

## Isolation of Fungi from Otitis Media patients in Erbil city

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### Abstract:

**Background:** Although otitis media (OM) incidence estimates from around world widely, it is clear that OM is a very common childhood disease. It is especially prevalent in children younger than 2 years of age. Furthermore, the earlier the first episode of OM, the greater the risk of subsequent recurrent OM and chronic otitis media with effusion. In addition, a number of other host, agent, and environmental factors have been associated with increased risk of otitis media. Environmental factors that favor the transmission of upper respiratory pathogens increase the risk of OM, recurrent OM, and chronic OM with effusion. Several factors suggest a genetic role in OM susceptibility, which needs further exploration.

**Objectives:** Isolation and identification of fungi from patients with chronic inflammation of the otitis media.

**Patients and Methods:** A total of 50 samples were collected from patients with a clinical suspicion of Otomycosis, which was based on different clinical symptoms like pruritus, chronic otorrhea, Otagia, impaired hearing, and on otoscopic findings like appearance of fungal mass or debris and blackish foul smelling discharge .

It was a retrospective study conducted at Outpatient Department of ENT (Ear, Nose and Throat) of Erbil Teaching Hospital & Rezgary Hospital in Erbil city.

**Results:** From 50 samples 23 (46%) were positive for fungus by using KOH exam & culture. Among positive patients, 13 (56.52%) were female and the rest 10 (43.48%) were male, candida and Aspergillus species were isolated from samples.

Positive cases from females were more than positive male cases of patients and age range was from 20 yrs to 65 yrs. The highest number of positive cases were following in age group of 51-60 years old (47.82%), followed by 21-30 years age group (30.43%).

**Key words:** Fungi, Otomycosis, ENT, otitis media patients.

### 1. Introduction:

Otomycosis is a fungal infection of the external auditory canal and its associated complications sometimes involving the middle ear(1,16). It occurs because the protective lipid/acid balance of the ear is lost(2,22) and fungal infection caused 10% of all cases of otitis externa(2,17). In recent years, opportunistic fungal infections have gained greater importance in human medicine, perhaps because of the increasing number of immunocompromised patients(3,36). However, such fungi may also produce infection in immunocompetent hosts(1,46). In immunocompromised patients, treatment of otomycosis

should be vigorous to prevent complications such as hearing loss and invasive temporal bone infection(4,5). Its prevalence is greatest in hot humid and dusty areas of the tropics and subtropics(31,35). Andrall and Gaverret were the first to describe fungal infections of the ear; although a wide spectrum of fungi are involved, *Aspergillus* and *Candida* are the most common species encountered(32,42,45). In 1960's studies by Graney and by Lakshmipathi and Murthy revealed that all cases observed by them had been caused by either *Aspergillus* or *Candida* species(23,52). Fungi are abundant in soil or sand that contains decomposing vegetable matter. This material is desiccated rapidly in tropical sun and blown in the wind as small dust particles (20,56). The airborne fungal spores are carried by water vapours, a fact that correlates the higher rates of infection with the monsoon, during which the relative humidity rises to 80%(28,43).

A fungal mass does not protrude from the EAC (External Auditory Canal), even in most chronic cases. This is because the fungus does not find its nutritional requirements outside the EAC. Patients with diabetes, lymphoma, or AIDS and patients undergoing or receiving chemotherapy or radiation therapy are at increased risk for potential complications for otomycosis (3,24). Pathologically, fungal infection of the EAC and TM (Tympanic Membrane) lead to small intradermal abscess. Hemorrhagic granulations can cause thrombosis of adjacent blood vessels leading to a vascular necrosis and perforation of TM (4,19). Otomycosis presents with nonspecific symptoms like pruritus, discomfort and pain in the ear, aural fullness, tinnitus, hearing impairment, and sometimes discharge, and also recurrence is common (7, 8, and 9). Predisposing factors for otomycosis include habitual instrumentation, dermatitis, unhygienic habits, immunocompromised individuals, pre-existing ear disease etc. (10, 11, 12). Several studies have revealed that there is an increase in the prevalence of otomycosis in the recent years that have been linked to the extensive use of antibiotic eardrops, (9, 10,57) widespread use of steroids, broad-spectrum antibiotics, and chemotherapeutic agents(18,54). There is substantial variation with respect to clinical features, presentation, and treatment outcome seen among immunocompetent and immunocompromised individuals (49,62).

