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Profile of Fungal Ear Infection among Patients Attending El-Manzala Central Hospital

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Abstract

Background: **Otomycosis is a fungal infection of the ear. Although it affects the external ear canal, the middle ear may be involved in the case of a perforated tympanic membrane. The mastoid cavity may be affected if an open cavity mastoid surgery is performed previously.** ***Aim:*** **This study aimed to contribute to the epidemiologic profile of fungal ear infections among patients in Manzala central hospital.** ***Patients and Methods:*** **This descriptive cross-sectional study was conducted on 88 patients with fungal ear infections attending the Otolaryngology clinic in El-Manzala central hospital (Dkahlia, Egypt). The studied patients were subjected to the following: detailed history taking, clinical examination, sterile cotton swabs, and Mycological analysis.** ***Results:*** **In the present study the most common fungi isolated from otomycosis cases during the present survey was A. niger (it was positively isolated from 60 cases), followed by A. flavus, which was isolated from 19 cases of otomycosis and Candida in 9 patients. Regarding the age and sex distribution in the present study, the disease was more prevalent among adults between 21-40 years where it was diagnosed in 45 patients (51.1% of total cases) with a statistically significant difference. as 10 cases according to age group as 5 patients ≤10 years old, 3 cases were detected in the age group of 11–20 years, 45 cases were detected in the age group of 21–40 years, 25 cases were detected in the age group of 40-60 years and 10 cases ≥61 years. Males showed a significantly higher incidence of otomycosis than females.** ***Conclusion:*** **In conclusion, the overall clinical mycological and epidemiological profile of otomycosis infection observed at El-Manzala Central Hospital, Egypt, does not differ significantly from those observed by previous researchers around the world, and any variation is probably due to the differences in climates of the different study populations.**

Keywords: *Otomycosis, Risk factors, Pattern of fungal ear infection, Egypt*

Introduction

Otomycosis is a fungal infection of the ear. Although it affects the external ear canal, the middle ear may be involved in the case of a perforated tympanic membrane. The mastoid cavity may be affected if an open cavity mastoid surgery is performed previously[1]. The presence of itching in the external ear canal must raise a high index of suspicion for otomycosis since it is the most frequent symptom. In some studies, itching

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was present in more than 90% of the patients[2]. Approximately 61 species of different fungi (molds, yeasts, dermatophyte, and Malassezia) have been identified as the cause of otomycosis. In the literature, several saprophytic fungi and Aspergillus niger were described as the most common agents for otomycosis. In addition, Aspergillus awamori and Aspergillus tubingensis are another black Aspergillus involved in otomycosis. Other agents include Aspergillus flavus, Aspergillus Scedosporium apiospermum (sexual state: Petriellidium boydii), Scopulariopsis, Penicillium, Chrysosporium, Rhizopus, Absidia, and Cryptococcus species[3]. Pigmented fungi, Alternaria, and Cladosporium species were also reported as causative agents. Although nearly all of the saprophytic fungi are presented in the atmosphere as airborne fungi, their concentration differs according to location, altitude, time of day, season, and climatic conditions. In addition, endogenous organisms such as C. albicans, C. guillier-mondii, C. parapsilosis, Malassezia, and Rhodotorulaspecies contributed to the infection in some cases[4]. Wearing turban or other clothes on the head has been reported as a risk factor for otomycosis. Swimming is also reported as a risk factor for otomycosis. Perforation of the tympanic membrane and previous ear surgery have also been reported as important risk factors for otomycosis[5]. Otomycosis has a worldwide distribution, but the prevalence of infection is related to the geographical location, areas with tropical and subtropical climate showing higher prevalence rates. The prevalence of dermatomycoses in patients with otomycosis was 36.5% in Turkey and 51% in India[5]. In Egypt, knowledge on otomycosis is still very limited. The present study aims at identifying fungal species involved in otomycosis as well as incidence of fungal infections among patients in El-Manzala central hospital. The aim of the current study was to describe the epidemiologic profile of otomycosis among patients in El-manzala hospital.

