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RESEARCH PAPER

Identification of fungi from Otomycosis patients in Duhok city and their in vitro antifungal susceptibility testing

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A B S T R A C T:

The aim of this study was to determine the most common causative agents of otomycosis in Duhok city, Iraq; and subsequently study their sensitivity to the most commonly prescribed Drugs. From August 2021 till October 2021, a total of 90 patients (46 females and 44 males) who attended the outpatient clinic of ear, nose and throat (ENT) department at Azadi teaching hospital; were clinically examined for mycotic otitis. Ear debris was collected by sterile swabs and transferred to the laboratory for direct microscopic and macroscopic examination by culturing on sabouraud dextrose agar, potato dextrose agar and CHROM Agar Candida. Antifungal drugs were dissolved with (dimethyl sulfoxide) DMSO and used for evaluation of antifungal sensitivity by agar well diffusion method against commonly used antifungal drugs namely; Fluconazole, Itraconazole, Terbinafine, Nystatin, Amphotericin B, and Clotrimazole. In this study positive fungal infections were found in 88 (97.8%) of the collected samples, and it was more common among patients that aged 40 to 49 years. Mycological examination revealed the isolation of 24 genera and species with one variety. The most common fungal isolates were *Candida* (88.9%), followed by *Aspergillus* (28.8%) and non-identified yeast (25%), *Penicillium* (8.9%). Among the identified species, *Candida krusei* (44.4%) and *Candida albicans* (16.7%) followed by *Aspergillus niger* (15.6%) were the predominant species isolates.

KEY WORDS: Otomycosis, ENT, Fungi, Yeasts, Antifungal sensitivity test
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1. INTRODUCTION:

Fungi are responsible for some of the most severe and intractable human illnesses. They also give rise to a slew of less serious illnesses that are almost untreatable due to a lack of adequate treatment or the inability to deliver antifungal medicine in sufficient concentrations on site. Otomycosis is one of these instances. Otomycosis is a fungal infection that usually affects the medial aspect of the external ear canal. This disease can occur as a standalone infection or in combination with other bacterial infections (Barati *et al.*, 2011, Agarwal and Devi, 2017).

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Despite the fact that the disease is rarely life threatening, it is a difficult and challenging entity for both patients and otolaryngologists because it often necessitates long-term treatment and follow-up (Prasad *et al.*, 2014). Otomycosis can be subacute or acute, with symptoms such as pruritus, tinnitus, discomfort, otorrhea, otalgia, scaling, malodorous discharge, hearing impairment, sensation that something is in the ear canal and a blocking sensation (Agarwal and Devi, 2017, Pontes *et al.*, 2009).

The patients can be predisposed to otomycosis through numerous factors such as extensive use of topical antibiotic ear drops for the treatment of otitis media and otitis externa, poor health, wearing hearing aids, maceration of external auditory canal, minor inflammation or injury,

swimming pools, exposure to hot and humid climates such as those found in tropical and sub-tropical regions, and use of ear steroid drops in immunocompromised individuals (Agarwal and Devi, 2017, Abdelazeem *et al.*, 2015, Ozcan *et al.*, 2003, Yavo *et al.*, 2004).

Antifungal drug resistance results from improper antifungal drug administration because the clinical symptoms of otomycosis are similar to those of other ear infections (Jia *et al.*, 2012). In the ear canal, fungal organisms have a distinct appearance, mainly when magnified the filaments and spores of fungi resemble molds when growing on rotted food. There are numerous species of fungi that occur in otomycosis. include different types of fungi, such as hyaline saprophytic mold, dematiaceous saprophytic mold, yeasts, and, rarely, pathogenic molds like dermatophytes. *Aspergillus and Candida* spp. are the most frequently recovered organisms (Gharaghani *et al.*, 2015).