Otomycosis may be refractory to the treatment prescribed hence challenges the clinician to determine whether it is an isolated entity or related to any other systemic disorder or the result of an underlying immunodeficiency disorder (26,40,66). Accurate diagnosis of otomycosis requires a high index of suspicion. The diagnosis is clinical and accompanied by microbiological confirmation. Blotting paper appearance of matted mycelia can be seen on otoscopic examination while characteristic appearance of fruiting bodies or conidiophores can be seen on microscopy. But mere microscopic evidence can become an evidence of negativity for fungal presence and should be accompanied by culture(21,59,61). Mainstay of treatment involves removal of debris, thorough toileting of the external ear and the use of antimycotic agents such as clotrimazole. Clotrimazole is a broad-spectrum antifungal agent and effectively controls fungal isolates attributed to otomycosis

(*Aspergillus* and *Candida*) (13, 14, 15). It is worth stressing that there are no reports on the toxicity of antifungal drugs in literature(53,55). Other components of treatment include keeping the ear dry and aiming to restore the physiology by avoiding maneuvers in the EAC (1,25,39). In this study, we describe the commonest mode of presentation, predisposing factors, the spectrum of fungi, sex distribution, complications, and treatment outcome of otomycosis both in immunocompetent and immunocompromised individuals.

## 1- Patients and Methods

It was a retrospective study conducted at Outpatient Department of ENT (Ear, Nose and Throat) of Erbil teaching hospital & Rezgary Hospital in Erbil city, duration between October 2016 and March 2017. A total of 50 samples were collected from patients with a clinical suspicion of Otomycosis, which was based on different clinical symptoms like pruritus, chronic otorrhea, Otagia, impaired hearing, and on otoscopic findings like appearance of fungal mass or debris, blackish foul smelling discharge. Patients on anti-fungal drugs were excluded from the study. Samples were collected using sterile cotton swabs followed by detail clinical history & clinical examination. All received samples were transported to Mycology laboratory immediately, with maintenance of sterile condition, to know the presenting form of fungi responsible for infection in received sample. For fungus isolation all collected samples were processed for direct KOH preparation each sample was inoculated on Sabouraud's Dextrose agar(SDA), inoculated media were incubated at 25 C and 37 C for four weeks. For isolation of fungi, growth on media was confirmed by Lacto Phenol Cotton Blue wet preparation (LPCB stain) (75,76)

### 2-1 LABORATORY DIAGNOSIS:

#### 2-1-1 Direct Microscopic Examination

- Specimens for examinations can be skin scrapings, Cotton swabs, from external and internal sites of ear.
- The specimen is treated directly with 1-2 drops of 10-20% KOH.
- The presence of the budding yeast cells and filamentous fungi are considered as the positive results(5,6).
- Most isolates of *Candida albicans* produce a pseudohyphae growth consist from Blastospores when they are suspended in serum at 37°C for 2-3 hours.