Patients and Methods

This descriptive cross-sectional study was conducted on 88 patients attending the Otolaryngology clinic in El-Manzala central hospital (Dkahlia, Egypt). Patients with any age, presenting with symptoms of Otomycosis like itching, pain, ear discharge, feeling of foreign body in the ear, hearing loss, headache were included. While patients with uncontrolled systemic illness were excluded.

Methods

The studied patients were subjected to the following: Detailed history taking name, age, sex, occupation, complaint in patient's language (onset: sudden or gradual, course: progressive slow or rapid increasing, duration: continuous or intermittent, unilateral or bilateral, factors aggravating or relieving events, since when and where). Clinical examination: for swelling. discharge color and odor, flaky skin, narrowing of external ear canal, plugged ear , two ears or one ear, there is wax or not. Cotton swabs were used for collecting debris and earwax from the external ear canal of patients showing symptoms of otomycosis. Samples were immediately transferred for further analysis at El Fouad medical laboratory.

Mycological analysis

a) Direct microscopic examination: Direct smears from swabs were prepared and examined using Lactophenol Cotton Blue stain

(LPCB) as recommended by Ellis et al.[6].

b) Culturing of samples: Swabs were streaked on Sabouraud's dextrose agar medium (SDA) with the composition of (g/l): peptone, 15; dextrose, 40 and agar, 20[6] Cultures were incubated at 28°C for 7-15 days until fungal colonies appear. Cultures were also preserved on SDA slant agar at 4°C for further studies.

c) Identification of fungi: Phenotypic identification: Fungi were identified on the basis of macro-and microscopic features[7]. Enzymatic activities of otomycotic fungi:

1. Proteolytic activity: Test tubes containing modified casein hydrolysis medium was used. The medium comprised (g/l): KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.1; 15% skimmed milk, 25 ml; glucose, 10; and agar, 20. After inoculation with the tested fungi, cultures were incubated at 25°C for 7 days. Degradation of milk protein was measured as depth of clear zone (in mm).
2. Lipolytic activity: The medium of Wadhwani[8] was used which has the following composition (g/l): peptone, 10; $MgSO_4$.$7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.2; and agar, 20). The medium was sterilized by autoclaving at 121°C for 20 minutes. Tween 80 (10 ml) was autoclaved separately and added to the sterile and cooled basal medium. The medium was dispensed aseptically in test tubes (10 ml/tube) followed by inoculation by fungal isolates. After incubation at 25°C for 7 days, the lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of precipitate (in mm) was measured.
3. Urease Activity: Christensen urea medium described by Ellis et al.[6] was employed with some modifications. The medium was prepared in 2 separate parts. The first part contained (g/l): peptone, 1; KH_2PO_4, 1; KCl, 0.05; yeast extract, 1; and phenol red, 0.012. These components were dissolved in 800 ml distilled water. The second part was composed of (g/l): glucose, 5; $MgSO_4.7H_2O$, 0.5; and distilled water, 200 ml. The two parts were mixed after autoclaving and cooled to 50°C. Aliquots of 5 ml of 40% solution of sterilized urea will be added to each 100 ml of the medium which is then poured into sterile 5 ml test tubes (3 ml for each). After inoculation, cultures were incubated at 25°C for 3-5 days. Results will be recorded as positive after appearance of a deep pink color in the broth medium.

Screening for aflatoxin production

a. Culturing of selected fungi: Thirty-six fungal isolates belonging to A. flavus (26 isolates), A. flavus var. columnaris (9) and A. parasiticus (1) were cultivated in 250 ml conical flasks containing 50 ml of Potato Dextrose Broth (PDB) followed by sterilization, inoculation, and incubation at 28C for 7 days.
b. Extraction and detection of aflatoxins: Chloroform was the solvent for mycotoxin extraction. Pre-coated TLC silica plates were used for separation of mycotoxins followed by visualization under UV light (254 or

365 nm). Aflatoxins B and G when present fluoresce blue and greenish blue respectively. The intensity of the sample spots will be compared with that of the standard aflatoxins.

