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Isolation and identification of some fungi that cause otomycosis in the Misan Governorate

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Abstract
Otomycosis specimens were collected from 25 (9 is negative and 16 postive) patients aged 6 months to 55 years 6 males and 10 females) at Al-Sadr General Teaching Hospital, Massan Province, between 01/01/2021 and 01/02/2022. Data, such as age and sex, were also recorded. The collected specimens were grown on Sabouraud's dextrose agar (SDA), which contained chloramphinicol (0.05 g), in addition to chrom candid agar for *Candida* sp. And 16 specimens were positive for culture (53%). The highest rate of infection was observed among females (62.5%) compared to males (37.5%), whereas the 31-40 year age range in both sexes showed a higher prevalence rate (37.5%), and the age ranges of 6–10 years and 21-30 years in both sexes showed a lower prevalence rate (6.25%). *Aspergillus* sp. and *Candida* sp. were the most frequent etiological factors of otomycosis and the results showed that *Aspergillus niger* had the highest incidence (37.5%) (6 isolates), followed by *Candida krusei* (18.7%) (3 isolates) and *A. fumigatus*, *C. parapsilosis*, *C. ablicans*, *C. tropicalis* and *A. lentulus* (6.25%) (one isolate for each fungus).

Keywords: Otomycosis, yeast, *Aspergillus*, *Candida*

Introduction
Numerous and various eukaryotic, heterotrophic, spore-producing organisms called "fungi" include unicellular or multicellular organisms supported by breaking down absorbing the organic substances they encounter as they develop, produce exoenzymes, and obtain nutrients by absorption. Although there are more than 1.5 million kinds of fungus on the planet, yet only 300 of them are known to infect people [1]. Costs to patients, communities, and healthcare facilities [2].

Human fungal diseases are classified according to the affected site or site on the body Regarding the pathogen's virulence: cutaneous, when confined to the epidermis; subcutaneous, if the infection penetrates significantly under the skin; systemic, when the illness has migrated to internal organs or is widespread throughout the body; and opportunistic when the physiological condition of the host is changed (immunodeficient host) [3-4].

External ear fungal infections involve inflammation of the outer part of the ear [1]. Otomycosis is an external auditory canal fungal infection that is just superficial. The infection is often unilateral, subacute, or chronic.Otomycosis is sometimes referred to as Singapore's ear or swimmer's ear [5]. A fungus called otomycosis often infects the external ear canal [6].

Otomycosis, a superficial outer ear infection caused by fungal pathogens, is a common disease in tropical and subtropical regions and is usually associated with symptoms such as itching, ear discharge, ear pain, hearing loss and ear congestion. Without a functioning immune system, pathogens can enter the inner ear and lead to serious complications [7-8].

Many fungi are involved in the clinical course of the disease and 61 different species have been identified in selected studies.Pathogens range from temperate to tropical climates, with *Aspergillus*, *Candida* and *Penicillium* being the most common [9].

Aspergillus and *Candida* are the most prevalent causal factors. of otomycosis. Among *Aspergillus* species, *A. niger* is generally the dominant species, followed by *A.flavus*, *A. fumigatus* and *A. terreus*. Among the *Candida* species, *C. albicans* is the most prevalent isolate, and then *C.parapsilosis*, *C. glabrata*, *C. guilliermondii* and *C. krusei*. Other fungi such as *Penicillium*, *Mucor*, Rhizopus, *Cladosporium*, *Chrysosporium* and *Absidia* also participate [10-12].

According to research, otomycosis is thought to be the root cause of between 5 and 30% of instances of otitis externa [13]. The prevalence of otomycosis correlates with geographic range, including tropical and subtropical areas having higher incidence rates [14]. Over 54% of tropical areas have a high incidence, whereas only 9% of temperate areas do [15]. Incidence of otomycosis is shown to be greater during the wetter months. following summer, as heat and humidity provide a very favorable environment for fungal growth [16]. Otomycosis is usually chronic with acute episodes and intermittent remissions [17]. Yeast infections can manifest as a gentle, creamy, white substance that lines the ear canal [18]. In severe cases, the ear canal is inflamed and swollen Figure 1.