#### 2-1-2 Culture media:-

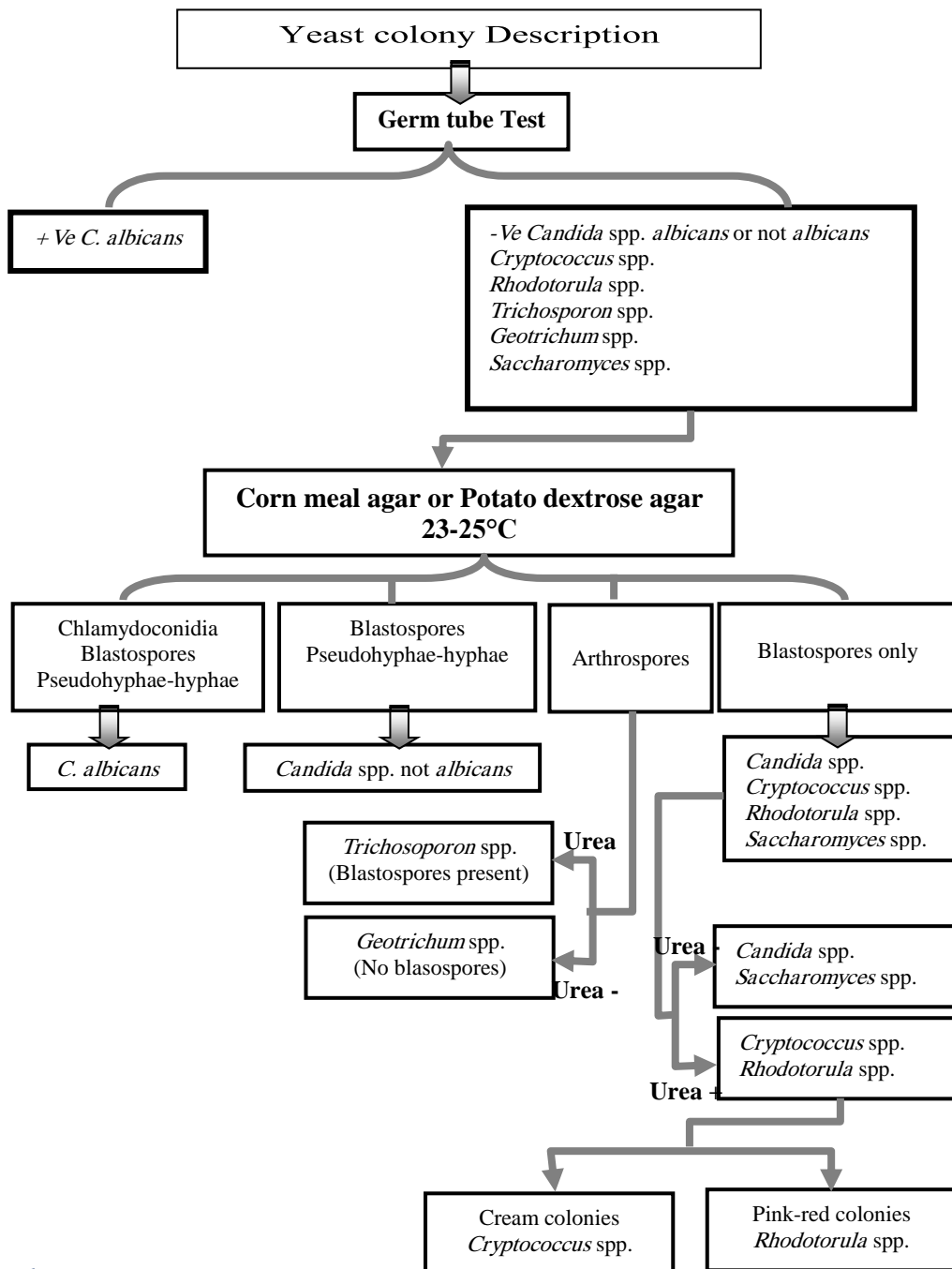
- SDA (Sabouraud Dextrose Agar) at either room temperature or at 37°C

### 2-2 Germ Tube Test

The germ tube test provides a simple, reliable and economical procedure for the presumptive identification of *Candida albicans*.(63,64,65) About 95% of the clinical isolates produce germ tubes when incubated in serum at 37C for 2.5-3 hours. A germ tube represents the initiation of a hypha directly from

the yeast cell. They have parallel walls at their point of origin. Germ tube formation is influenced by the medium, inoculum size and temperature of incubation.

Figure 1 ---Yeast Identification----- (58,60)

