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Otomycosis among Patients in Otorhinolaryngology Clinic Taiz City- Yemen

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إصابات الاذن الفطرية بين المرضي في عيادات الاذن والانف والحنجرة في محافظه تعز- اليمن

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الملخص

اصابات الاذن الفطرية تعتبر واحده من الاصابات الفطرية التي تصيب الاذن الخارجية والتي توثر بشكل اساسي على قناة السمع وطبله الاذن والاذن الوسطي. **هدف الدراسة:** تهدف الدراسة الي معرفه انتشار اصابات الاذن الفطرية والعوامل المؤثرة بين مرضي عيادات الاذن والانف والحنجرة في محافظه تعز. اليمن بالإضافة الي معرفه حساسيه الفطريات المعزولة تجاه المضادات الفطرية. **الطريقة:** خلال الدراسة تم جمع 300 عينه من المرضي باستخدام تقنيه مسحه الاذن وتم تعريفها بالطرق المكروبيولوجية. **النتائج:** اوضحت الدراسة ان اصابات الاذن الفطرية تنتشر في النساء (51.7%) أكثر من الرجال (48.3%) وأكثر الفئات العمرية تأثرا بين0–اقل من 10 سنوات وما بين 50–اقل من 60 سنه بنسبه من12.3 %و11.4% بالتتابع واظهرت علاقة إحصائية بين اصابات الاذن الفطرية والعمر (P=0.022) ولم تظهر علاقة إحصائية بين اصابات الاذن الفطرية والجنس (P=0.874). الم الاذن والحكة في الاذن هما أكثر الاعراض شيوعا وتوجد علاقة إحصائية بينها وبين اصابات الاذن الفطرية (P=0.00) وتعتبر الرطوبة من أكثر العوامل شيوعا لانتشار الإصابة. الفطريات الخيطية هي أكثر الفطريات المعزولة شيوعا 180(60%) والاسبرجلس فلافس هو الجنس الاكثر شيوعا وخمائر الكنديدا هي ثاني أكثر الفطريات شيوعا وتمثل 89 (29.67%) و38(12.7%) بالتتابع واظهرت الدراسة ان التراكنزوال وكيتاكونازوال اعطت أكثر حساسية ضد الفطريات الخيطية بينما الفلوكانازوال والتراكنزوال وكلوترازوال اعطت أكثر حساسية ضد الكنديدا.. **الاستنتاجات:** الفطريات الخيطية هي أكثر الفطريات المعزولة من المرضي وان المضادات الفطرية لها تأثير ملحوظ

الكلمات المفتاحية: الفطريات الخيطية، الكنديدا، اصابات الاذن الفطرية.

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Abstract:

Background: Otomycosis is considered one of the fungal infections of external ear which basically affects auditory duct, ear drum and middle ear. **Objectives**: The present study aimed to find out the prevalence and predisposing factors for Otomycosis among patients of Otorhinolaryngology in Taiz city, Yemen. In addition, it aimed to determine the sensitivity of the isolated otomycosis to some antifungal drugs . **Methods**: During this study, a total of (300) specimen with otomycosis have been collected from clinically diagnosed patients by using the ear swab technique and identified by microbiological procedures. **Results**: Distribution of otomycosis has been noticed to be more in females (51.7%) than males (48.3%). The highest infected age groups were between 0-<10 years and 50-<60 accounted for 12.3% and 11.4% respectively and there was a statistically significant association between age and otomycosis (P=0.022), while there was no statistical significance with sex (P=.874). The most common symptoms were pain and itching of the ear with a statistically significant association between them and otomycosis (P=0.00). Humidity highly occurred representing 12% of the tested number of samples. Most of the isolates were filamentous fungi representing 180(60%) of the total isolates. *Aspergillus flavus* was the most common pathogenic agent and yeast of *Candida sp* was the second most common representing 89 (29.67%), 38 (12.7%) respectively. Itraconazole and ketoconazole were the most effective antifungal against most filamentous fungi isolates, while Fluconazole, Itraconazole and Clotrimazole were more effective against *Candida sp*. **Conclusions**: Filamentous fungi were the most prevalent isolates from infected patients. Antifungal drugs showed reliable activity in *vitro.*

Keywords: Filamentous fung, Candida sp, Otomycosis.