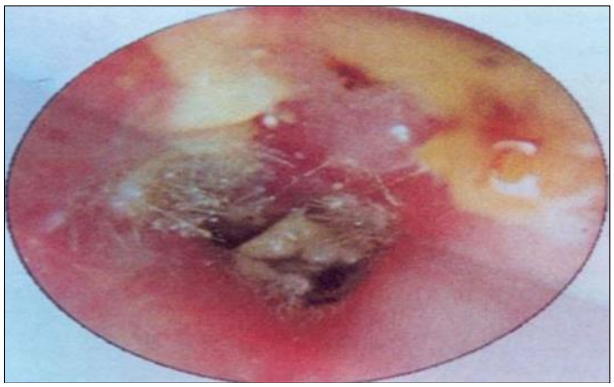

Fig 1: Otoscopic image of the ear canal with otomycosis [19].

Aim

Isolate, identify and characterize fungus obtained from individuals with a clinical suspicion of otomycosis

Methods

Specimens Collection

Patients' selection

Patients with a clinically confirmed diagnosis of otomycosis who took part in our research department underwent an experimental descriptive investigation. at Al-Sadr General Teaching Hospital and some private clinics in Masan Governorate between 1/11/2021 and 1/2/2022. These patients reported a variety of complaints, including ear pain, itching, and ear discharge, physical inspection indicated erythema, fungal debris, and creamy or blackish ear discharge, together with or without hearing loss. Subjects who had taken topical antifungal drugs in the recent past were excluded from our study.

Preparation and processing of specimens

After the clinical diagnosis was made, samples were obtained from each patient's external auditory canal. under aseptic conditions using sterile cotton swabs. The sample was placed on the surface of two plates with (SDA) supplemented with 0.05 mg/ml chloramphenicol (AppliChem GmbH, Darmstadt, Germany) and incubated at 30 °C. This medium was prepared according to the manufacturer's recommendations (Himedia) by combining 1000 litres of distilled water with 65 g of SDA powder (with autoclaving) and pouring it into plastic dishes with a diameter of 9 mm, which were used for primary isolation and diagnosis [20]. Plates were incubated for 7 days, with inspection everyday for the first week, then twice per week for the following three weeks, or until colonies started to develop or stopped growing.. The growing fungi were stored in SDA slant samples for further mycological studies, which were utilised to fully identify the species of *Candida*. We were using CHROM Candida agar. To create this medium, 49.5 g of chrome agar were dissolved in 1000 ml of D.W. by dissolving the powder for 15 minutes on the hot plate while stirring (without autoclaving), then pouring into petri dishes. was used to distinguish yeasts based on color [21].

Fungi were diagnosed based on the phenotypic characteristics of the fungal colonies on the nutrient medium, conidia, and their microscopic properties, based on the following sources [22-23].

Isolation and of Identification of fungi causing otomycosis

The isolated fungi were diagnosed based regarding the cultural traits of the expanding colonies, such as color, size, nature of the culture, backside of the plate, as well as its microscopic features such as the shape of the fungi. They were isolated in 16 out of a total 25 samples at a rate of 64%, and nine species belonging to two genera, *Aspergillus* and *Candida*, were identified.

A. niger showed the highest prevalence with 37.5% (6 isolates), followed by *C. krusei* with 18.7% (3 isolates) and *A. fumigatus*, *C. parapsilosis*, *C.ablicans*, *C. tropicalalis* and *A. lentulus*. The minimum incidence was 6.25% (one isolate for each fungus) as shown in Table 1.

