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MYCOTIC INCIDENCE OF EAR INFECTION AMONG PRIMARY SCHOOL STUDENTS AT TAIZ - CITY, YEMEN

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Abstract:-

The prevalence and distribution of fungi involved in otomycosis in children of primary school at Taiz city were studied using two types of isolation media. The obtained results revealed the confirmation of fungal otomycosis in 76. 7 % of suspected patients. Ear infection in relation to age the results showed that the group with age between 10- 15 years were highly infected with fungi and the female patients were more affected than male. Patients with excellent and good healthy state were less infected. Also, the majority of ear infection were found in children of poor occupation and decreased with raising of the family occupation and disappeared in rich families. On the other hands, there are no relationship between ear infection and the level of family education.

Twelve species and one variety belonging to 5 genera were isolated from 60 children patients on Czapeck›s and Sabouraud›s dextrose agar at 28°C. Members of Aspergillus were the most prevalent. From the genus 8 species were identified of which A. niger, A. sydowii and A. versicolor were the most predominant. The second incidence fungi in children ears was represented by Penicillium. The other 3 genera identified, represented each by one species, wereisolated in less frequency of occurrence at least on one isolation medium.

About 17 isolates appertaining to 10 species related to 5 genera were tested for their abilities to produce lipase and protease enzymes on solid medium. The results indicated that 12 isolates could produce the two enzymes.

Isolates related to A. flavus, A. niger, A. sydowii, Penicillium sp. were the most producers for lipase and protease enzymes. Thus, critical diagnosis of the causative agent by employing aseptic and proper culture techniques and susceptibility testing for proper treatment of this disease is the need of the hour.

Key words: - *Otomycosis, fungi, ear diseases, enzymes, otitis.*

INTRODUCTION

Otomycosis (Gr.oto = ear +mycosis = fungal infection) is often an infection of the pinna , the external auditory meatus, however, the disease may occur in the middle ear if the tympanic membrane is perforated (Anaissie *et al.* 2003, Ho *et al.* 2006, Munguia and Daniel 2008, Aneja *et al.* 2010). It is mainly characterized by pruritus , otalgia aural fullness , hearing impairment and tinnitus (Kaur *et al.* 2000, Fasunla *et al.* 2007) .

Otomycosis is a superficial, subacute or chronic infection of the outer ear canal characterized by inflammation, pruitus, pain and scaling. Otitis externa (OE) and otitis media (OM) in children are very common pediatric disorders (Koupari *et al.* 2000, Amigot *et al.* 2003, Fasunla *et al.* 2007). Otomycosis is worldwide in distribution but it is more common in tropical and subtropical regions. In temperate regions, it is most frequently seen during the summer month (Kaur *et al.* 2000). The infection may be acute, subacute or chronic and usually present with itching of the ear, otalgia, aural fullness, hearting impairment and tinnitus. The accompanied inflammation is associated with superficial epithelial exfoliation and formation of masses of debris containing hyphae, which further worsen the discomfort and sometimes culminate in to frank suppuration in the affected ear (Kaya and Kiraz 2007).

Most infections are present in patients who previously had medical treatment of the external canal and in those who have undergone surgical procedures. Where local lesions, such as congestion, increased vascular permeability, higher temperature and acid pH, create favorable conditions for the growth of fungi (Sohnle *et al.* 1976). The prevalence of otomycosis is influenced by several predisposing factors such as immunocompromised host, steroid usage , trauma, swimming , use of oils, hot promised host , and humid climate , instrumentation of ear , fungal infection elsewhere in the body like dermatomycosis and malnutrition in children. The etiological agent of otomycosis are commonly found in indoor and out door air ,in the soil and dust and on lysis of plant materials (Jackman *et al*. 2005, Aneja *et al*. 2010).

