
Isolation and Identification of Fungi Associated with Otomycosis.

Ammar H Khammas.
FICMS

Auroba K Abbas
PhD

Salma Ilabi
MSc

Amer R Al-Najjar.
PhD

Abstract:

Objective and Background: Laboratory diagnosis of predisposing factors of otomycosis which represent a significant percentage of clinical external otitis and is usually caused by *Candida*, *Aspergillus*; *penicillium*, clinical symptoms such as otorrhea, erythematic and stenosis of the external auditory canal.

Patients: Seventy-five patients were included in this study suffering from symptoms indicating otitis externa in Al-Yermouk teaching hospital, E.N.T. department during the period from November 2007 to April 2008.

Methods: The specimens were taken by sterile cotton swabs from bony portion of the external ear. All specimens were cultured on sabouraud Dextrose Agar with chloramphenical (0.05mg/ml). Mycological Cultures were incubated at (25-27) °C for 2-3 weeks and examined microscopically every 3 days.

Results: In this study 75 patients (44 Female and 31 male) with symptomatic otomycosis were confirmed by direct microscopy and cultures. Results revealed that (45) 60% of patients (38 female and 7 male) showed association of fungi and *Candida albicans*. Female susceptibility to fungal infection was more than male 82.23%. The most common fungal pathogens isolated from ear were *Aspergillus niger* (44.44%) followed by *Candida albicans* (33.33%), and *penicillium* spp. (22.22%).

Conclusion: In clinical finding of otitis externa, mycological examination could be very important in setting accurate diagnosis and appropriate therapy can be decided. In this study we found that otomycosis is common in female than male and *A. niger* is the major etiologic agent in ear infection.

Keywords: Otomycosis, *Aspergillus*, *Candida albicans*, *penicillium*.

Introduction:

Otomycosis or external otitis fungi are produced by yeast and filamentous fungi^[1]. Otomycosis is a superficial mycotic infection of the outer ear canal. The infection may be either sub-acute or acute and is characterized by inflammation, pruritus, scaling and severe inflammation, superficial epithelial masses of debris containing hyphae, suppuration, and pain^[2].

The most characteristic finding on ear examination is the presence of grayish white thick debris called as (wet blotting paper)^[3].

Fungal external otitis (otomycosis) is a common pathology throughout the world. Its frequency varies according to different geographic zones, in relation to environmental factors (temperature, relative humidity) and the time of year^[4]. Otomycosis has a worldwide distribution with a higher prevalence in the hot, humid and dusty climate of the tropical and subtropical region^[5].

In the last few years the participation of fungi in external otitis has increased, mainly because of the use of broad spectrum antibiotics for treatment of bacterial otitis and to factors relating changes in immunity. *Aspergillus* spp. and *candida* spp were the most common fungal pathogen causing otomycosis^[6]. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*,

penicillium spp, *Candida albicans*, *Candida parapsilosis* and *Rizopus* spp also has been reported as the most common fungal pathogen in otomycosis^[7]. In this study, fungal causative agents & predisposing factors were investigated.

Patients & Methods:

Seventy-five patients were included in this study, clinically diagnosed as otitis externa in Al-Yermouk teaching hospital, department of E.N.T. during the period extending between Novembers 2007 and April 2008. Patients were 44 females (58.67%) and 31 males (41.34%). The ages of the patients ranged between 1-75 years. Secretion, discharge and pus were collected from the infected ear using two sterile cotton wool swabs. One swab was used for direct microscopy and the other for culture examination. Direct examination of the smear with methylene blue and Gram techniques was done. Otomycosis was confirmed by the presence of aseptate mycelium, septate mycelium, *Aspergillus* Conidia, fruiting bodies, yeast and pseudohyphae in culture. The presence of fungal elements in stained smears was re-confirmed by fungal culture and fungal colonies. Any kind of clinical materials, especially liquid samples (swabs, pus) was examined as quickly as possible. Swabs were rolled and inoculated over the surface of Sabouraud's Dextrose Agar with chloramphenical (0.05 mg/ml). Cultures were

incubated at (25-27°C for molds and 37°C for yeast) for 2-3 weeks, aerobically and examined every 3 days. Fungal isolates were identified on the basis of colonial morphology and slide cultures. Yeast colonies, also detected by germ tube test, production of chlamydo-spore on corn meal agar, fermentation reaction tests, and carbon-nitrate assimilation tests. [8, 9].