Table 1: Various species were isolated for this investigation. (n = 16)

Fungus isolated	Number	Percentage%
Aspergillus niger	6	37.5%
Candida krusei	3	18.7%
A. fumigatus	1	6.25%
C. parapsilosis	1	6.25%
C. ablicans	1	6.25%
C. tropicalis	1	6.25%
A. flavus	2	12.5%
A. lentulus	1	6.25%
Total	16	100%

The present study's findings revealed that A*spergillus* had the greatest prevalence of infection, which is in accordance with other studies by [14, 24, 21, 25]. However, many investigations have shown that *A. flavus* and other fungi are the main causes of otomycosis [26]. *A. fumigatus* (Panchal *et al*., 2013), [27] and *Candida* [7]. We ascribed this variation to the spatial distribution's unpredictability and environmental conditions [28]. Additionally, these variations could result from the geographic distribution of fungus in various regions, although there is a dearth of epidemiological information to compare [29].

The results showed that 16 specimens were positive for culture at a rate of 53%, which is considered a small percentage. The reason for the low percentage in this study is due to several factors, including the location from which

the study samples were collected, the cultural differences of the infected people, the climatic factors of the study area, and the time of specimen collection and the seasonal changes that accompany it [30].

Regarding the negative culture results for certain specimens, this may be because those who have fungus infections used an antibiotic without first seeing a specialist doctor in order to relieve the uncomfortable symptoms of otomycosis infection [31]. the kind of culture media used, the fact that there was inadequate vaccine administered, or the fact that the sample size taken was insufficient to provide a positive result are possible causes for this [32] or as a consequence of improper sample storage techniques, such as placing samples in damp containers, which causes contamination of the sample and produces unfavourable results [33]. The topical use of corticosteroid antibiotics by infected people is the most frequent cause of the appearance of a bad consequence [34].

Classification of fungi causing otomycosis

***A. niger*:** One of the most prevalent and readily recognised species of the genus *Aspergillus* is *A. niger*, which has a surface of the mycelial culture that ranges from white to yellow and subsequently bears black conidia. This species is the most frequent otomycosis-causing agent and is prevalent in aspergillomas. It is a frequent source of contamination in laboratories.

Morphological identification

Colonies were made up of a thick layer of dark brown to black conidial tips on top of a solid white or yellow basal mat. The conidial heads are large (up to 3 mm x 15–20 µm in diameter), spherical, dark brown, radial, and tend to divide into several loose columns with age.

Conidiophore stalks are hyaline and smooth or darker toward the follicle. The heads of the conidia are in two rows with phialides attached to brown, often septate, metuli. The conidia were spherical to subglobular (3.5-5 µm in diameter), rough walls, a dark brown to black colour Figure 2.

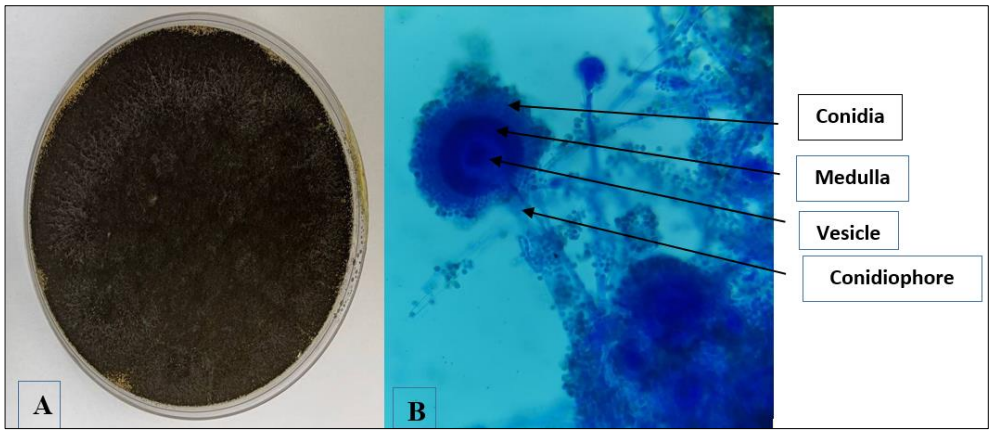

Fig 2A: *A. niger* colonies on SDA. B: Macroscopic appearance of *A. niger*

A. fumigatus

Morphological descriptions

Colonies in culture are often blue-green, with a suede-like surface made of thick conidiophore felt. Most conidial heads are columnar and arranged in a row, measuring up to 400 µm in height. The stalks of the Short, smooth-walled conidiophores have conical terminal vesicles that are two-thirds of the way up and contain a single row of phialids. Conidia arise after the basidia in spherical to subglobular in shape and have lengthy chains (2,5–3 µm in diameter), green and very rough. Figure 3.

