

Prevalence and Antifungal Susceptibility of Fungal Isolates Associated with Otomycotic Patients in Lafia

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Abstract

This study assessed the fungal pathogens associated with otomycosis and their antifungal profile. A cross-sectional study was done to collect, examine and culture 177 exudate swabs from patients presenting with otomycosis at the Dalhatu Araf Specialist Hospital for a period of three months. Fungal isolates were identified using their morphological and microscopic structure and characteristics. Mycological assessments showed that 20.90% were positive for fungal agents. Males (70.27%) had more positive cases than females (29.73%). Age group 31-40 had the most predominant number of cases (49.95%) than any other age group. *Aspergillus niger* (51.35%) was the most predominant, followed by *Candida* spp. (32.43%), while *A. flavus* (16.22%) was the least prevalent. The month of May had the most positive cases (16; 43.24%), and March had the least (24.32%). Antifungal assay showed varied susceptibility of the isolates to the antifungal drugs. *A. flavus* showed no resistance to Itraconazole. However, multiple drug resistance was observed amongst the samples, with *A. niger* showing high resistance to ketoconazole and fluconazole. This study showed that *A. niger*, *A. flavus* and *Candida* spp. are etiological agents associated with otomycosis in Lafia. Caution also needs to be taken in managing this infection as multiple drug resistance was also observed.

Keywords: Otomycosis, *Aspergillus*, *Candida*, prevalence, antifungal

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Introduction

The fungal infection of the external auditory canal, including the auricle, auditory duct, eardrum, and middle ear, is known as otomycosis or fungus otitis externa [1]. Otomycosis causes 10% of all cases of otitis externa, with saprophytic filamentous fungi, yeast and dermatophytes amongst the diversity of fungi associated with the infection [1–3]. Despite the fact that the illness is seldom life-threatening and has a low mortality rate, it is a problematic and frustrating ailment since it often requires long-term therapy and follow-up [4].

Otomycosis may manifest as an acute, subacute, or prolonged (chronic) disease with symptoms such as inflammation and exudate. Aching of the ear and secretion of exudates characterizes acute otomycosis, while subacute infection is characterized by dry dermatitis and itching with no exudates. Chronic otomycosis presents with little exudates with no foul smell. It however presents with scaly itching, accompanied by inflammation and erythema of the external ear, resulting in the clumping of fungal epithelial cell wax, and mycelial that blocks the outer ear canal and causes hearing impairment or deafness [2, 3, 5, 6].

Mugliston and O'Donoghue [7] found that otomycosis is a frequent disease seen in general otolaryngology clinics and that it is prevalent among patients who have otitis externa symptoms. *Candida* and *Aspergillus* are the most prevalent fungal species, and it is a pathogenic condition [8]. However, it's unclear if these fungi are

genuine otomycosis infective organisms or just colonization species as a consequence of local host immunity being weakened due to bacterial infection. A humid environment, the presence of cerumen, ear instrumentation, increased use of topical antibiotics/steroid preparations, an immunocompromised host, individuals who have had an open cavity mastoidectomy, and those who use hearing aids with an occlusive ear mould are all risk factors. Inflammatory pruritis, scaling, and otalgia are common symptoms [9–11].

This study, therefore, evaluated the prevalence and antifungal susceptibility profiles of fungal isolates from patients presenting with otomycosis in Lafia, a community that is characterized by hot/warm and humid climate, which is a predisposing factor to the infection.

Materials and Methods

Sample size

A total of 177 samples – determined using a 13.3% [5] prevalence rate – were collected from Dalhatu Araf Specialist Hospital in Lafia using the Fischer formula;

Fischer formula (n)

$$= \frac{pqz^2}{d^2} = \frac{0.133(0.8670)(1.96^2)}{0.05^2} = 177.19 \sim 177$$

Where: n = minimum sample size required; p = proportion of the target population estimated to have particular problem; z = 1.96 (level of precision at 95% confidence level); q = 1-p; d = 0.05 (degree of accuracy desired)