Although, the most common causative agent is the genus *Aspergillus*, especially *Aspergillus niger*. Additional fungi involved are species of; *Candida*, *Penicillium*, *Fusarium*, *Malassezia*, *Alternaria*, *Cladosporium*, *Mucor* spp., *Paecilomyces* spp, *Bipolaris* spp, *Rhodotorula* spp, *Rhizopus* spp and also various dermatophytes (Agarwal and Devi, 2017, Aneja *et al.*, 2010). Otomycosis is a unilateral infection that affects people of all ages. However, the majority of cases occur in young adults, in comparison to females, there is a slight male preponderance (Moharram *et al.*, 2013). Due to the importance of the subject and the lack of studies of otomycosis.

In Duhok governorate the present is carried out and aimed at isolation and identification of fungal species involved in otomycosis and determining the antifungal susceptibility patterns of the isolates in order to select the appropriate antifungals for MIC determination.

2. MATERIALS AND METHODS

2.1 PATIEETS

Ninety patients who attended the outpatient clinic of Otolaryngology Department, Azadi teaching Hospital were clinically examined for otomycosis during the period from August to October 2021. Examined patients were those who showed different symptoms such as: aural pain, itching and ear discharge; whether they had hearing loss or not. Also, erythema, fungal debris, and a creamy or blackish aural discharge were discovered during their examination. Those patients who had recently used antifungal topical medication were not included in our research.

2.2 Sample Collection and processing:

First of all, the outer ear canal was cleaned by a moist swab. To collect debris, fungal elements, and earwax from the external ear canal, sterile cotton swabs were used. Patients age groups were from-3 to 70 years and of both sex whom showing symptoms of otomycosis were included. All collected swabs were transported to the laboratory in appropriate media within 30 mins to the microbiology laboratory / College of Health Sciences/ University of Duhok.

2.3 Mycological analysis

A) Direct microscopic examination**:** The collected samples were directly examined microscopically using 10% KOH slide preparation and Lactophenol Cotton Blue stain (LPCB) as recommended by Ellis *et al.* (2007).

B) Culturing of samples*:* the samples were cultured by streaking the swabs on Sabouraud's dextrose agar medium (SDA), Potato dextrose agar PDA supplemented with 0.05 mg/mL of chloramphenicol (AppliChem GmbH, Darmstadt, Germany) to inhibit bacterial growth, and ChromAgar. Seven petridishes were used for each sample; one for each of SDA and PDA were incubated at 25°C, while two for each of SDA, two PDA, with one ChromAgar were incubated at 37°C. All plates were incubated for 4 -7 days with daily examination until colonies appeared or the culture revealed no growth.

2.4 Identification of fungi

Colony characterization and microscopic examination with KOH and Lactophenol with cotton blue staining were used to identify all species. And the characterization was carried out using the keys and descriptions provided by the authors, Ellis *et al*., (2007), Klich, (2002), Raper and Fennell, (1965), and Samson *et al.*, (2007).

The Isolation Frequency (Fr) of detected species in samples was calculated according to Gonzalez *et al.,* (1995) as follows:

$$\text{Isolation frequency FR \%} = \frac{\textbf{Number of samples on which a fungus appeared}}{\textbf{Total Number of Samples}} \times 100$$

2.5 Yeast Identification

All yeast colonies obtained from primary isolations were sub-cultured on a surface of Chromogenic Candida agar using a sterilized loop at 37°C for 48 hours. CHROM agar Candida is a specific culture medium that allows for the selective isolation of Candida. Then incubated for identifying Candida albicans, Candida tropicalis, Candida krusei, and Candida glabrata colonies due to LAB-M (AL019 HarlequinTM Candida Chromogenic Agar) color changes on the CHROMagar.