Statistical Analysis

Statistical analysis was performed using statistical Package for the social sciences (SPSS) software version 24. Fisher exact test was used to identify the possible association between the categorical variables. Mann-Whitney U test was used to identify the possible association between the numerical variables. Results were considered statistically significant at a P-value < 0.05.

Ethics consideration

The participants gave informed written consent. The protocol was approved by the local ethical committee of the Suez Canal University, Faculty of Medicine. Privacy and confidentiality of the obtained data was ensured for all participants. Laboratory results were given to all tested members. All results were used for research purposes only. All data was saved, only the researcher had access to it. No stored samples were shipped out of the country. The subjects had the right to withdraw samples at any time.

Results

This descriptive cross-sectional study was conducted on 88 patients attending the Otolaryngology clinic in El-Manzala central hospital (Dkahlia, Egypt). The study shows that patients had mean age of 37.3±10.8 years ranged from 10-70 years. Most of patients were male (68.2%). It also shows the distribution of otomycosis among different age groups was as follows: 10 cases according to age group 5 patients ≤10 years old, 3 cases in the age group of 11–20 years, 45 cases in the age group of 21–40 years, 25 cases in the age group of 40-60 years and 10 cases ≥61 years.

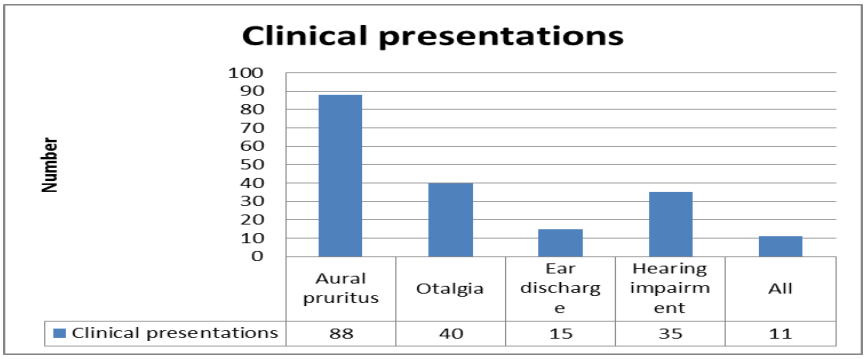

Figure 1: Clinical presentations of patients (N=88).

Figure 1 shows that the predominant complaint of the patients was itching, followed by otalgia and hearing impairment, and the least common complaint was ear discharge. Eleven percent of patients had all complaints. Figure 2 shows that the most common predisposing factor was traumatic injury to the ear canal by frequent picking, followed by water entry, antibiotic ear drop treatment and systemic diseases. Wax was absent in 89.8% of cases and present in 10.2% of cases. So, absence of wax is a very

important predisposing risk factor for otomycosis. This study shows that the most common fungi isolated from otomycosis cases during the present survey was A. nigri (it was positively isolated from 60 cases), followed by A. flavi, which was isolated from 19 cases of otomycosis and Candida in 9 patients. Table 1 shows that Lipolytic activity: Out of 88 isolates, 74(84%) were able to produce lipase but with variable capabilities (Table 1).

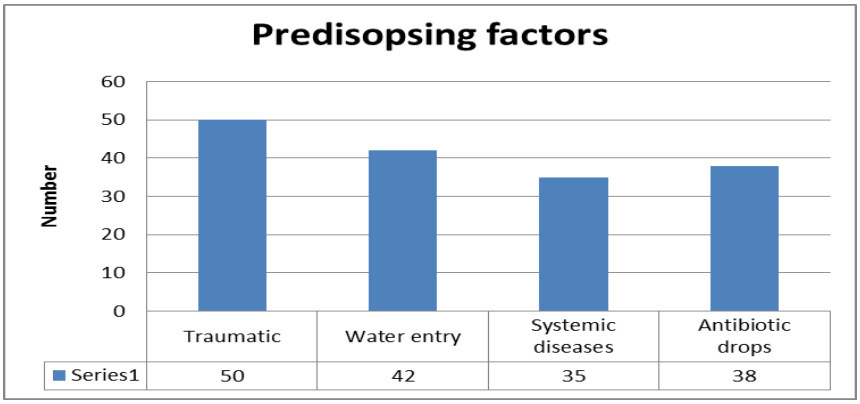

Figure 2: Predisposing factors of patients (N=88).

