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Isolation and identification of opportunistic fungi that causes for Otitis

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Abstract

The purpose of this study was to isolate and characterize Aspergillus niger. Isolated from patients with otitis media using various identification techniques, such as B. direct examination and laboratory culture. Used to observe yeast hyphae, large and small spores and chlamydospores . A total of 30 swabs were taken from patients with otitis throughout the months of June through November 2022 while they were receiving care at AL- Sadder Medical City (specialist ENT). The samples were taken as 30 ear swabs from otitis patients in AL-Najaf Governorate. The findings of this study indicate that Aspergillus niger and other opportunistic fungus caused more cases of otitis than Aspergillus sp.. The findings of the current study show that Aspergillus niger was more common in otitis patients than Candida albicans, with prevalence rates of 17 (77.27%) and 5 (22.72%), respectively. The results show the presence of 22 isolates belonging to the genera Aspergillus niger & Candida albicans, This phenomenon was observed in both female and male patients after culturing ear specimens on the primary separation medium Sabouraud dextrose agar (SDA) and secondary medium on additional potatokdextrose agar (PDA).

Keywords: *Aspergillus niger, opportunistic fungi, Candida albicans, Otitis.*

Introduction

Aspergillus is a large genus consisting of more thanh180 recognized species of anamorphic species (1). Aspergillus species remain significant cause of life-threatening infections in immunocompromised patients (2). Opportunistic fungi consist of 1. Saprophytic - from the environment and 2. Endogenous - a commensal organism. In immunocompromised pediatric cancer patients, fungi are a major source of morbidity and death. (3). The following factors (4) contribute to the continuing rise in the significance of fungi infections: (1) The fungi that cause the mycoses are widely dispersed in the environment and hence highly challenging to remove. (2) A fungal infection-related illness's clinical presentation might vary greatly. (3) It can be challenging to interpret the extremely varied clinical images that occur in people with colonization, infection, and/or illness, making the diagnosis of these disorders challenging. (4) There are currently no vaccinations available to protect against these illnesses. (5) Treatment is difficult since there are fewer antifungal options available than antibacterial ones. There are now extremely few and considerably more difficult to produce drugs available.

Otomycosis, also known as fungal otitis externa, is primarily characterized as a fungal infection of the external auditory canal with sporadic middle ear problems. The condition is difficult and irritating for both patients and otolaryngologists, even though it seldom

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poses a life-threatening hazard. This is because it frequently need ongoing therapy and monitoring . Despite this, recurrences are still a possibility. Otomycosis is one of the most common illnesses noticed in a general otolaryngology clinic, with prevalence estimates ranging from 9% (5) to 27.2% (6,7) among patients with otitis externa symptoms and up to 30% (4-6) in patients with ear discharge. It is widespeard throughout the world but is more common in the hot, dusty, and tropical and subtropical regions (8, 9, 10, 11). Otomycosis is a widespread medical issue in India, according to a review of the literature (12, 13).Infections caused by fungi can either be secondary to bacterial infections or the main culprit. Most patients with early otomycosis have intense itching, which frequently progresses to pain, hearing loss, and other symptoms. (13)(14).

Opportunistic fungal infections have grown in significance in human medicine recently, possibly as a result of the rise in patients with immune system impairments (15). Treatment of otomycosis in immunocompromised patients should be aggressive to avoid side effects such hearing loss and invasive temporal bone infection (16,17). Due to their method of action-typically fungistatic, with fungicidal impact being dosage dependent-lower tissue penetration, and the latent nature of the infection, antifungals are less effective than antibacterials (18). Botanist Christine Marie Berkhout identified the genus Candida, particularly Candida albicans, in her PhD dissertation at the University of Utrecht in 1923. The word Candida albicans also originates from Latin, where albicans means "to whiten" (19). These names reflect to the Candida species' typically white appearance when Candida albicans has traditionally been classified in the Deuteromycota (fungi imperfect) since the sexual phase of this fungus is unknown accurately (20,21, 22).Candidaoalbicans is the predominant cause of invasive fungal infections from yeast.(23, 24). In susceptible people, Candida albicans can cause surface as well as systemic infections. It is an opportunistic human pathogen with growing medical significance (25, 26, 27, 28, 29) , Aspergillus niger is an extremely common filamentous ascomycete fungus that has been linked to opportunistic human infection (29). The outer ear canal can get infected with Aspergillus niger, which can harm nearby skin (30, 31). The goal of the study is to isolate and identify opportunistic fungi from the ears of otitis patients using a traditional method (macroscopic and microscopic features). Aspergillus Niger is oneuof the most common causes of otomycosis (fungal ear infections),which can hurt, temporarily impair hearing, and, in severe cases, damage to the ear canal and tympanic membrane(32).

Materials and Methods

Equipment’s and apparatus

Table (1) contains a list of the tools and apparatus utilized during the study.