2.6 Antifungal susceptibility testing:

Well diffusion method was carried out for the antifungal susceptibility testing to determine the minimum inhibitory concentration (MIC), which is a simple, low-cost and easy to read technique Magaldi *et al*., (2004). The antifungal agents tested were: polyenes; (amphotericin B 50 mg and nystatin 100 units), azoles; (clotrimazole drop 1%, fluconazole 150mg and itraconazole 100 mg) and terbinafine 250 mg. Each antifungal drug's stock solution was made by dissolving 50 mg of the drug in 5 ml DMSO (dimethyl sulfoxide) in sterile screw-capped glass vials to achieve a concentration of 10000 µg /ml stock solution, then 1ml from it was transferred and prepared a serial dilution (25,50,75,100,125, 150,...), and the inhibition zone around each well was measured.

2.7 Statistical analysis

The STATA intercooled version 12.1 was used to analyze the data (StataCorp. LP, College Station, TX). The mean, standard deviation, median, and range were used to express quantitative data. Numbers and percentages were used to present qualitative data.

8-Preservation of samples

The growing fungi were kept in SDA slants and sterile Eppendorf tubes containing sterile glycerol in distilled water at a concentration of 20% for yeasts and 10% for filamentous fungi and were stored in the refrigerator at 4^{o}C for further mycological testing.

3. Results

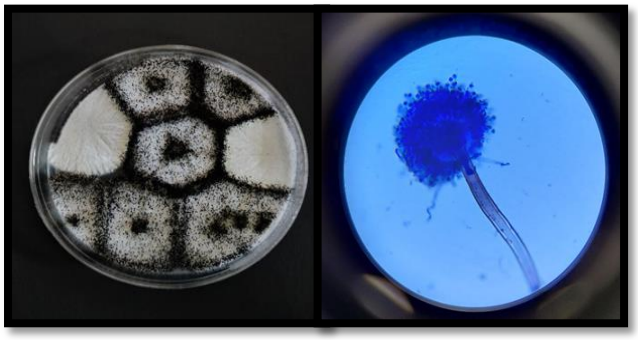
Out of 90 patients, 88 cases (97.8%) were positive for fungal growth. Among all 90 cases 44 were male (n=44; 48.9%) and 46 were female (n=46; 51.1%). Most frequent infected cases were at the age group 40-49 years old (n=28; 31.1%). Most of the studied patients have unilateral disease (n= 87; 96.7%) while only 3 patients (3.3%) have bilateral disease. The most common genus isolated in this study using the three media (PDA, SDA, and CHROMA) was *candida* (88.9%) followed by *Aspergillus* (28.8%) and non-identified yeast (25%) in addition to many other genera such as *Penicillium* (8.9%), *Alternaria* (3.3%), *Cladosporium* and *Geotrichum* (2.2%), with one variety which is *sterile mycelia* (3.3%) in addition to many other genera as it shows in table 1 and 2.

Table 1: Frequency percentage of isolated fungal genera

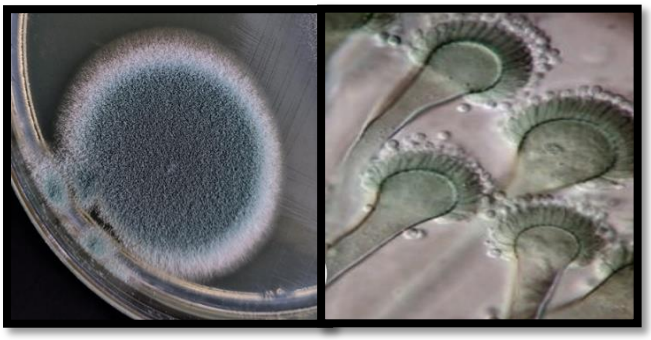
G. N	Frequency % of fungal genera on PDA, SDA, CHROME A	
	Fungal genera	Frequency %
1	*Candida*	88.9
2	*Aspergillus*	28.8
3	*Non-identified yeast*	25
4	*Penicillium*	8.9
5	*Alternaria*	3.3
6	*Sterillia mycelia*	3.3
7	*Cladosporium*	2.2
8	*Geotrichum*	2.2
9	*Dreschlera*	1.1
10	*Stachybotryus*	1.1
11	*Ulocladium*	1.1
12	*Chrysosporium*	1.1
13	*Rhodotorula*	1.1
14	*Rhizopus*	1.1
15	*Neoscytilidium*	1.1
16	*Neosartorya*	1.1