Table 1: Enzymatic activities of fungi recovered from otomycosis (N=88)

	Lipase					Protease					Urease				
	H	M	L	P	N	H	M	L	P	N	H	M	L	P	N
Aspergillus Flavi (n=19)	4	11	2	17	2	0	19	0	19	0	5	5	3	13	6
Aspergillus Nigri (n=60)	18	11	26	55	5	0	38	22	60	0	0	0	0	0	60
Candida species (n=9)	0	0	2	2	7	0	4	4	8	1	0	0	0	0	9

High lipase production was exhibited by 22 isolates which were mainly belonging to Aspergillus Flavi and Nigri. Candida isolates were lipolytic showing low activity. Proteolytic activity: Protease was produced by 87 out of 88 isolates which were almost of moderate proteolytic capabilities. All fungal isolates belonging to sections Flavi (19), Nigri (60) and Candida isolates (8). Urease activity: Urease was only produced by 13 fungal isolates which belong to Aspergillus Flavi (30 isolates). Table 2 shows that Aflatoxins were produced by 16 out of 19 isolates representing 89.5% of tested fungi. Isolates of A. flavus 11 were able to produce aflatoxins B1, B2 and G1 with variable levels. 5 isolates of A. flavus var. columnaris produced low to intermediate levels of aflatoxins B1, B2 and G1. A. parasiticus produced low levels of aflatoxin B1. Table 3 showed that Terbinafine (TER) followed by Clotrimazole (CC) and clove oil were the most effective antifungal agents. Results showed also most of isolates were resistant to Fluconazole (FLC). Aspergillus Flavi was more resistant to Fluconazole (FLC) and Amphoteicin B (AP), while Aspergillus Nigri was more resistant to Fluconazole (FLC), Amphoteicin B (AP) and Cetrimide (CET). Candida species were more resistant to SER:

Sertaconazole, TIO: Tioconazole, CO: Clove oil. Table 4 shows that the disease was more prevalent among adults between 21-40 years where it was diagnosed in 45 patients (51.1% of total cases) (p<0.001). Table 4 shows also that male had significantly higher incidence of otomycosis than females.

Table 2: Mycotoxins produced by otomycotic Aspergillus isolates (N=19).

	Aflatoxins	L	M	H	P	N
A. flavus (n=12)	B1	1	1	4	6	1
	B2	0	1	0	1	
	B1+B2	0	2	0	2	
	B1+G1	1	0	0	1	
	G1	1	0	0	1	
A. flavus var. columnaris (n=6)	B1	1	0	0	1	2
	B2	1	0	0	1	
	B1+B2	0	1	0	1	
	B1+G1	0	1	0	1	
	G1	1	0	0	1	
A. parasiticus (n=1)	B1	1	0	0	0	0

Aflatoxin (µg/L): L= Low (≤ 100), M= Moderate (>100- <500), H= High (≥ 500).

Table 3: Drug sensitivity among patients (N=88)

	DS	AP	CC	CET	FLC	IT	KT	TER	NS	SER	TIO	CO
Aspergillus Flavi	S	3	13	9	1	1	2	17	4	11	15	12
	I	6	6	9	0	12	9	2	5	7	2	6
	R	10	0	1	18	6	8	0	10	1	2	1
Aspergillus Nigri	S	10	20	12	0	1	3	36	20	10	22	42
	I	20	18	18	3	12	19	11	18	20	23	9
	R	30	22	30	57	47	28	13	22	30	15	9
Candida species	S	4	8	6	5	2	9	2	7	0	3	2
	I	4	1	0	0	6	0	3	2	2	0	2
	R	1	0	3	4	1	0	4	0	7	6	5

Degree of sensitivity (DS): S = Susceptible, I = Intermediate, R= Resistant of common otomycotic fungal isolates to different antifungal agents, AP: Amphotericine-B, CC: Clotrimazole, CET: Cetrimide, FLU: Fluconazole, IT: Itraconazole KT:Ketoconazole, TER: Terbinafine, NS: Nystatin, SER: Sertaconazole, TIO: Tioconazole, CO: Clove oil.