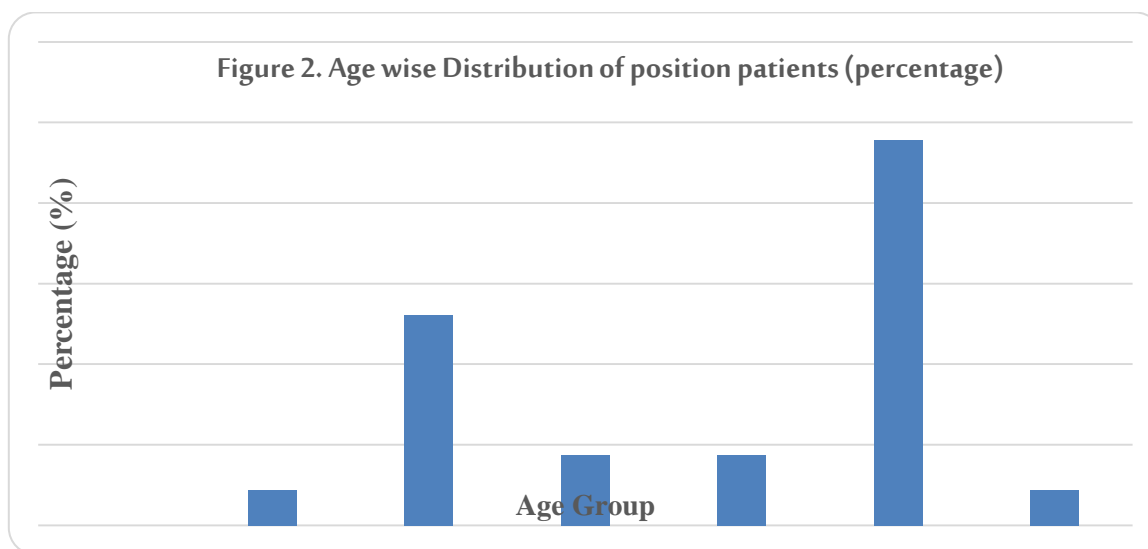


## 2. Results

On the basis of clinical suspicion a total of 50 patients were included in the study. From the 50 samples 23 (46%) were positive for fungus on KOH preparation & culture. Among the positive patients, 13 (56.52%) were female and rest 10 (43.48%) were male. The Age of patients were ranging from 20 yrs to 65 yrs and the highest number of positive cases were in the age group 51-60 yrs old (47.82%), followed by 21-30 yrs age group (30.43%). Age wise distribution of positive cases has been depicted in Table-1 and Fig 2.

Age group	Number of patients(n=23)	Percentage (%)
0-10	0	0
11-20	1	4.34
21-30	6	26.09
31-40	2	8.70
41-50	2	8.70
51-60	11	47.83
61-70	1	4.34