INTRODUCTION

Otomycosis is considered one of the fungal infection of external ear which basically affect auditory duct, ear drum and middle ear. It is a chronic or sub-chronic inflammation of the ear that seldom includes the middle ear. (Kiakojori *et al*., 2018). It is spreads worldwide, especially in tropical and subtropical regions and it has been estimated to cause 15–20% of external otitis. This is more common in warm, humid climates, individuals with poor hygiene and lower socioeconomic status (Garcı´a-Agudo *et al*., 2011). This is worldwide in distribution with prevalence ranging from 9% to 30% among patients presenting with signs and symptoms of otitis external and discharging ears in otolaryngology settings (pontes *et al*.,2009). It is challenging for both patients and otolaryngologist because it usually requires long-term treatment and follow-up. In spite of this, recurrences may occur, (Punia *et al*., 2019). Predisposing factors of otomycosis mainly include: heat, humidity, a poor personal hygiene, bathing or diving in fresh water or seawater, and previous antibiotic therapy for a bacterial otitis. (Pontes *et al*., 2009; Aneja *et al*., 2010& Sogebi *et al*.,2024). In addition to tympanic membrane perforation, the hearing aid usage and self-cleaning with cotton swabs are other predisposing factors. (Jackman *et al*., 2005; Munguia *et al*., 2008). The most genus that causes otomyosis are *Aspergillus* and *Candida albicans* (Alseaesdey *et al*.,2022). Other less frequently involved fungi include *Penicillium* and *Mucor species*. (Chauhan & Surender 2021). Fungi can be defined as the secondary pathogens that affect skin of canal only after it is exposed to bacterial toxin. Recent evidence confirms the notion that fungi can be primary pathogens. (Siddiqui *et al*., 2017). It is grow quickly on the rigid layer of the skin and cause swell to inflammation accompanied by unspecific symptoms: ear pain itching, scaling of the epithelium and poor of hearing. (Garcı´a-Agudo *et al*., 2011). There are two main groups of antifungal agents used to treat of otomycosis, the polyene and azole derivatives. (AL.astruey-Izquierdo *et al*., 2015).

In Taiz City, there is a problem in diagnosing of fungal ear infection and the diagnosing of bacterial ear infection that lead to deficiency in the treatment. In addition to, the prolonged use of topical antibiotics ear drops causes suppression of the bacterial flora with subsequent emergence of fungal flora, causing fungal super infection (Juyal *et al.*, 2014; Rachna *et*

al., 2014 and Deshmukh *et al.,* 2014). However, the studies that deal with incidence of otomycosis among patients of Otorhinolaryngology are limited in Taiz City, Yemen and which was one of the most important reasons for carrying out the present study.

MATERIALS & METHODS

Target population

A total of 300 patients suffering from otomycosis were enrolled in this study. Their age ranged from less than 1 year to 70 years (145 males and 155 females).

Data Collection

All patients were interviewed using a questionnaire which included: personal data, predisposing factors, clinical data and symptoms.

Study design

A cross sectional descriptive study was conducted during the period from October 2021to March 2023.

Study area

This study was conducted in three Otorhinolaryngology centers located in Taiz city (AL Mahmody center, AL Masni center, and AL Abdowadod AL.Samgy center).

Samples Collection

A total of 300 ear swab specimens were collected from patients with clinically suspected otomycosis. The samples were collected from Otorhinolaryngology clinics of Taiz City-Yemen. Another 20 health persons without ear infection symptoms were used as control. Samples were collected with sterile swab under aseptic conditions and were send to the laboratory.

Sample size estimation

The sample size (300) was calculated using the following formula: (Charan & Biswas, 2013).

Examination of the Specimens

Direct examination

Direct smear examination of ear swabs included 10% KOH on a glass slide (Talwar *et al*., 1988).

Isolation of Fungi

Ear swab inoculated into two Sabouraud dextrose agar supplemented with gentamycin 80%. Inoculated plates were then incubated at room temperature (25–28°C) and observed on daily basis for any fungal growth for up to 7 days and another incubation at 37°C, (Rippon, 1974). Then the molds inoculation (subculture) on Czapek Dox agar at room temperature (25–28°C) and observed on daily basis for any fungal growth for up to 7 days (Ali *et al*., 2017).