Table 2: Percentage of isolated fungal species in the present study**:**

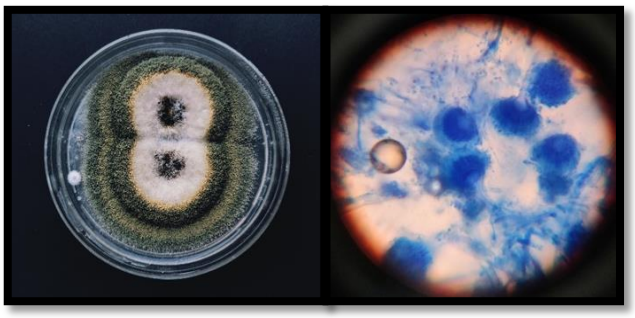
S.N.	**Frequency % of fungal species isolated from patients with otomycosis on PDA, CHROM Agar and SDA**	
	Fungal species	Frequency %
1	*Candida krusei*	44.4
2	*C.albicans*	16.7
3	*Aspergillus niger*	15.6
4	*A.flavus*	14.4
5	*C.glabrata*	14.4
6	*Penicillium marneffei*	7.8
7	*C.tropicalis*	4.4
8	*A. glaucus*	3.3
9	*A.parasiticus*	2.2
10	*Geotrichum candidum*	2.2
11	*A.fumigatus*	1.1
12	*A.niveus*	1.1
13	*Stachybotryus chartarum*	1.1
14	*A.foetidus*	1.1



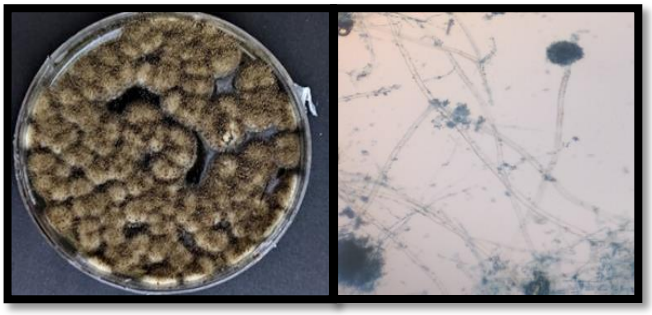
A **B**



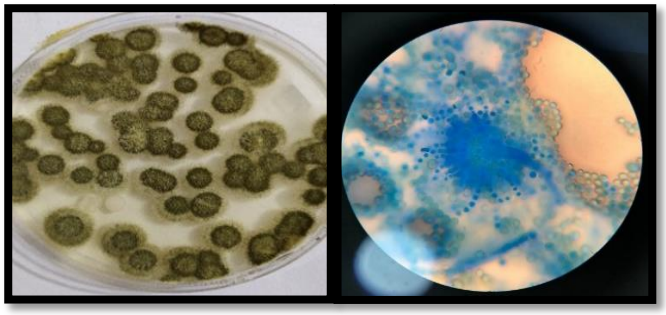
C **D**



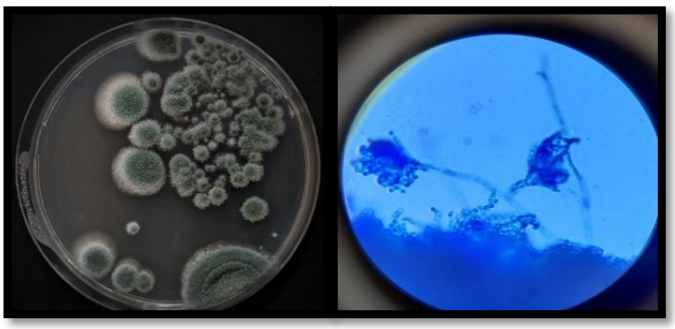
E **F**



G **H**



I **J**



K **L**

Figure 1 / Culture and prepared slide of isolated fungi in the present study: (A&B) *A.niger* (25 µm)/ (C&D) *A.fumigatus* (10 µm)/ (E&F) *A.flavus* (10µm)/(G&H) *A.foetidus* (10µm)/(I&J) *A.parasiticus* (20µm)/ (K&L) *P.marneffei* (20 µm)

