (D.M.E.), where the sample was treated on a microscopic slide with potassium hydroxide (KOH) solution at a concentration of 10% to detect fungal hyphae, spores, or yeasts. The second part was cultured on Sabouraud dextrose agar (S.D.A.) plates supplemented with 0.05 mg/mL of chloramphenicol for one week at 30°C, allowing the fungi to grow and form colonies, which were then examined for colony morphology. Additionally, the colonies were microscopically examined using Lactophenol Cotton Blue (LPCB) to clear the material and examined under 10X and 40X objectives for fungal elements. Each fungal species required pure isolates, which were recorded and stored in a refrigerator [4, 5, 14, 10, 16, 24].

3. Statistical Analysis

The data were statistically evaluated using SPSS (Version 23) software. The level of significance was set at $p < 0.05$. A chi-square test was employed to determine the prevalence of fungal species and the relationship between fungal species infection and diabetes mellitus in both infected and uninfected patients. Additionally, the chi-square test was used to compare the prevalence of fungal species between males and females, with $p < 0.05$ considered statistically significant.

4. Results

In this preliminary study, 205 clinically examined samples from different age groups were cultured. The overall prevalence of fungal infection was 86.34% (177/205), with no fungal growth observed in 13.66% (28/205) of cases. Furthermore, 72 specimens were infected by more than one species in the same case, as revealed in Fig. 1.

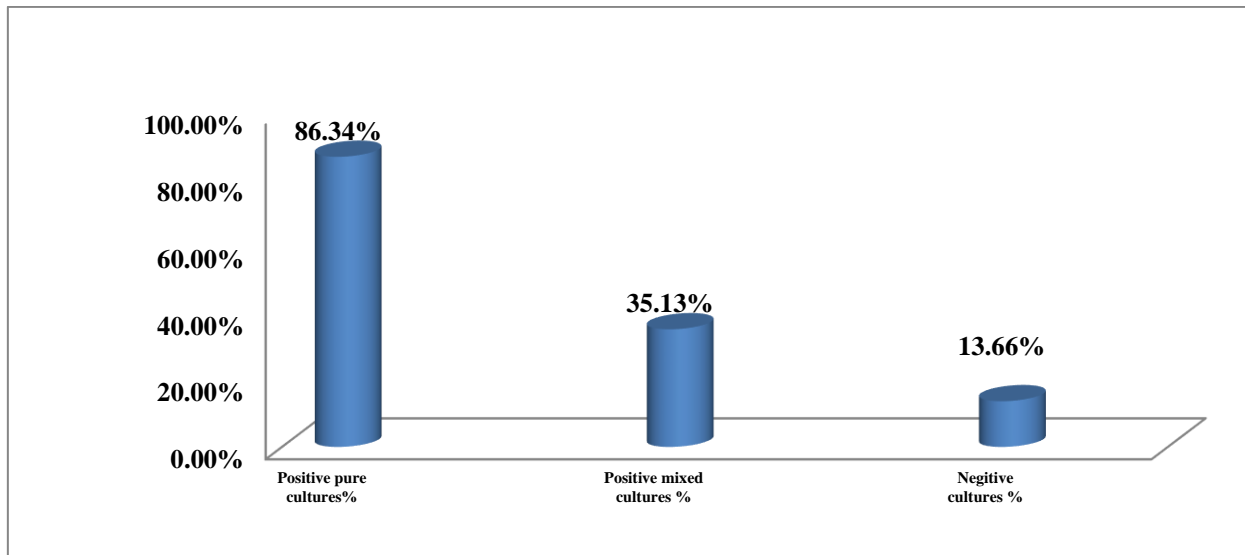


Figure1: prevalence percent of fungal growth in SDA cultures.

• Different letters indicates a significant difference between isolated fungal species. . p value< 0.05

As shown in table (1), there were 277 fungal isolates from 205-cultured sample. Healthy specimens showed the highest rate of fungal infection (57.40%) of the total specimens, followed by clinical samples with a rate of (29.25%), and the lowest prevalence recorded in non-clinical specimens (13.34%). Nine fungal species belonging to four fungal genera were isolated from them: *Aspergillus spp.*,

Alternaria spp., and *candida spp.* and *Penicillium spp.* *Aspergillus spp.* was the most prevalent type of fungal genus with a rate of 82.67%, while the genus *Penicillium spp.* the lowest incidence was at a rate of 2.17%. Since the calculated chi-square value (26.25, P < 0.05) is much greater than the critical value (7.81), this indicates a significant difference between the isolated fungal species.