Isolation and identification of fungal species

Fungi species associated with otomycosis were isolated and identified based on the methods described by Jayachitra [12]. Ear exudates samples were collected from patients presenting otomycosis at the Dalhatu Araf Specialist Hospital, Lafia. Each sample was divided into two (2); one part was examined directly while the other was inoculated on Sabouraud dextrose agar plates supplemented with chloramphenicol, incubated at room temperature (25±2°C) and observed for fungal growth for about two weeks. Fungal growths were examined morphologically (obverse and reverse pigmentation, texture and surface) and microscopically by staining with lactophenol cotton blue and observed under the microscope for their mycelia, hyphal septation and characteristics of reproductive structures.

A questionnaire was also given to each patient to collect data related to age and sex. Ethical clearance was collected from the Ethics Review Committee of the Dalhatu Araf Specialist Hospital.

Antifungal susceptibility testing

Using agar disc diffusion method, the antifungal susceptibility of the isolates was determined [13]. Antifungals tested were fluconazole, ketoconazole and itraconazole. Aliquots of Overnight cultures of each fungal isolate was standardized to 0.5 McFarland, and aliquots of each were inoculated and evenly spread on Muller Hinton agar plates and allowed to stand for 5 min. Each antifungal disc was aseptically placed onto each plate, allowed to stand for 30 min and incubated at 37°C for 72 h. Zones of inhibition were measured in millimetres (mm) and interpreted using the CLSI 2014 guidelines.

Results and Discussion

Over the period of 3 months (March to May), a total number of 177 samples (consisting of 98 males and 79 females) from otomycosis patients were collected and clinically analysed. A total number of 37 (20.90%) samples were positive for otomycosis, with male samples accounting for 70.27% (26/37) of all positive cases and female samples accounting for the remaining 29.73% (11/37). Based on age, most cases were observed in the 31-40 age group, accounting for almost half (45.95%; 17/37) of all cases, followed by 21-30 age group (35.14%; 13/37) with age group 51-60 accounting for the least number of positive cases (1/37; 2.70%) (Table 1).

Table 1: Prevalence and distribution of otomycosis by age and sex

Age (Years)	Male (%) n = 98	Female (%) n = 79	Total (%) n = 37
≤20 (n = 27)	2	1	3 (8.11)
21-30 (n =48)	9	4	13 (35.14)
31-40 (n =46)	13	4	17 (45.95)
41-50 (n =33)	1	2	3 (8.11)
51-60 (n =23)	1	0	1 (2.70)
Total	26 (70.27)	11 (29.73)	37

Table 2: Distribution of Fungi species isolated over 12weeks

Month	No of positive samples (%)	<i>A. niger</i>	<i>A. flavus</i>	<i>Candida</i> spp.
March	9(24.32)	7	0	2
April	12(32.43)	7	3	3
May	16(43.24)	5	3	7
Total (%)	37	19(51.35)	6(16.22)	12(32.43)

Table 2 shows the different fungal species isolated, their prevalence and distribution from the ear exudates over the span of 3 months. *Aspergillus niger* was the most prevalent (51.35%; 19/37) of the total isolates, followed by *Candida* spp. (32.43%; 12/37) and *Aspergillus flavus* (16.22%; 6/37) been the least prevalent. Based on sample months, the month of May had the highest number of positive cases (16/37; 43.24%) while March had the least number of positive cases (9/37; 24.32%).

The antifungal assay showed that all tested antibiotics were active against the fungal isolates. Itraconazole showed high activity against all isolates. 68.42% (13/19) of *A. niger* isolates were susceptible to Itraconazole, whereas 83.33% of *A. flavus* were susceptible to both Fluconazole and Itraconazole. More *Candida* isolates (66.67%) were most susceptible to both Fluconazole and Ketoconazole. *A. niger* showed high resistance of 47.37% and 36.84% to Ketoconazole and Fluconazole, respectively; however, no *A. flavus* isolate was resistant to Itraconazole (Table 3).