Results:

Of the seventy-five ear swabs obtained from patients clinically diagnosed as cases of otitis

externa who were included in this study, results revealed that 60% of patients showed positive culture for fungal elements. The age range was (1-75) years old, 44 patients were female and 31 male (Table -1).

The incidence of otomycosis according to sex was found to be 37 females and 8 males. The percentage of female infection approached 82.22% while in male it was 17.78 (Table- 2

Table1: Distribution & % of fungal positive isolates results in culture of patients with ear infection.

Age group (years)	No. of patients	sex No.	Number and percentage of fungal isolates in positive fungal culture
1-15	16	M 6	2
		F 10	6
16-30	24	M 7	3
		F 17	16
31-45	16	M 6	2
		F 10	8
46-60	14	M 9	1
		F 5	5
61-75	5	M 3	0
		F 2	2
Total %	75 100%	M 31(41.34%) F 44(58.67%)	45(60%)

Table 2:-Pathogenic fungal isolates associated with otomycosis

Fungal species	sex		Total	%
	M	F		
Aspergillus niger	4	16	20	44.44%
Candida albicans	0	5	15	33.33%
Penicillium spp	4	6	10	22.22%
Total No	8	37	45	100%
%	17.78%	82.22%	100%	

Table 3: Isolation and identification of fungi and monilia from different age.

Age group years	Sex No.	Fungal species		
		Aspergillus niger	(C.albicans)	Penicillium spp.
1-15	M 2	1	0	1
	F 6	2	4	0
16-30	M 3	2	0	1
	F 16	8	5	3
31-45	M 2	1	0	1
	F 8	3	3	2
64-60	M 1	0	0	1
	F 6	3	2	0
61-75	M 0	0	0	0
	F 2	0	1	1
Total No.	45	20	15	10

The present work demonstrates the isolation of the etiologic agent of fungal ear infection according to the available diagnostic methods, like direct examination of wet preparation or Grams techniques, culturing the fungus, examination of spores, hyphal structures, color of the colonies and the development of fungus, fruiting structures.

The most common fungal pathogens were *Aspergillus niger* (20 cases), which represented (44.44%) followed by *Candida albicans* (15 cases), which represented (33.33%) and *penicillium* spp. (10 cases), which represented (22.22%) (Table- 2,3).

Discussion:

Otomycosis is an entity frequently encountered by otolaryngologists can usually be diagnosed by clinical examination. However, the correct diagnosis requires microscopic examination and culture to specify the type of fungi^[10].

Otomycosis occurs more commonly in females than males and our finding confirmed the results of other researchers which had been reported^[7,11,12]. Out of 75 patient, 44 (58.66%) were females and 31 (41.33%) males. This may be due to the environmental factors (temperature, humidity), heavy clothing of female and hormonal changes that precipitate flaring up of the infection as seen during menstruation or pregnancy^[13]. Some workers have reported males and females were almost equally effected, a result which disagrees with our findings, further more demonstrated that environmental conditions, personal hygiene, occupational predisposition were few of the factors contributing to a large extent to the development of otomycosis in patients. More number of clinical cases is detected during the rainy season as compared to the summer months due to the moist and humid available conditions facilitating good fungal growth^[13].

Infectious mold agents are present in environment including: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* spp, *Rhizopus*, *Mucor*.^[14]

In the present study the fungal species *Aspergillus niger*, *Candida albicans* as the chief fungal species isolated.

This conclusion matches the results of other researchers^[7,11,13,15,16] and differs some what to those obtained by another study^[17] who reported the genus *Aspergillus* and *Candida* are equal in quantities. Other fungi that have

been associated with otomycosis are *penicillium* in the current study. Some studies have reported other organisms as causative isolates as *Acremonium* sp., *Fusarium* sp. other known species of *Candida* such as *C. parasilosis* and *C. guilliermondi* with varying percentage of isolates^[16, 18].

We conclude that otomycosis is a secondary infection of the ear and predisposing factors are responsible for the invasion of fungi. Secondary bacterial infection was one of the history of our patients followed previous antibiotic therapy for one to four months duration, use of oils, eardrops, steroids, swimming (wetness predisposes to fungal infection).

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*Department of E.N.T. Al-Yermouk Teaching Hospital.
Department of Microbiology, College of Medicine.
Al-Mustansiriya University.*