The distribution of fungal infection is not only affected geographically, but also changes with time and season. Identification of the factors predisposing for otomycosis is important in order to prevent the occurrence . These factors may differ from region to region owing to different weather condition and social habits. The prevalence and fungal genera depend greatly on the patient, geographic location (Kumar 2005). The highest prevalence of otomycosis occurs in the hot and humid climates .in temperate climates, such as where this study took place ,the prevalence decrease. Although a wide spectrum of fungal taxa, such as *Aspergillus , Penicillium , Mucor , Rhizopus , Scopulariopsis*, *Absidia* and *Candida* are involved, species of *Aspergillus* and *Candida* are the most common etiological agents (Mugliston and Donoghue 1985, Lucente 1993). These fungi are opportunistic and usually bear varied pathogenicity, being part of the normal microbiota from different body part. Species of *Aspergillus* and *candida* are the most commonly identified germs causing otomycosis. Studies found a greater prevalence of *Aspergillus (A. niger, A. fumigatus, A. flavus*) as otomycosis agents . Jaiswal (1990) and Navarrete and Elizalde (2000) found 46% and 35% of *Candida* spp, respectively.

Moulds and yeasts are common in the auditory canals of healthy peoples, but the percentage of isolated *Aspergillus* and *Candida* species is very low. Many authors have reported only *Aspergillus* isolates of patient with otomycosis. *Candida* and *Aspergillus* are both ubiquitous organisms and normal skin flora that can cause opportunistic infection in the EAC . When either the skin barriers to infection or the metabolic equilibrium of the skin flora in the EAC are altered, colonizing fungi and bacteria can proliferate and distrupt the normal floral hemostatsis (Jackman *et al*. 2005).

Fungal OE is sporadic and can be caused by a wide variety of fungi, the majority of which are saprobes such as *Aspergillus* spp. or fungal normal flora of the skin. Since *Candida* spp. is known to occur as a common on the skin of a healthy individual. The more recovery of fungus from the clinical samples cannot conclusively establish the diagnosis of mycotic disease . Therefore, the emphasis laid on possible virulence factors such as protease production, which is involved in the infection caused by *Candida* spp. The constitutive hydrolytic enzyme of *Candida* spp. avoid the invasion of host tissues. These findings suggest that protease production may play an important role in the pathogenesis of otomycosis caused by *Candida* spp. It is possible that protease enzyme enhance the ability of *Candida* spp. to colonize the skin and penetrate the host cells, which could be important in establishing the cause of the ear infection. It is known that secreted protease are an important (Negi *et al*. 1984, Jadhave *et al*. 2003, Trpkovic *et al*. 2004).

Aim of the work

The aim of our work was to characterize the prevalence of fungal agents, sex, age distribution and other predisposing factors involved in otomycosis in primary school students at Taiz City . Also, production of protease and lipase enzymes by isolated fungi was evaluated.

Materials and Methods

I- Clinical diagnosis and sampling:

This study was carried out during 3 months period starting from 12/2018 to 3/2019, Including 60 patients from students in primary school at Taiz city (32 females and 28 males).The specimens of the external ear were collected by cotton swabs (one swab for each ear). Swab specimens were placed in sterile tubes and transferred to the Microbiological laboratory to be processed in the same day.

II-Isolation of fungi:

Samples with material collected was inoculated directly over the agar surface of the following two culture media:-

a) Sabouraud's dextrose agar:
This medium was used mainly to isolate pathogenic fungi from the clinical specimens The composition of this medium is (g/L): Pepton, 20; Dextrose, 40; Agar agar, 20; pH, 5.2-5.6. Chloromphenicol, 50 mg/L and Cyclohexamide, 0.5 g were also added to inhibit the bacterial and saprophytic fungal growth. The medium was incubated after inoculation at 28°C for three to four weeks.

b) Czapek's glucose agar:
This medium was used mainly to isolate saprophytic fungi. The composition of this medium is (g/L): Glucose, 10; NaNO3, 3; KH2PO4, 1; MgSO4, 0.5; Agar agar, 15; pH, 5.6;. Rose Bengal (1:15000) and Chloramphenicol (500 mg/L) were also added as bacteriostatic agent. After inoculation the medium was incubated at 28°C for two weeks.

c) Identification of fungi:
The following references were used for identification of fungal genera and species based on macro and microscopic characteristics (Raper and Fennell 1965, Frey *et al*. 1979, Kwon-Chung and Bennett 1992, Cheesbrough 1992 and Moubasher 1993).