Table 3: Antifungal susceptibility pattern of isolates against various antifungal agents

Name	No	Drug sensitivity	FLU (%)	KET (%)	ITR (%)
<i>A. niger</i>	19	S	8(42.12)	10(52.63)	13(68.42)
		I	4 (21.05)	0 (0)	3 (15.79)
		R	7(36.84)	9 (47.37)	3 (15.79)
<i>A. flavus</i>	6	S	5 (83.33)	4 (66.67)	5 (83.33)
		I	0 (0)	1 (16.67)	1 (16.67)
		R	1(16.67)	1 (16.67)	0 (0)
<i>Candida</i> spp	12	S	8 (66.67)	8 (66.67)	7(58.33)
		I	1 (8.33)	2 (16.67)	3 (25)
		R	3 (25)	2 (16.67)	2 (16.67)

FLU= fluconazole; KET= ketoconazole; ITR= itraconazole; S= sensitive; I= intermediate; R= resistant

Otomycosis is a common infection in cities like Lafia which have tropical climates, characterized by warm and humid weather. This study found a high prevalence of otomycosis in Lafia, which is similar to previous studies of different scholars [5, 6, 14–16]. The dusty, temperate and humid climatic nature of Lafia, accompanied by poor environmental sanitation and personal hygiene with the high presence of immunocompromised individuals could be attributed to the observed otomycosis prevalence. Men were observed to have more cases (70.27%) than women (29.73%).

Frequent and regular cleaning of the ear and associated high hygienic level of women compared to men could be reason for the difference in prevalence rates observed between men and women [17, 18]. However, this (frequent cleaning of the ear) could be a predisposing factor to otomycosis, an aetiological 'mystery' yet to be unravelled about otomycosis. This finding, however, contradicts that of Saki *et al.*[6], who observed more cases in females than in males.

Although one or more cases were identified in all age groups, age groups 21-30 (13/37; 35.15%) and 31-40 (17/37; 49.95) jointly accounted for over 80% of all positive cases. This was in tandem with previous findings [6, 19, 20]. On the basis of month, May had the highest number of confirmed cases, followed by April and then March. A more humid environment characterizes the month of May due to rainfall than the other two months of April and March. This further supports the claim that warm and humid weather increases the risk of otomycosis.

Three fungal species belonging to two fungal genera – *Aspergillus* and *Candida* – were observed in this study. The fungi isolates identified were *A. niger*, *A. flavus* and *Candida* spp. Earlier reports [5, 6, 19, 21, 22] reported similar findings. The most prevalent fungi isolated was *A. niger* (19/37; 51.35%), followed by *Candida* spp. (12/37; 32.43%) and *A. flavus* was the least dominant (6/37; 16.22%). This concurs with recent findings [5, 6, 23, 24]. *Candida* is a leading cause of otomycosis in immunocompromised patients predisposed to dermatitis, frequent instrument use, dirt and an existing ear infection [22]. *A. niger* is ubiquitous in nature, mostly predominant in moist environments like farmlands and vegetations. Thereby predisposing most farmers and people of low socioeconomic class, who are common in Lafia. *A. flavus* also causes otomycosis in immunocompromised hosts exposed to environmental factors [21, 25].

This study showed that the isolates exhibited varying degrees of susceptibility to the antifungal drugs tested, with more susceptibility observed to Itraconazole. *A. niger* was highly susceptible to Itraconazole, *A. flavus* was highly susceptible to Itraconazole and Fluconazole, while *Candida* was highly susceptible to both Fluconazole and Ketoconazole. All *A. flavus* isolates were susceptible to Itraconazole. Results obtained, though similar to those reported by Sziget *et al.* [26], adds to varied antifungal profiles [19, 25–30] of fungi associated with otomycosis.

Conclusion

In this study, *Aspergillus* and *Candida* spp. were revealed to be the fungal pathogens associated with otomycosis, with *A. niger* being the most predominant and *A. flavus* being the least prevalent isolates.

Although all isolates showed moderate to high susceptibilities to the antifungals tested, *A. niger* showed high resistance to ketoconazole and fluconazole. This calls for caution, especially with regards to multiple drug resistance observed in almost all isolates.

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