Identification of isolated fungi

The mycological identification was based on macroscopic and microscopic examination of the culture isolates. (Campbell *et al*., 2013)**.**

Identification of filamentous fungi

The macroscopic examination of filamentous fungi was identified according to their colonial morphology (time of growth, texture, color, presence of fungal elements) Raper &Fennell (1965), Moubasher (1993) and Barnett *et al*., (2000).

Identification of fungi was based on microscopic characteristics as described by Raper & Fennell (1965) and Moubasher (1993), All filamentous fungi isolates were grown on Sabouraud dextrose agar plates, exanimated microscopically with lactophenol cotton blue and then examined under the microscope looking for two genes *Aspergillus sp* and *penicillium sp*. (Zafar *et al*., 2017)

Identification of yeast

The macroscopic examination of isolated species was characterized by duration of growth, surface morphology and pigment production on the reverse, (Campbell *et al*., 2013 and Kidd *et al*., 2016).

Yeasts isolates were showed white to creamy colored smooth colonies.

Yeasts isolates were examined under the microscope looking for yeast budding cells. Moreover, the isolates were stained with Gram stain to detect their reply to stain (Yan *et al*., 2013 and Saranya *et al*., 2014). Then examined the production of germ tube from yeast isolates under microscope. *C.albicans* was done for sprouting yeast cells, which were tube-like outgrowths from the cells (known as germ tubes). (Cheesbrough, 2006).

In vitro Antifungal Drugs Susceptibility Testing

A fungal isolate from otomycosis infection was tested for sensitivity against various antifungal agents using agar disc diffusion method. (Ellis,

2011). The used antifungals were fluconazole (10mcg), ketoconazole (30mcg), itraconazole (30mcg), clotrimazole (10mcg), and Nystatin (50mcg).

Data Analysis:

Data were subjected to analysis of variance by using the Statistical Package for the Social Science (SPSS) Version 21.

RESULTS AND DISCUSSION

Microbiological Analysis

Identification of otomycosis

A total of 300 ear swab specimens were identified from cases of otomycosis during the period of the study in Taiz city. 145 cases were males and 155 were females, their ages ranged from <1 to 70 years. 20 ear swabs were collected from healthy persons without ear infection symptoms as control. Basically, the morphological features on culture media (Sabouraud dextrose agar and Czapek's Agar), growth at 28 & 37°C, lactophenol cotton blue mount, wet preparation, gram stain and germ tube formation were performed to identify the isolates.

Most of the isolates were filamentous fungi representing 180 (60%) of the total ear samples. Yeast *Candida species* were the second most common isolates accounting for 38 (12.7%). The remaining of ear samples did not exhibit any growth (negative cases), representing in 82 (27.3%) **(Table 1).** So the results of the study showed that otomycosis caused by filamentous fungi was higher than otomycosis caused by yeast (*Candida sp*).

Table (1) Microbiological analysis of 300 cases investigated during the period of the study

Result of Identification	Identification Process						
	Direct Ex.	Growth on culture media at 28°C		LPCB	Growth on culture medium (SDA) at 37°C	Gram stain	Germ tube
	KOH 10%	SDA	CDA				
Filamentous fungi	180	180	180	180	0	0	0
Candida species	0	0	0	0	38	38	23
No growth	0	0	0	0	0	0	0
Total	180	180	180	180	38	38	23

KOH: potassium hydroxide SDA: Sabouraud Dextrose Agar CDA: Czapek Dox agar LPCB: Lactophenol cotton blue.

The results of microbiological identification of our study are similar to those obtained by Siddiqui *et al*., (2017) who cultured *Aspergillus* and *Candid* on SDA medium at two different incubation temperatures, 37 and 25°C. *Aspergillus* isolates were identified by colony morphology and LPCB wet mount, while *Candida species* isolates were identified by colony morphology, Gram staining and germ tube formation.

Distribution of otomycosis among the cases

A total of 300 samples were collected in the study. Among the examined specimens 218 samples (72.7 % of total specimens) showed growth on isolation media (positive results). The isolated fungi were *Aspergillus flavus* 89 (29.67%), *A. fumigatus* 41 (13.67%), *Candida species* 38 (12.67%), *A. niger* 34 (11.33%), *Penicillium sp* 10 (3.33%) and *A. terreus* 6 (2%), while the remaining of specimens 82 (27.33%) showed no growth (negative cases) (Figure 1).