Discussion

Otomycosis has been observed to be a common disease in patients applying to the outpatient clinic of El-Manzala hospital (EL-Dkahlia, Egypt). In the present study, a total of 88 cases were recorded with otomycosis. These study patients have mean age of 37.3±10.8 years ranged from 10-70 years. Most of patients were male (68.2%). The most common predisposing factor was traumatic injury to the ear canal, followed by water entry, antibiotic ear drops treatment and systemic diseases. Wax was absent in 89.8% of cases and present in 10.2% of cases. These findings were in accordance

with the study of Ahmed[9], who found 72% of cases due to trauma and 45% due to swimming. The lipid mantle layer formed by the cerumen in the external canal has long been considered as the key factor for the protection of the canal wall, and its removal by frequent irrigation of the external layer is thought to be the reason why frequent bathing in tropical climates is incriminated as a cause of recurrent otomycosis. In the present study, 65% of the patients had a history of the use of antibiotic drops.

Table 4: prevalence of otomycotic cases and causative fungi in relation to age of patients and sex of patients (N=88)

	Patient's age groups					Patient's sex	
	≤10 (n=5)	11-≤20 (n=3)	21-≤40 (n=45)	41-≤60 (n=25)	≥61 (n=10)	Males (n=60)	Females (n=28)
Aspergillus Flavi (n=19)	0	0	12	3	4	16	3
Aspergillus Nigri (n=60)	4	2	30	21	3	44	16
Candida species (n=9)	1	1	3	1	3	0	9
P-value	**<0.001*[1]**					**<0.001*[1]**	

*****=Statistically significant as p<0.05; 1= Fisher exact test used.*

This was in accordance with the study of Fasunla et al.[10] who found 42% of cases with the same predisposing factor. Abdel Azeem et al.[11] study found that, 91% of otomycotic cases in the present study had no cerumen in the external canal and this was in accordance with the study of Pontes et al.[12] who showed that ear wax contains numerous amino acids and lysozymes that have an inhibitory effect on fungi. In the present study the most common fungi isolated from otomycosis cases during the present survey was A. niger (it was positively isolated from 60 cases), followed by A. flavus, which was isolated from 19 cases of otomycosis and Candida in 9 patients. In agreement with Abdelazeem et al.[11] study in which the most common fungal species isolated from otomycosis cases was A. niger (91%), followed by A. flavus (9%). As regards enzymatic activities our study found that, out of 88 isolates, 74(84%) were able to produce lipase but with variable capabilities. High lipase production was exhibited by 22 isolates which were mainly belonging to Aspergillus Flavi and Nigri. Candida isolates were lipolytic showing low activity. In Gharamahet al.[13] study out of 101 isolates, 76 (75.2%) were able to produce lipase but with variable capabilities. High lipase production was exhibited by 40 isolates which were mainly belonging to Aspergillus sections Flavi and Nigri. Although 50% of A. terreus isolates were moderately able to produce lipase, only 13.3% of Candida isolates were lipolytic showing low activity. In the present study, protease was produced by 87 out of 88 isolates which were almost of moderate proteolytic capabilities. All fungal isolates belonging to sections Flavi (19), Nigri (60) and Candida isolates (8). Similar to Gharamahet al.[13] study in which protease was produced by 96 out of 101 isolates which were almost of moderate proteolytic capabilities. All fungal isolates belonging to sections Flavi (35), Nigri (39) and Terrei (4) were proteolytic. On the other hand, 10 out of 15 Candida isolates were