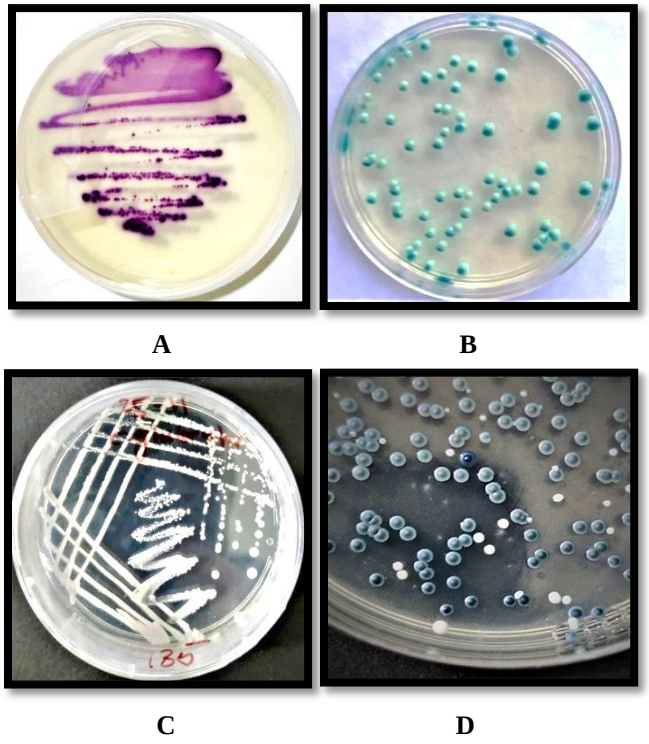


Figure 2: A *C.krusei* / B *C.albicans* / C *C.glabrata* / D *C. tropicalis*

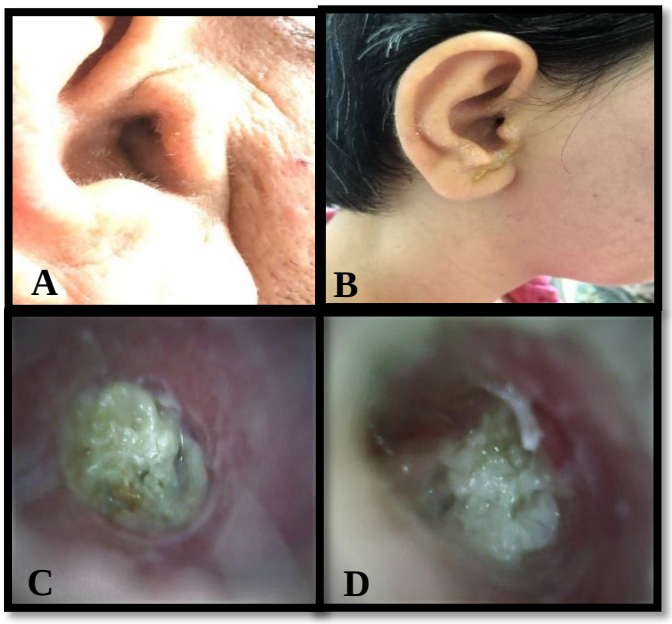


Figure 3: Ear of patients with otomycosis shows A. narrowing of external ear canal B. yellow fluid or pus draining from the outer ear. C. blockage of external ear canal D. hyphae of the fungus

Antifungal sensitivity test was accomplished using a well diffusion method for the most commonly used antifungal drugs such as polyenes; (amphotericin B 50 µg and nystatin 100 units), azoles; (clotrimazole drop 1%, fluconazole 150mg and itraconazole 100 mg) with terbinafine 250 mg. The minimum inhibitory concentration of each antifungal dilution is measured by observing the inhibition zone of fungi, as shown in table 3.