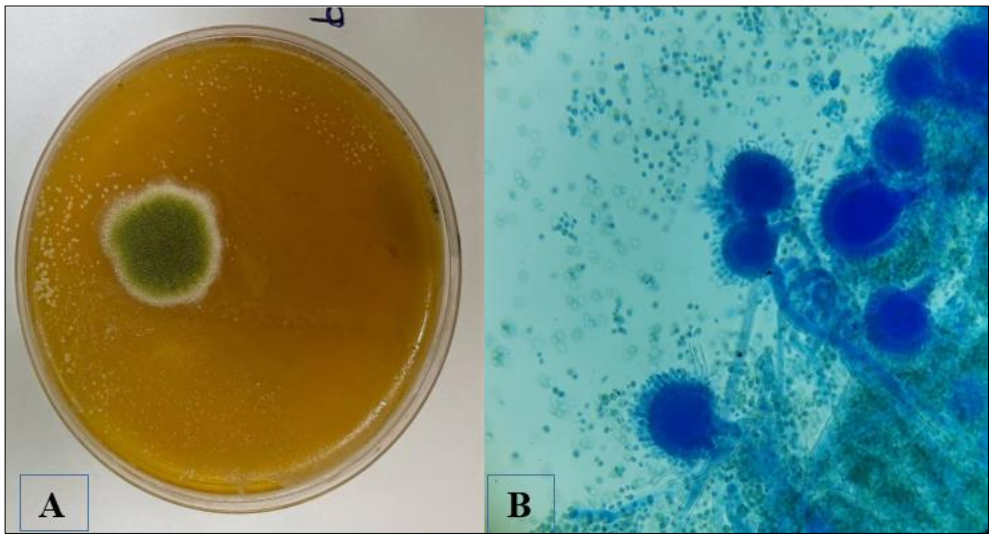

Fig 3A: *A. fumigatus* colonies on SDA. B: Macroscopic appearance of *A. fumigatus*

A. lentulus

It is now widely known that *A. lentulus* causes invasive aspergillosis in immunocompromised persons and that it is prevalent in soil. It belongs to the *A. fumigatus* family.

Description of the morphology

A. lentulus colonies are tawny to fluffy and white with greyish-green spots interspersed with conidia (conidia are slow or weak on most cultivars). The heads of the conidia are short, cylindrical and in a row. The conidiophore stalks have smooth walls., sometimes convoluted, and often narrowed at the neck. Typically, bubbles have a spherical form. The conidia were smooth to finely rough, spherical to mostly ellipsoidal, and 2–3 µm in diameter. Figure 4.

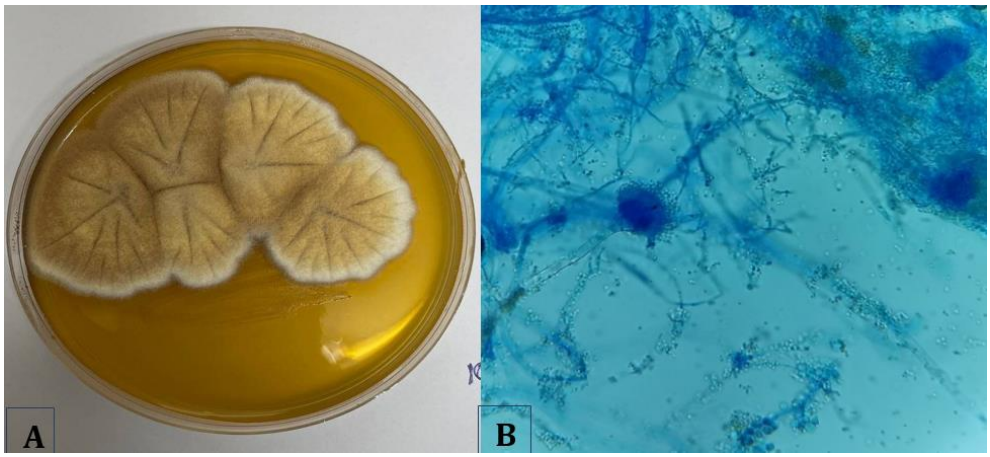

Fig 4A: *A. lentulus* colonies on SDA. B: Macroscopic appearance of *A. lentulus*