Table 1: Shows isolated fungal species and their prevalence among symptomatic patients ,non-symptomatic participants and health patient.

| Taxa | Fungal Isolates | | Clinical Samples | | Non-clinical samples | | Health | |
|-------------------------|-----------------|-------|------------------|-------|----------------------|-------|--------|-------|
| | I | I% | I | I% | I | I% | I | I% |
| <i>Aspergillus spp.</i> | 229 | 82.67 | 66 | 23.83 | 29 | 10.47 | 134 | 48.38 |
| <i>Alternaria spp.</i> | 33 | 11.91 | 8 | 2.89 | 5 | 1.80 | 20 | 7.22 |
| <i>Candida spp.</i> | 9 | 3.25 | 6 | 2.17 | 1 | 0.36 | 2 | 0.72 |
| <i>Penicillium spp.</i> | 6 | 2.17 | 1 | 0.36 | 2 | 0.72 | 3 | 1.08 |
| Total, I & I% | 277 | 100 | 81 | 29.25 | 37 | 13.35 | 159 | 57.40 |

I, Incidence. (I%), Incidence %.

No a significant difference between symptomatic patients, non-symptomatic participants and health patient. p value< 0.05.

In the present study, the isolated fungal species prevailed in the spread of *Aspergillus niger* at a rate of (41.16%), followed by *Aspergillus flavus* at a rate of (38.63%). The isolated species in these specimens, *Aspergillus fumigatus* was the least common (0.72%). The prevalence of different fungal species was higher in females than males at a rate of 62.81% of the total number of specimens, since the p-value (P < 0.05) is greater than 0.05, this means that there is no strong statistical evidence of a significant difference in the frequency of species between males and females in this data. Moreover,

the result showed that the fungus is present in the all age groups, where the highest fungal infection was in the age group from one

year to twenty at a rate of (39.35%), followed by the age group from 21 years to 40 years at a rate of (35.02%). While the elderly age range (61-80) revealed a lower prevalence of fungal infection in compare to other age groups (4.33%). *Aspergillus niger* was the dominant fungus in age groups (21-40; 41-60), followed by *Aspergillus flavus* (41.16%, 38.63%), The data shows a significant association between the age and the state, P < 0.05, as shown in Table No. (2).

Table 2: Prevalence of fungal species related to gender and age.

| Taxa | Gender | | years | | | | | | | | Total | |
|-------------------------|-------------|-------------|--------|-------|---------|-------|--------|-------|---------|------|-------|-------|
| | | | 1-20 * | | 21-40 * | | 41-60* | | 61-80 * | | | |
| | Male | Female | I | I% | I | I% | I | I% | I | I% | I | I% |
| <i>A. niger</i> | 47 | 67 | 50 | 18.05 | 37 | 13.35 | 23 | 8.30 | 4 | 1.45 | 114 | 41.16 |
| <i>A. flavus</i> | 34 | 73 | 40 | 14.44 | 41 | 14.80 | 23 | 8.30 | 3 | 1.08 | 107 | 38.63 |
| <i>Alternaria spp.</i> | 14 | 19 | 11 | 3.97 | 13 | 4.69 | 8 | 2.88 | 1 | 0.36 | 33 | 11.91 |
| <i>Candida spp.</i> | 4 | 5 | 4 | 1.45 | 2 | 0.72 | 1 | 0.36 | 2 | 0.72 | 9 | 3.25 |
| <i>Penicillium spp.</i> | 2 | 4 | 2 | 0.72 | 1 | 0.36 | 2 | 0.72 | 1 | 0.36 | 6 | 2.17 |
| <i>A. terreus</i> | 1 | 2 | 0 | 0.00 | 1 | 0.36 | 1 | 0.36 | 1 | 0.36 | 3 | 1.08 |
| <i>A. Fumigatus</i> | 1 | 1 | 1 | 0.36 | 1 | 0.36 | 0 | 0.00 | 0 | 0.00 | 2 | 0.72 |
| <i>A. ustus</i> | 0 | 2 | 0 | 0.00 | 1 | 0.36 | 1 | 0.36 | 0 | 0.00 | 2 | 0.72 |
| <i>A. aculeat</i> | 0 | 1 | 1 | (0.36 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 0.36 |
| Total, I & (I%) | 103(37.18%) | 174(62.82%) | 109 | 39.35 | 97 | 35.02 | 59 | 21.30 | 12 | 4.33 | 277 | 100 |

• An asterisk (*) indicates a significant difference between different years. p value< 0.05

• No a significant difference between male and female. p value< 0.05.

Distribution of fungal species according to regions showed that Zintan region (52.34%) has the highest spread of fungal infection followed by Yafran, Tripoli and Al-Rayina regions have (21.66%, 20.57%,

5.41%) respectively. The relationship between diabetic patients and fungus infection was found to be positive for all specimens taken with a 100% rate.