On performing antifungal sensitivity testing it was observed that all the isolates of *Candida* spp. were sensitive to nystatin and amphotericin B. however, one isolate (25%) was resistant to clotrimazole and 2 isolates (50%) were resistant to terbinafine.

Out of 7 *Aspergillus* spp. It was demonstrated that all the isolates were uniformly susceptible to all the tested antifungals. Out of other 13 fungal isolates, it was clear that all of them were sensitive to clotrimazole, nystatin, amphotericin B, and terbinafine. While one isolate (7.69%) was resistant to fluconazole, and two isolates (15.38%) were resistant to itraconazole.

Table 3/A: Minimal inhibitory concentrations (MIC) of the tested drugs against isolated fungal genus and species.

Fungal genera and species	MIC		
	Fluconazole µg /ml	Itraconazole µg /ml	Clotrimazole µg /ml
C.krusei	125	150	–
C.albicans	75	150	1
C.glabrata	100	150	0.25
C.tropicalis	25	75	0.25
A.niger	100	150	0.25
A.flavus	25	100	0.50
A.parasiticus	75	100	0.25
A.glaucus	75	–	0.25
A.foetidus	100	150	0.50
A.fumigatus	150	150	0.50
A.niveus	50	75	0.75
Rhizopus sp	100	150	0.25
P.marneffei	150	75	0.25
Rhodotorula sp.	150	–	0.25

St.chartarum	75	25	I
Cladosporium sp.	I	I	I
Drechslera sp.	150	150	0.25
Geotrichum candidum	150	150	0.50
Neoscytalidium sp.	125	125	0.25
Neosartorya sp.	125	150	0.75
Chrysosporium sp.	I	100	0.25
Alternaria sp.	I	I	0.25
Penicillium sp.	75	100	0.25
Ulocladium sp.	125	150	0.75

Table 3/B: Minimal inhibitory concentrations (MIC) of the tested drugs against isolated fungal genus and species.

Fungal genera and species	MIC		
	Nystatin µg /ml	Amphotericin B µg /ml	Terbinafine µg /ml
C.krusei	I	I	I
C.albicans	150	150	I
C.glabrata	150	75	175
C.tropicalis	25	75	175
A.niger	50	75	175
A.flavus	75	75	175
A.parasiticus	50	75	175
A.glaucus	25	100	25
A.foetidus	50	75	175
A.fumigatus	50	75	125
A.niveus	150	75	175
Rhizopus sp.	75	100	175
P.marneffei	25	25	50
Rhodotorula sp.	100	150	175
St.chartarum	I	I	I
Cladosporium sp.	150	I	75
Drechslera sp.	150	150	175
Geotrichum candidum	25	150	I
Neoscytalidium sp.	50	50	75
Neosartorya sp.	125	75	175
Chrysosporium sp.	150	150	125
Alternaria sp.	125	150	150
Penicillium sp.	25	25	175
Ulocladium sp.	75	75	175

Figure 4/ A: Minimum inhibitory concentration of Itraconazole against *Candida glabrata*

Figure 4/ B: Minimum inhibitory concentration of Clotrimazole against *Aspergillus niger*

Figure 4/ C: Minimum inhibitory concentration of terbinafine against *Aspergillus parasiticus*