III- Protease activity:
The production of protease enzymes by common fungal isolates was examined using a casein hydrolysis medium (Paterson and Bridge 1994). Hydrolysis of the casein results in a clear zone around the fungal colony.

IV- Lipase activity:
The lipolytic activity of fungal isolates identified in the current study was measured using the method of Ullman and Blasins (1974). The production of the enzyme by a colony was seen either as a visible precipitate due to the formation of crystals of calcium salt of oleic acid or as opaque zone surrounding the colony consisted of calcium salts of free fatty acid.

Results and Discussion
The results revealed that from 60 patients examined about 46 (76.7%) samples were infected with fungi (Table2). Our results are greatly similar to those obtained by Aneja *et al*. (2010). They observed that the mycological examination has revealed the confirmation of fungal otomycosis in 78% of the patients. It is estimated that otitis externa make up 5 to 20% of ear-related visits to ENTs, most of them caused by bacteria, and form the latter 9 to 25% are caused by fungi, called fungal otitis (otomycosis).
Mycological examination conducted by direct microscopy and fungal culture was performed on 139 specimens. Among these, 115 patients suffered from chronic otitis media with persisting tympanum perforation and otorrhea. A further 13 patient had clinical signs of an otitis externa. Out of 139 samples, fungi were identified in the auditory canal (n = 54), on the tympanic membrane (n = 5), and in the middle ear (n = 5). Two-thirds were as mould and one-third yeasts. The dominating species were *Aspergillus niger* and *Candida* (Vennewald *et al.* 2003). Many authors have reported about *Aspergillus* and *Candida* isolation in patients with fungal otitis externa. Other species such as *Mucor*, *Fusarium*, *Scedosporium*, *Hendersonula*, *Rhodotorula*, and *Cryptococcus* are very rare. Molds and yeasts are common in the auditory canals of healthy people, but most of the isolated fungi are *Penicillium* spp. and the percentage of isolated *Aspergillus* and *Candida* spp. is very low. The predominant molds are *A. niger*, *A. fumigatus*, *A. flavus* and *Candida parapsilosis* is the predominate yeast isolate (Vennewald *et al*. 2010).

1- **Incidence of ear infection in relation with age**:
Regarding to ear infection in relation to age, the results showed that the group with age between 10-15 years were highly infected with fungi. On the other side, the patients between 5-10 years were less infected with fungi. Whereas, the group of age above 15 years were considerable infected with fungi (Table 1). It has been found to be more prevalent in females than males in the age group of 31-40 years , higher incidence occurring in the rainy season. The women (60%) in the present study were more affected by otomycosis, and such figures were closer (65%) to those observed by Zaror *et al*.(1991). However, these data are in disagreement with the findings by Kaur *et al*. (2000), Ho *et al*.(2006) and Yehia *et al.*(1990), who found 60%, 56% and 52.5 %, respectively in males. Otomycosis was seen in patients aged between 2 and 66 years. Nonetheless, 50%of the cases were diagnosed in patients between 2 and 15 years of age, occurrence of 41.1% to 70 % were seen in patients within the age range of 16 to 30 years. Record were carefully examined for topical and systemic antibiotic usage before the diagnosis of otomycosis .

2- **Incidence of ear infection in relation to sex:**
The mycological analysis of children ear infection revealed that the female patients were the more infected cases. They were accounted for 45. 6 % and 54.4 % in female and male, respectively (Table 1). The recored were carefully examined for topical and systemic antibiotic usage before the diagnosis of otomycosis . These results were in accordance with those obtained by Fasunla *et al.* (2007). They examined 5784 patients with ear disease and noticed that 378 had otomycosis which consisted of 38.36% male and 61.64% female.