Table-1: Age wise Distribution of positive patients

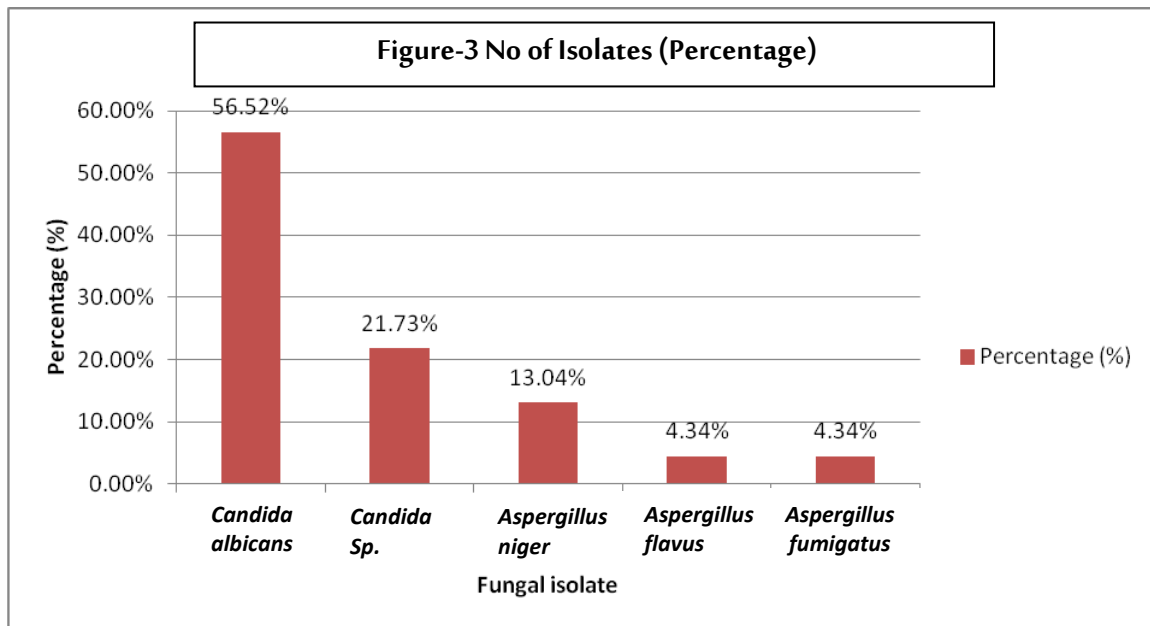


Clinical examination which showed various clinical signs like blackish discharge or sometimes whitish with tympanic perforation, congestion of canal, white cotton like or dry fungus debris in canal suggesting of Otitis Mycotic to which clinician rely on making probable clinical diagnosis; 46% samples were positive for fungus, no growth was seen in 54%. Table 2 and Fig 3 showed that *Aspergillus* & *Candida* sp. were the mainly isolated Fungus.

(Table-2)

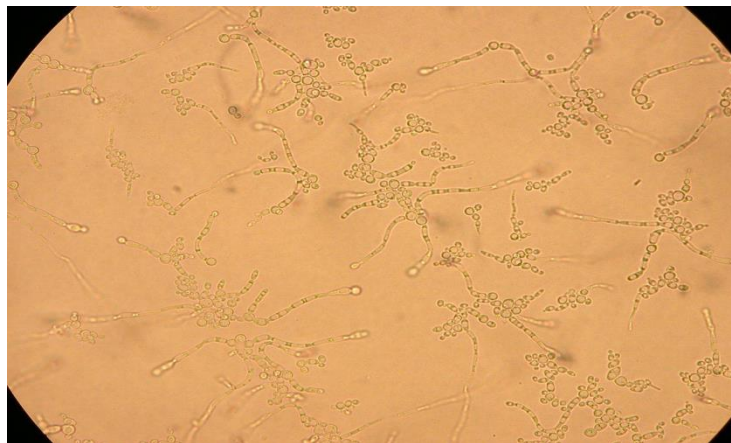
Fungal isolate	Number of isolates(n=23)	Percentage (%)
<i>Candida albicans</i>	13	56.52
<i>Candida sp.</i>	5	21.73
<i>Aspergillus niger</i>	3	13.04
<i>Aspergillus flavus</i>	1	4.34
<i>Aspergillus fumigates</i>	1	4.34