4. Discussion:

Otomycosis is a mycotic infection of the outer ear canal that affects 10% of patients with otitis externa. Symptoms of the illness, which can be subacute or acute, include inflammation, itching, scaling, and severe discomfort. Mycosis causes inflammation, superficial epithelial exfoliation, masses of debris containing hyphae, suppuration, and discomfort (Adoga and Iduh, 2014). In the present study that performed in Duhok-Iraq, Azadi teaching hospital, 90 patients suspected of otomycosis were examined, the percentage of women affected by otomycoses was (46%) which is higher than that of men (44%), this is comparable to many other studies (Ologe and Nawabuisi, 2002, Saki *et al.*, 2013). This could be due to the use of turban which increases the darkness, humidity, and heat, all of which are

conducive to the growth of fungi (Ologe and Nawabuisi, 2002). The age group that is most commonly affected in our study was 40-49 however Al-abbasi *et al*., (2011) who found that the commonly affected age group was 21-30. In our study the predominant causative fungi were *candida spp.* over the genus *Aspergillus spp.* which is comparable to a study done by Pontes *et al*., (2009), and differ to those obtained by Al-abbasi *et al*., (2011). *C.krusei* (44.4%) was the most common isolated genera of *Candida* followed by *C.albicans* (16.7%) which is differ from other studies (Jia *et al.,* 2012). The commonest isolates of *Aspergillus* was *A.niger* (15.6%) followed by *A.flavus* (14.4%).

This is in accordance with other studies (Satish *et al.,* 2013, Ghiacei, 2008). The most common presentation in our study were pain (70 patients), followed by inflammation (50 patients), then otorrhea (34 patients). However, in another study the most common presenting symptoms were otorrhea (98.66%), otalgia (18.06%), and hearing loss (6.35%) (Ismail *et al.,* 2017). In our study the observed range of MIC in *C.krusei* for fluconazole was 125 µg /ml, and for itraconazole was 150 µg /ml. in *C.albicans* the MIC for fluconazole was 75 µg /ml, and for nystatin and amphotericin B was 150 µg /ml. while in *A.niger* the MIC for fluconazole was 100 µg /ml, itraconazole 150 µg /ml, clotrimazole 0.25 µg /ml, nystatin 50 µg /ml, amphotericin B 75 µg /ml, and for terbinafine 175 µg /ml.

5. Conclusion:

Our study demonstrated that otomycosis was very prevalent in Duhok city (97.8%). The most common fungal isolates in our study are *Candida* and *Aspergillus*, and the predominant species identified was *C.krusei. Candida* showed the highest sensitivity to nystatin and amphotericin B, while *Aspergillus* isolates were sensitive to all tested antifungals. In order to avoid or reduce otomycosis, patients should avoid using cotton or metallic sticks to remove ear wax, avoid over use of broad-spectrum antibiotics and keep ear canal dry.

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Conflict of interest:

There is no conflict of interest.

References:

Abdelazeem, M., Gamea, A., Mubarak, H. and Elzawawy, N., 2015. Epidemiology, causative agents, and risk factors affecting humanotomycosis infections. *Turkish journal of medical sciences*, *45*(4), pp.820-826.

Adoga, A.S. and Iduh, A.A., 2014. Otomycosis in Jos: Predisposing factors and Management. *African journal of medicine and medical sciences*, *43*(Suppl 1), pp.209-213.

Agarwal, P. and Devi, L.S., 2017. Otomycosis in a rural community attending a tertiary care hospital: assessment of risk factors and identification of fungal and bacterial agents. *Journal of clinical and diagnostic research: JCDR*, *11*(6), p. DC14.

Al-Abbasi, A.M. and Sabbar, B.A., 2011. OTOMYCOSIS IN BASRAH-IRAQ قارعلا-ةرصبلا يف نذلأا راطف. *Journal of the Arab Board of Health Specializations Vol*, *12*(2).

Aneja, K.R., Sharma, C. and Joshi, R., 2010. Fungal infection of the ear: a common problem in the north eastern part of Haryana. *International journal of pediatric otorhinolaryngology*, *74*(6), pp.604-607.

Barati, B., Okhovvat, S.A.R., Goljanian, A. and Omrani, M., 2011. Otomycosis in central Iran: a clinical and mycological study. *Iranian red crescent medical journal*, *13*(12), p.873.