3- **Incidence of ear infection in relation to occupation:**
The results in table (1) indicated that the majority of ear infection were found in children of poor occupation. Also, the infection patients were considerably decreased with the raising of the family occupation and disappeared in rich families.

4- **Incidence of ear infection in relation to family education:**
The current results indicated that there are no relationship between ear infection and the level of family education. This may, due to tack caring the individual with self cleaning (Table 1).

5- **Incidence of ear infection in relation to ear inflammation:**
Data in table (1) showed that the patients with ear inflammation were more infected with fungal species.

6- **Incidence of ear infection in relation to healthy state:**
Regarding to ear infection in relation to healthy state, the results indicated that the patients with excellent and good healthy state were less infected with fungi. On the other hands, patients with low healthy state were highly infected with fungi (Table 1). Our results were greatly similar to those obtained by Vennewald *et al.* (2010). The incidence of fungi in ear infections of primary school children at Taiz - city was surveyed using both Czapek's and Sabouraud's dextrose agar at 28 °C. A total of 12 species and one variety related to 5 genera were isolated from the examined patients. Members of *Aspergillus* were the most prevalent. Otomycosis is most commonly caused by *Aspergillus* species particularly *A. niger*, *A. fumigatus*, *A. nidulans* and other moulds that have been implicated include *Penicillium* species and *Rhizopus* species (Fasunla *et al.* 2007, Aneja *et al.* 2010). Many authors have reported about *Aspergillus* and *Candida* isolation in patients with fungal otitis externa. Other species such as *Mucor*, *Fusarium*, *Scedosporium*, *Hendersonula*, *Rhodotorula* and *Cryptococcus* are very rare. Molds and yeasts are common in the auditory canals of healthy people, but most of the isolated fungi are *Penicillium* spp., and the percentage of isolated *Aspergillus* and *Candida* spp are very low. The predominant molds are *A. niger*,
A. fumigatus, *A. flavus* and *Candida parapsilosis* is the predominate yeast isolate (Vennewald *et al*. 2010). Mycological examination has revealed the confirmation of fungal otomycosis in 78% of the suspected patients. pruritus has been found as the wearing of traditional customary clothes followed by itching on other body parts and swimming (Aneja *et al*. 2010). The most common fungal species isolated from temperate climates are *Candida* and *Aspergillus.* The genus was found in 71.6 % and 23.3 % of the samples on both Czapek's and Sabouraud's dextrose agar at 28°C, respectively. Otomycosis is most commonly caused by *Aspergillus* species, particularly *A. fumigatus*, *A. niger*, *A. nidulans* and *A. flavus*. The fungi involved in otomycosis belonged to *Aspergillus niger , A. flavus , A. fumigatus , A. luchuensis , A. terreus , Candida albicans* and *Penicillium* sp. Of these , *A. niger* followed by *A. flavus* were the dominant fungi. *Aspergillus luchuensis* as the cause of otomycosis has been reported for the first time (Aneja *et al*. 2010).
Moulds of the genus *Aspergillus* are widespread in the environment, being found in the soil, in the air, on plants and on decomposing organic matter. In the home, these moulds are often found in dust and on food. Moulds of the genus *Aspergillus* are opportunistic pathogens, especially immunecompromised patients. Eight species from the genus were identified of which *A. niger* and *A. sydowii* were the commonest species. They were occurred in 30.0 % and 11.6 %, and 20.0 % and 3.3 % of the examined patients using the two isolation media, respectively. Also, *A. flavus* (1.6 % and 5.0 % of patients) was isolated in high incidence especially on Sabouraud's dextrose agar at 28 °C. On the other hand, *A. flavipes*, *A. flavus var. columnaris*, *A. oryzae*, *A. parasiticus* and *A. versicolor* were also, isolated in low occurrence using one or two isolation media. Species of *Aspergillus* and *candida* are the most commonly identified germs causing otomycosis. Studies found a greater prevalence of *Aspergillus (A. niger, A. fumigatus, A. flavus* and / or *Aspergillus* spp.) as otomycosis agents (Jaiswal 1990, Navarrete and Elizalde 2000). In Sao Paulo, Pontes *et al.* (2009) reported that there were 75% of *Aspergillus* and 20% of *Candida* species were identified.The data found in their study were of 55% isolates of *Candida (C. albicans, C. parapsilosis* and *C. tropicalis*) and 35% of *Aspergillus* (*A. niger , A. flavus, A. fumigatus*).