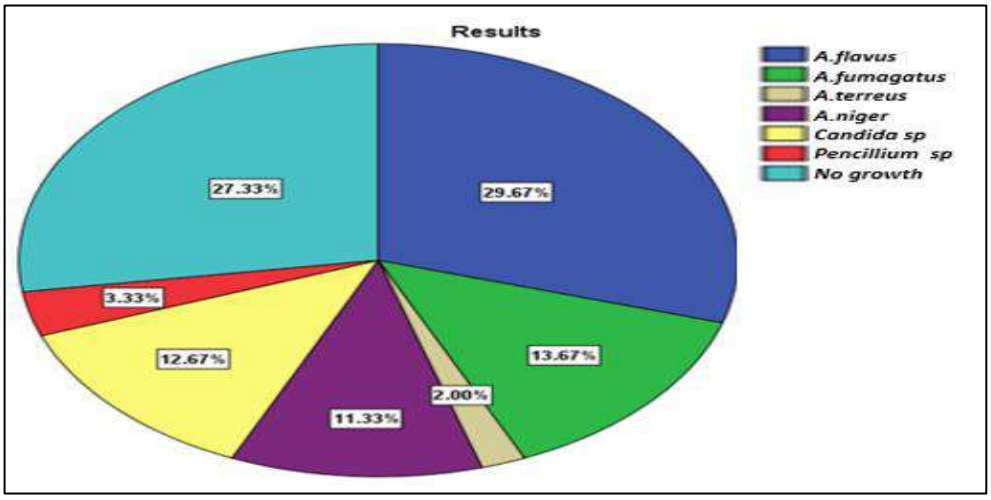

Figure (1) Distribution of otomycosis among the cases

The results of this investigation are similar to those obtained by Enweani *et al*., (1998); Tang *et al*.; (2006) and Mohammed *et al*., (2020) who reported highest prevalence of *Aspergillus flavus* among otomycosis patients. Mistry & Pathak (2020) found out that *Aspergillus species* was the commonest fungi involved in Otomycosis. In addition, Alam *et al.*, (2023) mentioned that *Aspergillus species* was the dominant species isolated, about 185 (91.1%) among 230 isolates, followed by Candida in 18 (8.9%) cases.

Vennewald *et al*., (2010) showed that *Aspergillus* and *Candida* spp were the most frequently isolated fungi in patients with otomycosis. On the other hand, these results are different from those reported by Mahmoudabadii (2006) who reported that *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus, penicillium spp, Candida albicans, Candida parapsilosis and Rizopus* spp. were the most common fungal pathogens in otomycosis. Gharaghani *et al*. (2020) isolated fungi causing otomycosis from 77 patients. *Aspergillus niger* complex (n=36) was found to be the most common agent, followed by *A. flavus*, *A. terreus*, and *A. nidulans* complexes. Nasser *et al*., (2019) carried out a study on 95 patients with otomycosis. After the clinical examination and laboratory tests, they showed that *candida albicans* was the main isolate accounting for 28 (29.4%) followed by *Aspergillus niger* 23 (24.2%) and *Aspergillus fumigatus* 18(18.9%).

Distribution of Otomycosis cases according to the sex of patients

The otomycosis cases in relation to sex are clarified in Figure (2). Out of 145 clinical cases in males, 104 were positive to fungal cultures and 41(13.67) were negative. 45 cases of *A. flavus* (15%), 16 cases of *A. niger* (5.33%), 3 cases of *A. terreus* (1%), 21 cases of *A. fumigatus* (7%), 5 cases of *Penicillium sp* (1.67%) and 14 cases of *Candida* sp. (4.67%). In females, out of 155 specimens, 114 were positive to fungal cultures, whereas 41(13.67) were negative. There were 44 cases of *A. flavus* (14.67%), 18 cases of *A. niger* (6%), 3 cases *A. terreus* (1%), 20 cases of *A. fumigatus* (6.67%), 5 cases of *Penicillium sp* (1.67%) and 24 cases of *Candida* sp.(8%).

The results of otomycosis infections indicated that females are highly affected than males, occurring in 51.7% and 48.3% respectively.

Results of chi-square test showed that there is no statistically significant association between sex of patients and otomycosis infections $P>0.05$ ($\chi^2=2.454$ $P=.874$).