Table-2: Fungal Isolates



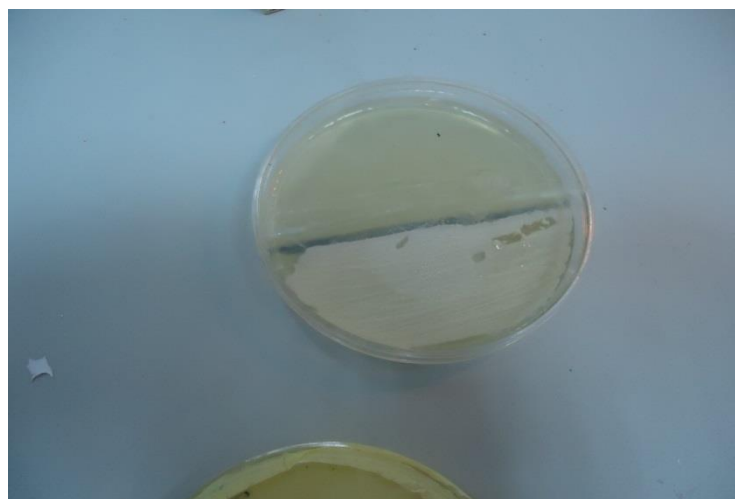
**The Fungi isolated images :-**

**1. *Candida albicans* detected by Germ tube test**



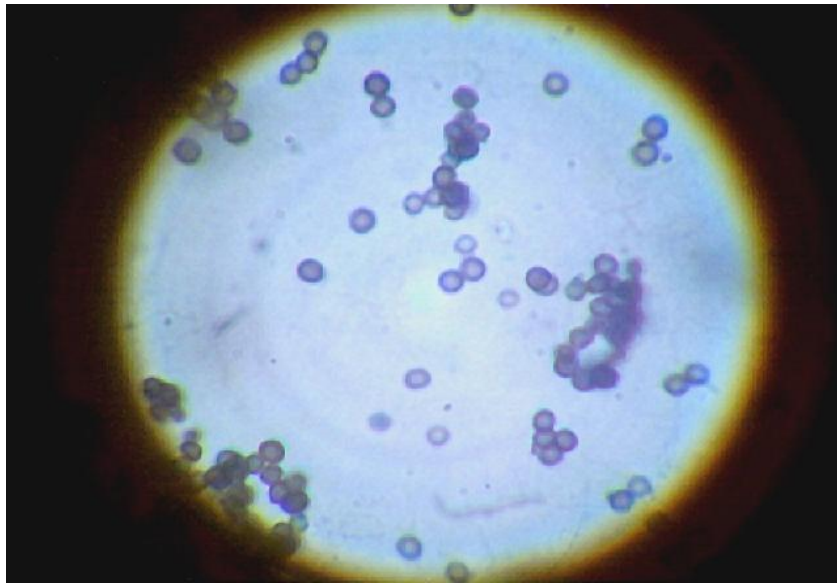
(3-1)

**2. *Candida sp.* detected by Germ tube test**



(3-2)

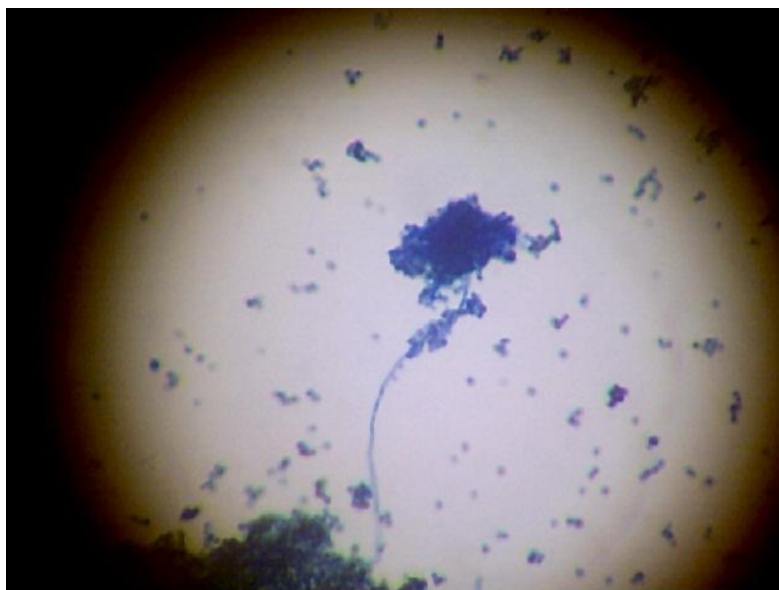
### 3. *Aspergillus niger*



(3-3)

Identification method for this isolate was performed based on macroscopic and microscopic examinations where it appeared hyphae. Hyphae are septate and hyaline and conidial heads are initially radiate, however, splitting into columns in age; *Aspergillus nigeris* biserial where in its vesicles form sterile cells called as metulae that support the conidiogenous phialides; conidiophores are hyaline, smooth-walled, with length ranging from 400 – 3,000  $\mu\text{m}$  long, and are becoming darker at the apex and terminating in a globose vesicle with size of 30 – 75  $\mu\text{m}$  in diameter; and metulae and phialides cover the entire surface of the vesicle; Conidia are globose, brown - to black - colored, very rough, and with size of 4 – 5  $\mu\text{m}$  in diameter.

### 4. *Aspergillus flavus*



(3-4)

Identification method for this isolate was performed based on macroscopic and microscopic examinations where it appeared conidial heads are mostly radiate with conidial masses splitting into blocky columns with age ;

conidiophores are with roughened walls especially near the vesicle, with size of 800  $\mu\text{m}$  long x 15 – 20  $\mu\text{m}$  wide; hyphae and septate and hyaline; vesicles are globose to sub – globose with size ranging from 20 – 45  $\mu\text{m}$ ; metulea cover the entire vesicle in biseriata species and with size ranging from 8 – 10 x 5 – 7  $\mu\text{m}$ . Some isolates may be uniseriate producing only phialides covering the vesicle, and with size ranging from 8 - 12 x 3 – 4  $\mu\text{m}$ ; and

canidia are round, globose to sub–globose with smooth to finely roughened walls, appeared in chain and with diameter size of 3 - 6  $\mu\text{m}$ .