Many authors have reported about *Aspergillus* and *Candida* isolation in patients with fungal otitis externa. Other species such as *Mucor*, *Fusarium*, *Scedosporium*, *Hendersonula*, *Rhodotorula* and *Cryptococcus* are very rare. Molds and yeasts are common in the auditory canals of healthy people, but most of the isolated fungi are *Penicillium* spp., and the percentage of isolated *Aspergillus* and *Candida* spp. is very low. The predominant molds are *A. niger*, *A. fumigatus*, *A. flavus*, and *Candida parapsilosis* is the predominate yeast isolate (Monod *et al.* 1989, Enveani and Iqunbor 1997, Harley *et al.* 1995, Vennewald *et al*. 2003, Martin *et al*. 2005, Ling and Sader 2008)
The secondary incidence fungi in ear children was represented by *Penicillium* emerged in 5.0 % and 3.3 % of tested patients on the two isolation media, respectively. From the genus tow species were identified on Czapek's agar represented each in 1.6 % of examined samples. Also, one unidentified species was isolated on Sabouraud's agar from 3.3 % of the samples. Also, this genus was isolated from ear and other parts of human body in moderate frequency of occurrence (Paulose *et al.* 1989, Amigot *et al.* 2003).
The remaining 3 genera (*Cladosprium herbarum*, *Geotrichum candidum* and *Phoma herbarum*) were isolated at least on one isolation medium represented in 1.6 % of total examined patients (Table 2). These species were previously isolated with different frequency of occurrence as reported by numerous authors (Monod *et al.* 1989, Enveani and Iqunbor 1997, Harley *et al.* 1993, Vennewald *et al*. 2003, Martin *et al*. 2005, Ling and Sader 2008). The diagnosis of otomycosis is usually confirmed from fungal culture of the ear swabs. The etiological agents of otomycosis found were *Aspergillus*

and *Candida* species with *Aspergillus niger* (48.35%) as the most preponderant. This agrees with previous similar studies although at variance with the study by kaur *et al.* (2000) which cited *Aspergillus fumigatus* as the most common mycotic agent. The prevalence of *Candida* species is much less in this study when compared with other similar studies carried out in other parts of Nigeria (Pradhan *et al*. 2003, Jackman *et al*. 2005, Martin *et al.* 2005). Otomycosis could be asymptomatic but if left untreated, may lead to morbidity like hearing loss. In our study, 56 (14.81%) patients had various degrees of conductive hearing impairments (Strauss 1991, Mgbor and Gugnam 2001, Ologe and Nwabuisi 2002, Fasunla *et al.* 2007).

Among 17 isolates related to 10 species, isolated from childrens suffering from otomycosis, 12 isolates have the ability to produce lipase and protease enzymes. The most active isolates for the enzymes production were those belonging to *A. flavus, A. flavus var. columnaris, A. niger, A. sydowii, Giotrichum candidum* and *Penicillium* sp. In this respect, Arsovic *et al*. (2005) studied protease activity by 8 isolates and noticed that 7 isolates were protease positive. The results indicated that the protease production may play an important role in the pathogenesis of otomycosis caused by these microorganisms. It is possible that protease enzymes enhance the ability of these fungi to colonize the skin and penetrate the host cells, which could be important in establishing the cause of the ear infection (Sohnle *et al*. 1976, Negi *et al*. 1984). Also, It is possible that the ability of fungi to adhere to which probably modifies the cell membranes of the host to accept the attachment of the fungus or modifies the surface of the fungal cell in a way that promotes attachment (Sohnle *et al*. 1976).There has been published research investigating protease production in fungal isolates from various sites on the body and this enzyme activity seems to be related to their virulence in the pathogenesis. The strains with higher proteolytic activity are considered more virulent (Hube 1996).