Similar findings were reported by Kiakojori *et al*., (2018) who showed that 11 (7.3%) cases had a recurrence of otomycosis. There is no significant relationship between the incidence of otomycosis and sex (P= 0.88), Ali *et al*., (2018) also noted that among 43 males, 25 were positive cases (40.9%) and among 57 females, 36 cases (59.01%) were positive.

Another investigation was conducted by Fasunla *et al*., (2007), they studied 5784 patients with ear diseases, 378 (6.54%) had otomycosis distributed between 145 (38.36%) males and 233 (61.64%) females. Panchal *et al*., (2013) also found out that females were commonly affected (56.52%). Yousif *et al;* (2024) as well reported a high incidence of otomycosis in females and However, our results are different from those reported by Enweani and Igumbor (1998) who indicated that a higher prevalence was observed in male samples than in female samples. This could be interpreted by differences in the environmental factors. Others also reported a high incidence of otomycosis in males, (Yassin *et al*., (1978); Kaur *et al*., (2000); Agrawal Priti *et al*., (2017) & Panigrahi *et al*., (2019)).

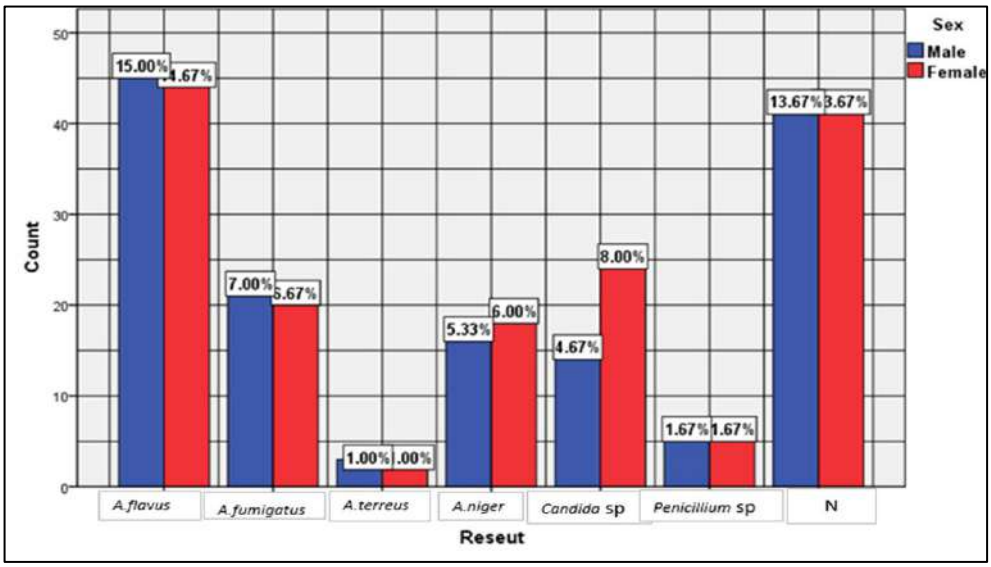

Figure (2) Distribution of otomycosis cases according to the sex of patients

Distribution of Otomycosis Cases According to the Age of Patients

The results indicated that the age group between 0-<10 and 50-<60 years were more susceptible to the infection and constituted 12.3% and 11.4% from the total cases respectively, followed by age group of 40-<50 years which occurred in 10.9%. The ages between 10-<20, 30-<40, 20-<30 accounted for 10.7%, 10.3% and 9.6 % respectively and the age group of 60-<70 years were less susceptible representing in 7.3%. (Table 2).

Table (2) Distribution of otomycosis cases according to the age of patients

Isolated fungi	0-<10		10<20		20-<30		30-<40		40<50		50<60		60<70	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
A. flavus	18	6	11	3.7	13	4.3	16	5.3	12	4	13	4.3	6	2
A. niger	1	0.3	6	2	4	1.3	8	2.7	7	2.3	5	1.7	3	1
A. terreus	0	0.0	0	0.0	2	0.7	1	0.3	0	0	3	1	0	0
A. fumigatus	4	1.3	9	3	1	0.3	2	0.7	6	2	11	3.7	8	2.7
Penicillium sp.	2	0.7	0	0	2	0.7	1	0.3	4	1.3	0	0	1	0.3
Candida sp.	12	4	6	2	7	2.3	3	1	4	1.3	2	0.7	4	1.3
Total	37	12.3	32	10.7	29	9.6	31	10.3	33	10.9	34	11.4	22	7.3

Results of chi-square test from Figure (3) show that there is a statistically significant association between different age groups of patients and otomycosis infections (χ^2=55.1 p=0.022).