### 5. *Aspergillus fumigatus*



(3-5)

Identification method for this isolate was performed based on macroscopic and microscopic examinations where it appeared conidial heads are in the form of compact columns in an undisturbed culture;

conidiophores are smooth – walled, often tinted greenish, up to 300  $\mu\text{m}$  long, and terminate in a dome – shaped vesicle with a diameter of 20 – 30  $\mu\text{m}$  long. Hyphae and septate and hyaline; The species is uniseriate producing a closely compacted phialides with size ranging from 5 - 10 x 2 – 3  $\mu\text{m}$ , and only occurring on the upper portion of the vesicle; and conidia are round to sub – globose, smooth to finely roughened, and with diameter size of 2 – 3.5  $\mu\text{m}$



### 3. Discussion

Otomycosis is described as superficial mycotic infection of the EAC with infrequent complications involving the middle ear(6,30). As in our study this infection can be acute or sub-acute, and is characterized by itching, earache, blocking sensation and discomfort(21,29). and the fungal infection results in inflammation, exfoliation, and accumulation of debris mass containing fungal elements, suppuration, and pain. This infection is worldwide in distribution, but it is more common in tropical and subtropical regions. (50,65) Otomycosis is sporadic and caused by a wide variety of fungi, most of which are saprobes occurring in diverse types of environmental material(67,68) like to our study in the isolation of *Aspergillus fumigates*, *Aspergillus flavus* and *Aspergillus niger*.

In our study an analysis of the age group revealed that otomycosis can affect any age from one year through 70 years. But the incidence was found to be low below 20 years and above 65 years. In our study the incidence was high in the age-group 20-30 and 50-60 years. The higher incidence in these patients may be due to the fact that these people are more exposed to the mycelia due to occupational exposure, travelling etc., where the older and younger age groups are not as exposed to these pathogens. The highest incidence was among the age group 40-50 years which may considered as immunocompromised patients and easily exposed to fungal infection; however, younger age group are less susceptible due active immune system. Overview of literature revealed that otomycosis is more common in females than in males.

In the present study, the *Aspergillus* growth rate was found to be higher at the temperature of 37 C, a fact that is clinically supported by the predilection of fungi to grow in the inner one-third of the EAC (External Auditory Canal).

Like to our study the common symptoms of otomycosis are itching and ear discharge followed by ear pain, blocking sensation, decreased hearing, and tinnitus. Itching and ear discharge were seen more in the immunocompetent patients than in the immunocompromised patients, while ear pain was present more in the immunocompromised patients(27,33). Our study revealed also ablocked sensation, decreased hearing, and tinnitus were seen more in the immunocompromised group. Overview of literature showed that among the fungus isolates, *Candida* and *Aspergillus niger* and were the most common species causing otomycosis worldwide(34,37). This is consistent with our study because in this study *Candida albicans* was isolated in 13 patients (56.52%), followed by *Candida sp.* in 5 patients (21.73%), *Aspergillus niger* in 3 patients(13.04%) and one-one patient with *Aspergillus fumigates* & *Aspergillus flavus* individually

Tympanic membrane perforation may occur as a complication of otomycosis that starts in an ear with an intact eardrum(41,48), the incidence of tympanic perforation in our otomycosis patients was found to be 11%. may be because perforations were more common with otomycosis caused by *Candida albicans*. Most of the perforations were behind the handle of malleus because of perforation was

attributed to mycotic thrombosis of the tympanic membrane blood vessels, resulting in avascular necrosis of the tympanic membrane this is exactly what happened with six patients in our immunocompromised group experienced tympanic membrane perforation. The perforations were small and situated in the posterior quadrant of the tympanic membrane. They healed spontaneously with medical treatment. Rarely, fungi can cause invasive otitis external, especially in immunocompromised patients (44,47). Aggressive systemic antifungal therapy is required in these patients, and a high rate of mortality is associated with this condition. (38,51,66)

#### 4. Conclusion

Otomycosis is fungal infection of the EAC that is frequently encountered in patients attending otolaryngology clinics. It presents with predominant symptoms of itching, ear discharge, ear ache, blocking sensation, followed by hearing impairment and tinnitus. In our study, we found the disease to be more common in females. The major predisposing factors for otomycosis are trauma to the EAC, use eardrops, unsterile oil, and immunocompromised status. The disease is predominately unilateral, but bilateral involvement is seen more in the immunocompromised group. Though the disease can be diagnosed clinically, microscopic examination and fungal culture is required for confirmation of the diagnosis. *Aspergillus niger* was the predominant fungal species isolated in immunocompetent patients while *Candida* was predominantly seen in immunocompromised patients.