Finally we can say higher incidence of otomycosis may be due to high degree of humidity, warm and dusty environment. Critical diagnosis of the causative agent by employing aseptic and proper culture techniques and susceptibility testing for proper treatment of this disease is the need of the hour.

Recommendations

Fungal ear infection can be preventable disease, where many factors that enhance the disease could be control or reduced by:

1- Enhancing public health education.
2- Good personal hygiene is very important to control this disease.
3- Early treatment with a proper antibiotic will be of a great advantage.
4- Prevention of fungal infection requires major commitment and efforts from governmental agencies, private organization, schools and individuals.
5- Further study to cover other agents causing ear infection in Yemen will be of a great value to the Yemeni society.
6- Polluted water is related to bacterial and fungal otitis externa. To prevent acute otitis externa, it is important that patient avoid swimming or use protective devices, including commercial rubber or silicon earplugs. Frequent cleaning and preventing moisture by drying the ear canal with a hair dryer after each period of swimming are strongly recommended.
7- Cleaning the ear canal with cotton tip applicators should be avoided because it traumatizes the skin and the eardrum and compromises the mechanical barrier of the ear canal.
8- Further investigation is necessary to clarify the contribution of enzymes production to fungal virulence associated with otomycosis and to use this information in the development of new therapeutic interventions.

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Table(1): Ear infecation in primary school students at Taiz city.

Sample No.	No..of genera	Age (years)	Sex	Occupation	Level of family education	Healthy state	Antibiotic used	Ear inflammation
1	2	5	Female	Good	University graduate	Good	Use	Yes
2	1	9	Female	moderate	Read and write	Accepted	No use	NO
3	0	11	Female	Good	Read and write	Excellent	No use	NO
4	0	12	Male	Good	University graduate	Good	Use	NO
5	0	8	Female	moderate	Secondary	Accepted	No use	NO
6	2	10	Female	Good	University graduate	Good	No use	NO
7	1	7	Male	Good	Basic	Excellent	No use	Yes
8	0	7	Female	Rich	Read and write	Accepted	Use	NO
9	0	6	Female	Good	Secondary	Excellent	No use	Yes
10	0	9	Male	Rich	Illiterate	Accepted	Use	Yes
11	0	7	Male	moderate	Read and write	Good	No use	NO
12	1	10	Male	moderate	Read and write	Accepted	No use	NO
13	1	10	Male	Good	University graduate	Good	Use	Yes
14	0	12	Female	Good	Read and write	Good	Use	Yes

Table (1): cont.

15	1	12	Male	moderate	Secondary	Good	Use	NO
16	1	6	Male	moderate	illiterate	Good	No use	NO
17	1	6	Female	Good	Read and write	Good	No use	Yes
18	1	10	Female	Good	Secondary	Excellent	No use	NO
19	0	10	Male	Good	Read and write	Good	Use	Yes
20	1	12	Female	Good	Read and write	Excellent	No use	NO
21	1	12	Male	Rich	University graduate	Accepted	Use	Yes
22	2	10	Female	moderate	Illiterate	Accepted	No use	Yes
23	1	11	Male	Good	University graduate	Good	Use	Yes
24	2	9	Male	Good	University graduate	Good	No use	Yes
25	2	10	Female	moderate	Read and write	Good	No use	NO
26	0	9	Female	moderate	Read and write	Good	Use	Yes
27	0	8	Female	good	Read and write	Excellent	Use	Yes
28	1	15	Female	Rich	University graduate	Excellent	Use	Yes
29	1	28	Male	moderate	Basic	Good	Use	Yes
30	3	11	Male	Good	University graduate	Good	No use	Yes
31	1	11	Male	moderate	Read and write	Good	Use	Yes

Table (1): Cont.