Similar results are mentioned in a cohort study carried out by Rathnaraajan *et al*., (2019). They indicated that ear diseases were more common at 6 years (15.60%) and less common at 14 years (7.51%). Osazuwa *et al*., (2011) also indicated that patients of 5 years or younger had significantly higher prevalence of otitis media compared with other children and adults. Panchal *et al*., (2013) found out that the most common age group involving in the infection was 51-60 years old (47.82%). However, our results are different from the results obtained by Kiakojori *et al*., (2018) and Ali *et al*., (2018). The former showed that there was no significant relationship between the incidence of otomycosis and age (P=0.86) and the latter also mentioned that no significant association between age and ear infection (p = 0.094).

Distribution of Otomycosis Cases According to Predisposing Factors

The results indicated that the humidity is the highest factor affecting the developing of otomycosis infections and represented in 12% of the tested cases, followed by previous antibiotic therapy in 4.6%. Malnutrition, chronic diseases and poor personal hygiene accounted for (3.4%, 3.2% and 1.3%) respectively, whereas swimming is the least affecting factor occurring in 0.8%. (Table 3).

Results of chi-square test showed that there is a statistically significant association between different predisposing factors and otomycosis infections (P=0.00).

Table (3) Distribution of otomycosis cases according to predisposing factors

predisposing factors	Yes	No	P-value
Humidity	216(12%)	84(4.7%)	0.00
Previous antibiotic	82(4.6%)	218(12.1%)	
malnutrition	62(3.4)	238(13.2)	
Swimming	14(0.8%)	286(15.9)	
Chronic disease	57(3.2%)	243(13.5%)	
Poor personal	24(1.3%)	276(15.3%)	

These findings are similar to those obtained by Kaur *et al.,* (2000) and Prasad *et al*. (2014) who noted that humidity and high environmental temperature are the most important risk factors for developing otitis externa. On the other hand, our results disagreed with those reported by Darko *et al*. (2004), who found out that the most common predisposing factors for otomycosis were swimming in public pools, baths, or diabetes mellitus. Juyal *et al*. (2014) and Rachna *et al*. (2014), noted that prolonged use of topical antibiotics or steroids ear drops causes suppression of the bacterial flora with subsequent emergence of fungal flora, causing fungal super infection. Moreover, Deshmukh *et al*. (2014), indicated that predisposing factors leading to *Aspergillus* infections were prolonged using of topical steroid and antibiotic ear drops.

Distribution of Otomycosis Cases According to Symptoms

The results indicated that ear pain is highly available in otomycosis represented in 19.9% of the tested number, followed by ear itching in 19.6% of cases, recurrence of ear infection and scaling of the epithelium (7.9% and 4%) respectively, whereas poor hearing is less existing accounting for 2.7%.

Results of chi-square test showed that there is a statistically significant association between different symptoms and otomycosis infections (P=0.00)

Similar observations were reported by Panchal *et al*., (2013) who stated that the most frequent symptom was chronic ottorhea (ear pain). Yahia *et al*., (2021) also noticed that ear pain is the most important symptom associated 194 patients clinically diagnosed of otomycosis

These results are dissimilar to those reported by Itabangi *et al*., (2018) who indicated that there are no statistically significant symptoms associated with otomycosis. Sheahan *et al*., (2002) and Caylan (2006) found out that

poor hearing or hearing loss is the most important symptom associated with otitis media in children at various ages.

Antifungal activity

Five antifungal drugs commonly used in sensitivity tests of fungal infections [Itraconazole (30 mcg), Ketaconazole (30 mcg), Nystatin (50 mcg), Fluconazole (10 mcg) and clotrimazole (10 mcg)] have been tested against 218 isolates of otomycosis in the study; *Aspergillus flavus* 89, *A. fumigatus* 41, *Candida species* 38, *A. niger* 34, *A. terreus* 6 and *Penicillium* sp 10. (Figure 3)