A. flavus

A. flavus is found worldwide and is commonly found Nevertheless, it is also a known human and animal disease. It lives as a saprophyte in soil and on many different forms of decomposing organic materials. In individuals with impaired immune systems, it may lead to otitis, keratitis, acute and chronic invasive sinusitis, as well as pulmonary and systemic infections. In terms of causing invasive aspergillosis in people, *A. flavus* is second only to *A. fumigatus* [35].

Description of Morphology

In culture, colonies are granular, flat, often radially striated, initially yellow but rapidly turning light green to dark yellow-green with age. Typically radial, the conidial heads separate to produce loose, bilinear columns that are 300-400 µm in diameter, but some heads contain phialids that float directly on the vesicle (unilinear).

The stalks of the conidiophores are hyaline, coarsely scaberulous, and often more prominent near the follicle. Conidia are spherical to hemispherical (3–6 µm in diameter), light green in color and distinctly echinaceous. Brownish sclerotia are produced by certain strains. Figure 5.

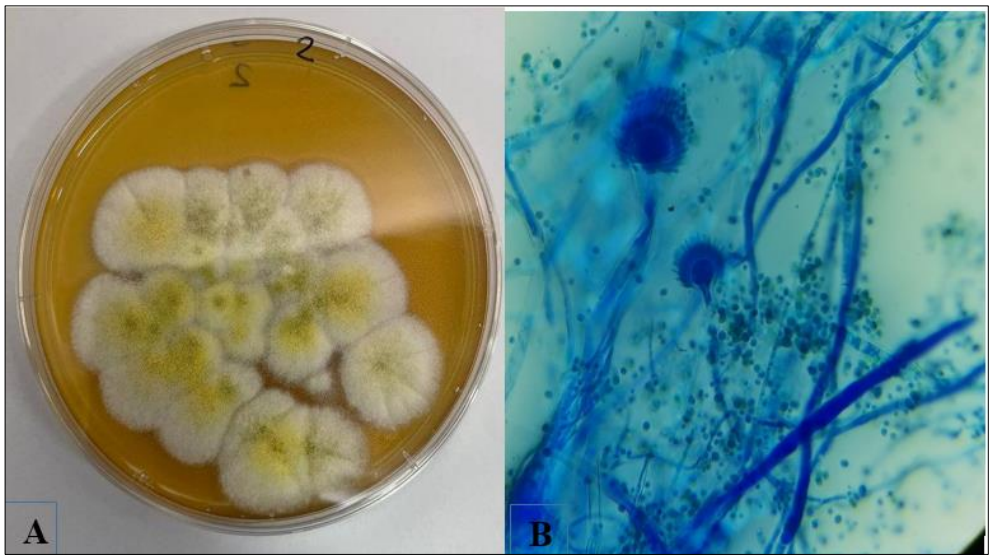

Fig 5A: *A. flavus* colonies on SDA B: Macroscopic appearance of *A. flavus*

C. albicans

C. albicans is a commensal of the digestive system and mucous membranes. Sources polluted with human or animal faeces, including as contaminated water, soil, air, and plants, were used to produce environmental isolates Figure 6.

Fig 6: *Candida* sp colonies on SDA

Culture
Colonies (SDA) are white to off-white, smooth, hairless, and yeasty.

Microscopy
Sprouting blastoconidia that are spherical or subspherical and measure 2-7 x 3-8 µm.

C. parapsilosis
Culture
Colonies (SDA) are white to off-white, smooth, glabrous and yeasty.

Microscopy
A few bigger oblong-shaped sprouting blastoconidia are spheroid to hemispherical, measuring 2-5 x 3-7 µm.