protease producers. In the current study urease was only produced by 13 fungal isolates which belong to Aspergillus Flavi (30 isolates). In agreement to Gharamahet al.[13] study in which urease was only produced by 37.6 % fungal isolates which belong to Aspergillus sections Flavi (30 isolates) and Terrei (3) as well as the tested isolates of Phoma, Mucor and Stemphylium. Urease was not detected in fungal cultures related to Aspergillus sections Nigri and Candida. In this study, aflatoxins were produced by 17 out of 19 isolates representing 89.5% of tested fungi. Isolates of A. flavus 11 were able to produce aflatoxins B1, B2 and G1 with variable levels. Four isolates of A. flavus var. columnaris produced low to intermediate levels of aflatoxins B1, B2 and G1. A. parasiticus produced low levels of aflatoxin B1. In agreement to Gharamahet al.[13] study aflatoxins were produced by 26 out of 36 isolates representing 72.2% of tested fungi. Isolates of A. flavus 19 (73%) were able to produce aflatoxins B1, B2 and G1 with variable levels. Production of these toxins by fungi isolated from human corneal ulcers in Egypt has been demonstrated by Gharamah et al.[13]. In the present study, Terbinafine (TER) followed by Clotrimazole (CC) and clove oil were the most effective antifungal agents. Results showed also most of isolates were resistant to Fluconazole (FLC). Aspergillus Flavi was more resistant to Fluconazole (FLC) and Amphoteicin B (AP), while Aspergillus Nigri was more resistant to Fluconazole (FLC), Amphoteicin B (AP) and Cetrimide (CET). Candida species were more resistant to SER: Sertaconazole, TIO: Tioconazole, CO: Clove oil. Gharamahet al.[13] study found that, Terbinafine (TER) followed by Clotrimazole (CC) and clove oil were the most effective antifungal agents where 55.1% – 68.3 % of total tested isolates were sensitive to these compounds. Results showed also that 90.8% of isolates were resistant to Fluconazole (FLC). Also, 50 - 54 % of isolates were resistant to Cetrimide (CET), Itraconazole (IT) and Amphotericin B (AP). Aspergillus species belonging to sections Flavi, Nigri and Terrei were sensitive to Clotrimazole, Cetrimide, Terbinaine, Tioconazole and clove oil. On the other hand, most of these fungi showed resistance to Amphotericin B, Fluconazole, Itraconazole, Ketoconazole and Nystatin. Candida spp. showed variable degrees of sensitivity to Amphotericin B, Clotrimazole, Fluconazole, Ketoconazole and Nystatin. Regarding the age and sex distribution in the present study, the disease was more prevalent among adults between 21-40 years where it was diagnosed in 45 patients (51.1% of total cases) with statistically significant difference as 10 cases according to age group as 5 patients ≤10 years old, 3 cases were detected in the age group of 11–20 years, 45 cases were detected in the age group of 21–40 years, 25 cases were detected in the age group of 40-60 years and 10 cases ≥61 years. Male showed significantly higher incidence of otomycosis than females. Abdelazeem et al.[11] survey revealed that otomycosis was more common among patients between 21 and 40 years of age (45.4%) and higher in males (63.6%) than females (36.4%). That could be explained by the increased outdoor activities of males compared to females in Egypt; thus, males are more exposed to the risks of diseases. This result could also be attributed to the difference in surface lipids between males and females, as surface lipids are under the control of sex hormones. These findings were relatively close to those of a previous study in Texas,

which recorded that the incidence of otomycosis in males (56%) was higher than that in females (44%) aged up to 30 years old[14].

Conclusion

The overall clinicomycological and epidemiological profile of otomycosis infection observed at El-Manzala Hospital. Egypt does not differ significantly from those observed by previous researchers around the world, and any variation is probably due to the differences in climates of the different study populations. It is advised to establish a continuous collaboration between otolaryngologists and microbiologists for characterization of the pathogenic fungal isolates involved in otomycosis.

Study limitations

Methodological limitations of this study included its small sample size, and lack of a control group.

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