Moist environment, poor hygiene, turban usage conditions are the primary predisposing factors for the development of otomycosis. More number of clinical cases is detected during the summer months as compared to the rainy season due to the moist and humid available conditions facilitating good fungal growth. Mere microscopic examination cannot be taken as evidence of negativity for fungal presence and has to be authenticated with a culture investigation of the specimen. The present study reiterates that *Candida albicans* and *Aspergillus* sp. is one of the most common causative organisms implicated in the causation of otomycosis, which also show resistance to various anti-fungal agents. The low detection rate in this study was perhaps due to the usage of topical antibiotics before the samples could be taken. Simultaneous treatment of concomitant infections such as otomycosis and dermatophytoses is mandatory to prevent the recurrence of both.

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## الملخص:

خلفية :- على الرغم من التهاب الاذن الوسطى كثير الظهور وفي جميع انحاء العالم وعلى نطاق واسع ، فمن الواضح انه مرض شائع جدا في مرحلة الطفولة . وهو سائد بشكل خاص في الاطفال الذين تقل اعمارهم عن سنتين .

وعلاوة على ذلك فان الظهور المبكر لحالة التهاب الاذن الوسطى يزيد من خطر اعادة ظهورها فيما بعد وكذلك تحولها لحالة التهاب مزمنة مع تدفق الخراج من الاذن. بالاضافة الى ذلك فان عدد من العوامل الاخرى، عوامل الشخص المصاب وعوامل بيئية تتعلق بزيادة خطر مرض التهاب الاذن الوسطى. العوامل البيئية المساعدة لانتقال الامراض للمجاري التنفسية العليا تزيد من خطر الاصابة بمرض التهاب الاذن الوسطى المتكرر وكذلك حدوث الاصابات المزمنة الشديدة المترتبة مع تدفق الخراج من الاذن. وتشير عدة عوامل الى دور وراثي لحدوث حالات التهاب الاذن الوسطى الامر الذي يحتاج الى مزيد من الاستكشاف.

الاهداف :- عزل وتشخيص الفطريات من مرضى حالات التهاب الاذن الوسطى المزمنة.

المرضى وطرق العمل :- جمعت 50 عينة من مرضى التهابات الاذن المتسببة عن الفطريات اعتمادا على اعراض سريرية مختلفة وهي الحكمة، سيلان الافرازات المزمنة من الاذن ، ألم الاذن ، ضعف السمع ، وكذلك ملاحظة بعض الاعراض المعتمدة على الفحص بمنظار الاذن مثل ظهور الكتلة الفطرية او الحطام الفطري والافرازات السوداء ذات الرائحة الكريهة .

اجريت هذه الدراسة ذات الاثر الرجعي على المرضى المراجعين لقسم الانف والاذن والحنجرة في مستشفى اربيل التعليمي وكذلك مستشفى رزكري في مدينة اربيل .

النتائج:- من 50 عينة تم تحديد 23 عينة (46%) كانت ايجابية للعزل الفطري بأجراء الفحص المباشر باستخدام هيدروكسيد البوتاسيوم وكذلك زرع العينات الفطرية. من بين عينات المرضى الايجابية كان هناك 13(56,52%) كانوا اناث والعدد المتبقي 10(43و48%) كانوا ذكور .

قد عزلت من عينات المرضى. *Aspergillus*Sp. *Candida*Sp.

كانت الحالات الايجابية من الاناث اكثر من الاحالات الايجابية للذكور من المرضى وكانت الاعمار تتراوح بين 20 سنة الى 65 سنة وكان العدد الاكبر من الحالات الايجابية في الفئة العمرية من 51-60 سنة (47,82%) تليها 21-30 سنة (30,43%).

الكلمات المفتاحية :- الفطريات ، اصابات الاذن الفطرية ، الانف والحنجرة، مرض التهاب الاذن الوسطى.