Ellis, D. H., Davis, S., Alexiou, H., Handke, R., & Bartley, R. (2007). *Descriptions of medical fungi* (Vol.2). Adelaide: University of Adelaide.

Gharaghani, M., Seifi, Z. and Mahmoudabadi, A.Z., 2015. Otomycosis in Iran: a review. *Mycopathologia*, *179*(5-6), pp.415-424. doi: 10.1007/s11046-015-9864-7. Epub 2015 Jan 30.

Ghiacei S. (2008): Survey of Otomycosis in north-western area of Iran. Med J Mashhad Uni Med Sci;(43): 85-7.

Gonzalez, H.H.L., Resnik, S.L., Boca, R.T. and Marasas, W.F.O., 1995. Mycoflora of Argentinian corn harvested in the main production area in 1990. *Mycopathologia*, *130*(1), pp.29-36.

Ismail, M.T., Al-Kafri, A. and Ismail, M., 2017. Otomycosis in Damascus, Syria: Etiology and clinical features. *Current medical mycology*, *3*(3), p.27.

Jia, X., Liang, Q., Chi, F. and Cao, W., 2012. Otomycosis in Shanghai: aetiology, clinical features and therapy. *Mycoses*, *55*(5), pp.404-409.

Klich, M.A., 2002. *Identification of common Aspergillus species*. CBS.

Magaldi, S., Mata-Essayag, S., De Capriles, C.H., Perez, C., Colella, M.T., Olaizola, C. and Ontiveros, Y., 2004. Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*, *8*(1), pp.39-45.

Moharram¹, A.M., Ahmed, H.E. and Nasr¹, S.A., 2013. Otomycosis in Assiut, Egypt. *Journal of Basic & Applied Mycology (Egypt)*, *4*, pp.1-11.

Ologe FE, Nwabuisi C. Treatment outcome of otomycosis in Ilorin, Nigeria. West Afr J Med 2002; 21:34–6.

Ozcan, K.M., Ozcan, M., Karaarslan, A. and Karaarslan, F., 2003. Otomycosis in Turkey: predisposing factors, aetiology and therapy. *The Journal of Laryngology & Otology*, *117*(1), pp.39-42.

Pontes, Z.B.V.D.S., Silva, A.D.F., Lima, E.D.O., Guerra, M.D.H., Oliveira, N.M.C., Carvalho, M.D.F.F.P. and Guerra, F.S.Q., 2009. Otomycosis: a retrospective study. *Brazilian Journal of otorhinolaryngology*, *75*, pp.367-370.

Prasad, S.C., Kotigadde, S., Shekhar, M., Thada, N.D., Prabhu, P., D'Souza, T. and Prasad, K.C., 2014. Primary otomycosis in the Indian subcontinent: predisposing factors, microbiology, and classification. *International journal of microbiology*, *2014*.

Raper, K.B. and Fennell, D.I., 1965. The genus Aspergillus. *The genus Aspergillus.*

Saki, N., Rafiei, A., Nikakhlagh, S., Amirrajab, N. and Saki, S., 2013. Prevalence of otomycosis in Khouzestan Province, south-west Iran. *The Journal of Laryngology & Otology*, *127*(1), pp.25-27.

Satish H.S., Viswanatha B. and Manjuladevi M. (2013): A Clinical Study of Otomycosis. IOSR-JDMS; (5.2): 57-62.

Yavo, W., Kassi, R.R., Kiki-Barro, P.C., Bamba, A., Kple, T., Menan, E.I., Ehouo, F. and Koné, M., 2004. Prevalence and risk factors for otomycosis treated in the hospital setting in Abidjan (Ivory Coast). *Médecine tropicale: revue du Corps de santé colonial*, *64*(1), pp.39-42.

Article highlights:

1. Isolation of causative fungi from otomycosis patients.
2. Identification of isolated fungi.
3. Determining the sensitivity of isolated fungi against commonly used antifungal agents.
4. Observing the MIC range of each fungus.