Table (1): The equipment and apparatus utilized in this study

Equipment & Apparatusesl	**Manufacture's countryn**
Autoclave	Harayma (Japan)
Bunsen burner	Iraqi
Cameram	Canon –Japan
Conical flasks	BBL- USA
Disposable Petri dishes	BBL- USA
Gloves TG medical	(Malaysia)
Hood Incubator	Lab TECH Memmert , Germany
Light microscope	Olympus (Japan)

Refrigerator	Concord , Italy
Sensitive balance	Sartorius (Germany)
Slides & cover slides	BBL- USA
Sterile cotton swabs	Himedia(India)
Sterile syringes	Iraqi
Water distiller	GFL(Germany)

Chemical and Biological Material

The chemical and biological materials that used throughout the study were summarized in table (2):

Table (2): The Biological and Chemical Materials were employed in the current study

Materials	**Manufacture's country**
Chloramphenicol Normal saline	Iraq Haidylena (Egypt)
Lacto phenol cotton blue	Himedia – India

Culture media

The following culture media in the table (3) were employed for proceeding of experiments and tests in this study:-

Table (3): The Culture media were used in the present study

Potato Dextrose Agar (PDA)	Himedia – India
Sabouraud`s dextrose Agar (SDA)	Himedia – India

Methods

Patients

Within June and November 2022, a total of 22 specimens were obtained from 300samples of randomly selected otitis patients who had a history of aspergillosis and were between the ages of 5 and 65.All samples were gathered in the AL-Sadder Medical City (specialist ENT) in the governorate of AL-Najaf. Using a private data, all patient information was captured.

Collection of specimens

The samples were extracted from the middle ear using sterile cotton swabs. For observable development of Aspergillus niger & Candida albicans colonies, they were subcultured on Potato dextrose agar (PDA) and incubated at225C for 1-57days, other Aspergillus were eliminated as a negative.

Preparation ofmculture8media

Every culture medium was made in accordance with the manufacturer's instructions, which were printed on the container. They were autoclaved at 15 psi/inch2 in 1218C for815 minutes to sanitize them.

Sabouraud’skdextrose agar (SDA) with chloramphenicol

This medium agartwas made as directed by the manufacturer by dissolving 658 gm of SDA powderdin51000 mlyof distilled water, adjusting the pH to 6.87, and adding

2509mg/L of the antibiotic chloramphenicol before autoclaving (48). Chloramphenicol, a broad-spectrum antibiotic inhibitory to a wide variety of Gram-negative and Gram-positive bacteria, was utilized in this medium for the cultivation of commensal and pathogenic yeasts and fungi.

Potato dextrose agar (PDA) with chloramphenicol

According to the manufacturer's instructions, potato dextrose agar was made by suspending 397gm of PDA powder in91000 ml of distilled water, adjusting the pH to 6.88, and adding7250 mg/L of the antibiotic chloramphenicol before being autoclaved(48).

Preparation of Stains and Solution

cotton blue

This stain, which is used to color hyphae and conidia, was kept in a clean, dark container. (49).

Identification of Aspergillus niger isolates

Based on their morphological characteristics on culture media, Aspergillus niger isolates were recognized as the following:

Colonial morphology

The plates were incubated at 25Ckfor 1 to 5 days while the isolates were cultured on Sabouraudodextrose agar. to separate out pure Aspergillus niger colonies and analyze their consistency, size, shape, and color. High color dependency on Aspergillus.

Microscopic Examination

A sample of colonies was taken out of the culture, put on a plate, and stained with lactopphenol cotton bluet for examination under a light microscope (50). Before moving on to the examination by microscope, and Aspergillus niger were streaked on the Sabouraud dextrose agaro(SDA)rplate and cultured atl250C for 59days. Results and Discussion

Patients

The current study included collection 22specimen's infection fromt30 samplestwere collected from (ENT) Consulting. From in AL- Sadder Medical City.In AL-Najaf Governorate. The specimens were included: - Aspergillus niger which was 17 (77.27%) and 5 (22.72%) respectively. Were collected from females &males . as shows in table (4).

Patients with otitis frequently develop invasive middle ears (1)(33). Patients with otitis often have fungal infections, primarily Aspergillus, and are typically profoundly and persistently neutropenic or receiving long-term steroid treatment. (34). In immunocompromised individuals, Aspergillus species continue to be a significant source of infections that can be fatal(2).In immunocompromised pediatric cancer patients, fungi are a major source of morbidity and mortality(3).

Furthermore, wide-spectrum antibiotic usage, corticosteroid and cytostatic therapy, and invasive surgical procedures frequently put youngsters at risk for developing fungus infections..(35).

In human medicine, opportunistic fungal infections are becoming more significant due to the potentially enormous number of immunocompromised patients(36). Treatment of otomycosis in immunocompromised individuals must be aggressive in order to reduce consequences including hearing loss and tympanic membrane perforations(4).An immunosuppressed host becomes more vulnerable to fungus infections as a result. One of the host'skdefense mechanisms against fungus infections is the normal bacterial flora.

Patients who take antibiotic ear drops have changed this process, which results in otomycosis (5). Pathogenesis may also be influenced by ear cleaning practices. (37).