32	2	12	Female	moderate	Read and write	Good	No use	Yes
33	1	12	Female	Good	illiterate	Good	Use	Yes
34	1	10	Female	Good	Secondary	Good	Use	Yes
35	1	11	Female	poor	Read and write	Accepted	Use	Yes
36	2	14	Female	Good	Read and write	Good	Use	Yes
37	2	13	Female	poor	Read and write	Accepted	Use	Yes
38	1	11	Male	Good	Read and write	Good	No use	Yes
39	1	13	Male	Good	University graduate	Good	Use	Yes
40	0	15	Male	Rich	Read and write	Excellent	No use	NO
41	S.M	9	Female	Rich	Read and write	Excellent	No use	NO
42	0	11	Male	Good	Illiterate	Good	Use	Yes
43	1	7	Male	moderate	Illiterate	Accepted	Use	Yes
44	1	8	Male	moderate	Read and write	Accepted	Use	Yes
45	1	7	Male	moderate	Basic	Good	No use	Yes
46	1	15	Male	moderate	Read and write	Accepted	Use	Yes
47	2	15	Male	moderate	Illiterate	Accepted	No use	NO
48	1	12	Male	Good	Basic	Good	Use	Yes
49	1	16	Male	Good	university graduate	Accepted	Use	Yes
50	1	13	Male	Rich	university graduate	Accepted	Use	Yes
51	2	13	Female	Moderate	Basic	Good	No use	Yes
52	1	13	Female	Moderate	Read and write	Accepted	No use	Yes
53	1	10	Female	moderate	Secondary	Accepted	No use	Yes
54	1	10	Female	Moderate	Basic	Accepted	No use	Yes
55	1	8	Female	Moderate	Basic	Excellent	No use	NO
56	2	7	Female	moderate	Secondary	Good	No use	Yes
57	1	12	Female	Good	University graduate	Accepted	Use	NO
58	0	11	Female	Good	Basic	Accepted	Use	Yes
59	1	6	Female	Moderate	Read and write	Accepted	No use	Yes
60	2	14	Female	Good	Illiterate	Accepted	No use	Yes

S. M. = Sterile mycelia

Table (2): Percentage frequency (F%) of fungal genera and species recovered from ear infection specimens on Czapk's and sabouraud's agar at 28°C.

Genera and Species	Czapek's agar (F%)	Sabouraud's agar (F%)
Aspergillus	71.6	23.3
A. flavipes	1.6	-Ve
A. flavus	1.6	5.0
A. flavus var.columnaris	1.6	-Ve
A. niger	30.0	11.6
A. oryzae	1.6	-Ve
A. parasiticus	1.6	1.6
A. sydowii	20.0	3.3
A. versicolor	-Ve	3.3
Cladosporium herbarum	1.6	-Ve
Geotrichum candidum	-Ve	1.6
Penicillium	5.0	3.3
Penicillium sp.	-Ve	3.3
P. brevicompactum	1.6	-Ve
P. requfortii	1.6	-Ve
Phoma herbarum	1.6	-Ve

Table (3): Lipase and protease enzymes production by isolated fungi.

Fungal of species	Lipase			Protease		
	NIT	NIP	NIN	NIT	NIP	NIN
Aspergillus flavus	5	4	1	5	4	1
A.flavus var columnaris	1	1	_	1	1	_
A.niger	3	3	_	3	_	3
A.oryzae	1	_	1	1	1	_
A.parasiticus	1	_	1	1	1	_
A.sydowii	1	1	_	1	1	_
Cladospurium herbarum	1	_	1	1	1	_
Geotrichum candidum	1	1	_	1	1	_
Penicillium sp	2	1	1	2	1	1
Phoma harbarum	1	1	_	1	1	_
Total isolates	11	12	5	11	12	5

NIT = number of isolates tested.
NIP = number of isolates positive.
NIN = number of isolates negative.