C. tropicalis
The main factor causing sepsis and widespread candidiasis is *C. tropicalis*. Additionally, it is a natural component of the mucosal flora of humans, and environmental isolates have been found in soil, prawns, kefir, and human excrement.

Culture
Colonies (SDA) have smooth, hairless skin that ranges in colour from white to off-white. Microscopy reveals the formation of 3.5-7 x 5.5-10 µm blastoconids, which are spherical or subspherical yeast-like cells.

C. krusei
Culture
Colonies (SDA) are smooth, glabrous, and white to off-white in colour.

Microscopy
Blastoconidia mostly small, elongated to oval, sprouting, 2.0-5.5 x 4.0-15.0 µm.

Fig 7: Differentiation of Candida isolates according to colony color on Chrom Candida Agar *C. albicans* (1), *C. parapsilosis* (2), *C. krusei* (3), *C. tropicalis* (4)

Infection with fungi that cause otomycosis according to sex and age group
The present study's findings revealed that females infected with otomycosis had a higher percentage (62.5%) than males (37.5%), as the highest percentage of infected females was found within the age group 31–40 years, at a rate of 25%. While no infection appeared in the age group 21–30 years, in males, The age range of 31 to 40 years had the greatest proportion, with a rate of 12.5%, while no infection appeared in the age group 6 months-10 years, which is shown in Table 2.
The results also showed that the age group 31–40 years was more susceptible to infection with fungi, at a rate of 37.5%, followed by the two age groups 11–20 and older than 50 years, at a rate of 18.75% for each age group. The least affected age groups were 6–10 years and 21–30 years; the infection rate in each group was 6.25%, as shown in Table 2.

Table 2: Percent of each age group affected by the fungi that cause otomycosis.

Age group	Sex		Total	Percentage %		
	Female	Male		Male	Female	Total
6 months-10 years	1	0	1	6.25	0.0	6.25
11-20 years	2	1	3	12.5	6.25	18.75
21-30 years	0	1	1	0.0	6.25	6.25
31-40 years	4	2	6	25	12.5	37.5
41-50 years	1	1	2	6.25	6.25	12.5
50 years >	2	1	3	12.5	6.25	18.75
Total	10	6	16	62.5	37.5	100 *%*

In the present study, otomycosis was more commonly diagnosed in female patients, which is in agreement with other reports, including those by [10, 36, 37]. This differs from many other studies, including those of [38-41].

This may be due to women's better self-care and their visits to ENT clinics. Also, headscarves and "hijab", which are commonly worn by women in Missan and lead to impaired air circulation and moisture accumulation in the ear canal, may be another factor attributed to the higher incidence of otomycosis in women [42]. In addition, they are exposed to hot, humid climates in households, cotton buds, and various oils (coconut oils) [43].

According to the results obtained by the study on the percentage of infection with fungi in different age groups, the highest percentage of infection in this study was found within the age group of 6 months to 9 years. These results are similar to the outcomes results of many other studies, including [44-45]. This differs from the results of other studies, including that by [14].

Conclusions

- *A. niger* was the main fungus in our study, closely followed by *C. krusei.*
- The incidence of otomycosis was higher in women than in men.
- The incidence was higher in middle-aged and young adults, and both extremes were in different age groups.
- Ear discharge, itching and pain in the ears were the main complaints in all cases.

Recommendations

In light of the conclusions of this study, we recommend the following:

- Conducting more studies on the incidence of otomycosis.
- Molecular identification of otomycosis and comparison with a GenBank
- Conducting awareness campaigns about otomycosis, urging people to maintain personal hygiene, not use antibiotics without consulting a doctor, and to visit hospitals when the infection appears.

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Conflict of Interest

The authors declare that there is no conflict of interest.

AuthorS' Contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Funding

None.

Data Availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics Statement

Permission to conduct this study was issued by the Health institutional; Al-Sadr teaching hospital and the Swabbing from patients was carried out by a public health technician.

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