Table 3: Distribution of fungal species according to regions and diabetes mellitus.

| Fungal species | Area | | | | Diabetes | | | |
|-------------------------|--------|--------|---------|--------|----------|------|------------|-------|
| | Zintan | Yfrane | Tripoli | Rayina | infected | | uninfected | |
| | | | | | I | I% | I | I% |
| <i>A. aculeatus</i> | 0 | 0 | 1 | 0 | 0 | 0.00 | 1 | 0.36 |
| <i>A. niger</i> | 60 | 25 | 22 | 7 | 5 | 1.81 | 109 | 39.35 |
| <i>A. flavus</i> | 58 | 22 | 21 | 6 | 3 | 1.08 | 104 | 37.55 |
| <i>Alternaria spp.</i> | 11 | 9 | 11 | 2 | 1 | 0.36 | 32 | 11.55 |
| <i>Candida spp.</i> | 7 | 1 | 1 | 0 | 0 | 0.00 | 9 | 3.25 |
| <i>Penicillium spp.</i> | 5 | 1 | 0 | 0 | 0 | 0.00 | 6 | 2.16 |
| <i>A. terreus</i> | 2 | 0 | 1 | 0 | 0 | 0.00 | 3 | 1.08 |
| <i>A. Fumigatus</i> | 0 | 2 | 0 | 0 | 0 | 0.00 | 2 | 0.72 |
| <i>A. ustus</i> | 2 | 0 | 0 | 0 | 0 | 0.00 | 2 | 0.72 |
| Total | 145 | 60 | 57 | 15 | 9 | 3.25 | 268 | 96.75 |

- There is no significant difference between uninfected and infected patients' diabetes mellitus. p value < 0.05.

5. Discussion

Fungal Fungal otomycosis is a superficial fungal infection that often affects the auditory canal of the external ear, with rare complications affecting the inner ear if the eardrum is penetrated [6, 14]. The infection may be mild, acute, or chronic and is accompanied by itching, ear pain, diminished hearing, ringing in the ear, and a feeling of fullness in the ear [11]. The infection may develop into ear swelling, with or without the release of purulent discharge or inflammatory exudates from the ear. The incidence of otomycosis is particularly influenced by the geographical climate of the patient's residence, especially in tropical regions with high humidity, heat, and dusty conditions [3, 4, 10, 12].

According to our study, *Aspergillus* spp. was considered the primary cause of otomycosis, at a rate of 82.67%, with *A. niger* dominating at a rate of 41.16% of the total number of isolated fungal species. This is consistent with several previous studies, including those conducted by Jing et al. in northern China [18], the study by Abu Talib et al. in Isfahan, Iran [5], and the findings of Saki et al. in southwest Iran [25]. However, the study by Kaur et al. [9] showed *Aspergillus* spp. as the dominant genus at a rate of 83%, but with *A. fumigatus*, not *A. niger*, being the most common species, which contrasts with the outcome of our current study. There are also two studies, one conducted in Spain [8] and another in Brazil [13], which found that *Candida* spp. is the most common and primary cause of ear fungus. This finding contradicts our results, possibly due to differences in the environment, humidity, temperature, and geographical distribution of the fungus [12].

Our results also showed that the highest percentage of fungal ear infections occurred among healthy individuals, accounting for 48.38% of the total sample count. This high percentage may be attributed to a lack of interest in visiting an ear, nose, and throat specialist, misdiagnosis, or receiving inappropriate treatment. Patients might also ignore symptoms, perceiving no need to seek medical advice for such cases. Fungal infections were more prevalent among women, at a rate of 62.82%. This could be due to women wearing hair covers, which prevent air from reaching the ears, creating a dark and moist environment conducive to fungal growth. Another reason may be that women are more likely to seek medical help than men. This result is consistent with similar studies, including one conducted in Iran, where the female infection rate was about 72.2% [5], and a study by Pontes et al. with an infection rate of 60% [13]. Other studies [6, 15, 16] also align with these results. However, some studies show higher fungal infection rates in males than in females [4, 8, 9, 10].

All age groups were affected by otomycosis, but the age group from 1 to 20 years was the most affected, followed by the age group from 21 to 40 years. These results are similar to other studies, which indicated that individuals between 21 and 40 years of age are more susceptible to infection, possibly due to greater exposure to environmental conditions such as heat, high humidity, and wind compared to the elderly [4, 10]. However, this result contradicts the findings of a study conducted in Spain, where infection was most common among the elderly (24.5%). Contrary to our study, the age group from 61 to 80 years had the lowest prevalence of fungi (4.33%). *A. flavus* rather than *A. niger* was more prevalent in that study. In our results, *A. niger* was dominant across genders, regions, and diabetic patients.

Regarding regional distribution, the results showed that Zintan had the highest prevalence of fungal infection at 52.34%, followed by Yafran

at 21.66%, Tripoli at 20.58%, and Al-Rayina with the lowest rate at 5.42%. These findings are supported by other studies conducted in Niger [9, 11]. Our results also indicated that diabetic individuals showed a higher proliferation and growth of fungi in all samples, consistent with many studies that suggest diabetics are predisposed to otomycosis, such as the study conducted in Shanghai investigating otomycosis pathogens [16, 26].

6. Conclusions:

This study demonstrated that otomycosis is widely prevalent in western Libya, with *Aspergillus niger* identified as the predominant etiological agent. Accurate diagnosis requires thorough clinical examinations and mycological studies, given the nonspecific nature of otomycosis symptoms. There is a critical need for additional research in this area.

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