Aspergillus is the most often reported isolate of otomycosis worldwide and is regarded as a main colonizer of the ear canal (7).

About 1 in 8 otitis externa infections are fungal in origin. Approximately 90% of these are caused by Aspergillus spp. (38).

Ingimmunocompromised individuals, Aspergillusmspecies frequently causelinvasive fungal infections.tThey are also linked to allergic bronchopulmonary illnesses, mycoticlkeratitis, otomycosis, and nasal sinusitiso(14)

Table (4) type of opportunistic fungi and percentage of specimens of otitis patients.

Type of opportunistic fungi		**No .positive**
Aspergillus niger	***Candida albicans***	
17 (72.27%)	**5 (27.72%)**	**22(100%)**

Collection and identification of opportunistic fungi isolation

Only 22% of the total 30 specimens under examination showed growth of Aspergillus niger and Candida albicans isolates and were thus declared positive specimens utilized in the phenotypic diagnosis. They were, in brief, the following: Aspergillus was isolated and screened from 22fsamples obtained from cases of diseases, and it was identified by morphological characteristics including color of the colony and growth pattern on culture media. 18 (60%) samples of Aspergillus niger and 12 (40%) samples of Candida albicans were isolates from middle ear. Using a light microscope, certain microscopic properties were studied.

The two primary species that are usually identified from immunocompromised individuals are Aspergillus and Candida spp. (39, 40, and 41). According to the table. (5)

The distinctive conidiophore of Aspergillus makes it easy to identify the genus, however identifying and differentiating species is difficult since it is usually done using a variety of physical characteristics. Conidial and mycelial color, colony diameter, colony reverse color, and exudate production are macromorphological parameters that are taken into account.

Characterizing micromorphology mostly depends on seriation, vesicle size and form, conidia, and stipe morphology(42,43)

Table (5) Distribution of opportunistic fungi isolates according to type.

Nature of infection	***Aspergillus niger***		***Candida albicans***		**Total**
	Positive	**negative**	**positive**	**negative**	
Ear	**12**	**6**	**8**	**4**	**30**
Total	**18**		**12**		**30**

Identification of Aspergillus Niger isolates: - including

Morphological and culturing features

Microscopy and cultural techniques continue to be widely used and crucial instruments for the identification of Aspergillus and Candida. All obtained swabs were grown on SDA. Aspergillus niger colonies are found in:

1- On Saboraud-Dextrose Agar, the mycelia quickly expanded from a white to yellow felt-like mat.

2- Conida rapidly become black as they mature and produce the aspergillin pigment. Reverse keeps its light tones of white. As shown in Figure (1).

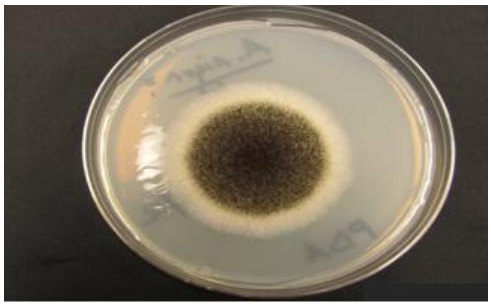

Figure (1) the colony of Aspergillus niger growth on (SDA) (at525˚C for879days).

Macroscopic Features

1. Aspergillus niger produces colonies made of white or yellow felt coated in black spores generated asexually.

2.Transparent, septally split mycelial or threadlike hyphae. Conidiophores (asexually generated fungal spores) of A. niger typically have globose (globular) vesicles that are 40–60 m in diameter and range in length from 900–1600 m. Biseriate phialides, whichfare protrusions from the conidiophore of A. niger, completely cover each globose vesicle.

3.The brown metulae, where conidiogenous cells are produced, are where these phialides emerge. The phialides produce globose mitospores with a diameter ranging from 3 to 5 m by a process known as blastic basipetal conidiogenesis (44).

Microscopic Features

Conidia size, shape, and roughness, stipes, vesicles form and seriation, conidia size, shape, and length, and phialides length and width of Aspergillus were microscopic criteria for identification. (45). As shown in Figure (2).

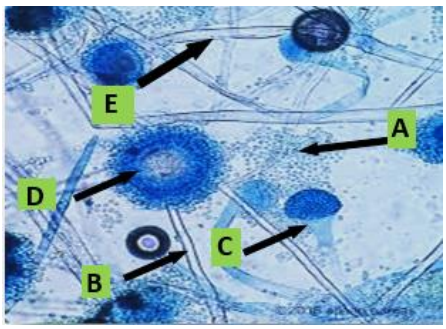

Figure (2) Micrographs showing light microscopic structure´s of Aspergillus niger A-conidia B- conidiophore C- vesicle D- phialides E-hypha and all stained with lactophenol (940x).

Morphological and culturing features of Candida albicans

The colonies of Candida spp. are cream to yellowish in color, develop quickly to maturity in three days, and vary in texture according on the species. All obtained swabs were cultured on SDA. These results were agreed with (46)(47). As shown in Figure (3).

Figure (3) the colony of Candida albicans growth on (SDA) (at725˚C for 85 days).

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