The results indicated that the susceptibility of *Aspergillus flavus* isolates towards itraconazole was recorded to have a mean of inhibition zone diameter 21mm, followed by ketaconazole, nystatin, clotrimazole with means of 21, 16, 15 mm respectively, whereas there was not any sensitivity towards fluconazole. The findings also indicated that the susceptibility of *A. fumigatus* towards itraconazole was recorded to have a mean of inhibition zone diameter 20 mm, followed by ketaconazole, nystatin, clotrimazole with means of 19, 18, 15 mm respectively, whereas fluconazole had no effect against the same isolates. In addition, the results revealed that *A. niger* isolates showed sensitivity to clotrimazole with a mean of inhibition zone diameter 25 mm, followed by fluconazole, nystatin, itraconazole and ketaconazole with means of 20,17,15,10 mm respectively, *A. terreus* isolates of this study also exhibited sensitivity towards clotrimazole with a mean of inhibition zone diameter 22 mm, followed by itraconazole and ketaconazole with means of 10, 9 respectively, although they showed resistance towards fluconazole and nystatin. Moreover, the results clarified that itraconazole and fluconazole showed their efficacy against *Candida* species isolates with identical mean of inhibition zone diameter 47 mm, followed by clotrimazole, ketaconazole and nystatin with means of 40, 39, 25 mm respectively. The results also indicated high susceptibility to ketaconazole towards *Penicillium sp* with a mean of inhibition zone diameter 38 mm, followed by nystatin, itraconazole, fluconazole and clotrimazole with means of 31, 25, 23, 21 mm respectively.

These results are different from those reported by El-Tahtawi *et al.*, (2019) who indicated that *Candida albicans* and *Aspergillus flavus* were very sensitive to fluconazole.

Nemati *et al*., (2013) indicated that *Aspergillus fumigatus* showed high sensitivity to clotrimazole followed by fluconazole (28.57 %) and ketoconazole (28.57 %) and indicated that *Candida albicans* was sensitive to clotrimazole, fluconazole, ketoconazole and nystatin. Fluconazole showed the highest effectiveness compared with other drugs. While *Penicillium sp* had sensitivity to clotrimazole only.

Sultan & Saadullah (2023), mentioned that *Aspergillus niger* was sensitive to clotrimazole, fluconazole and itraconazole.

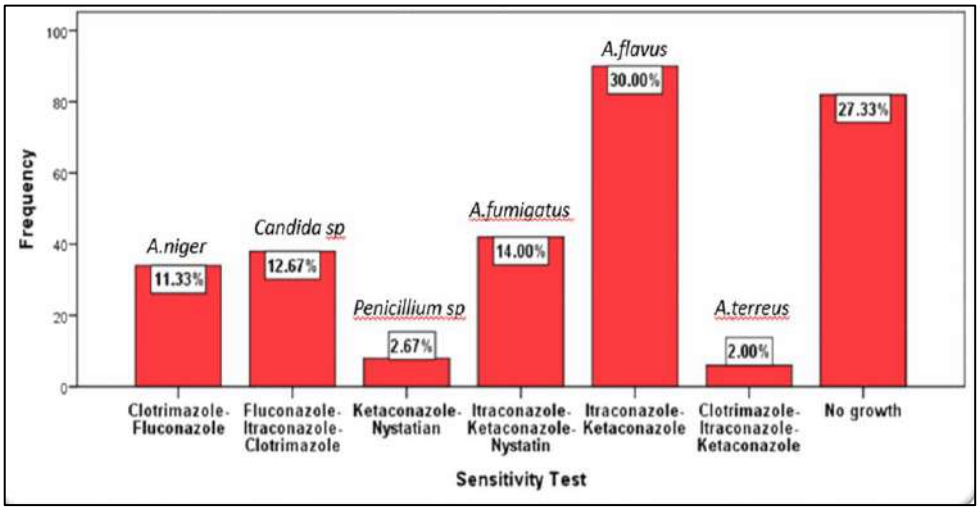

Figure (3) Effect of antifungal drugs on otomycosis

CONCLUSION

In this study a total of 300 patients were examined. 218 patients showed positive results and only 82 patients showed negative results. The data analysis showed the following:

1- The distribution of otomycosis was higher in female patients than in male patients; and the higher distribution of otomycosis was found in the age group 0-<10 years and 50-<60 years.
2- Pain and itching of the ear were the most common symptoms associated with otomycosis.
3- The highest prevalence of otomycosis was found among patients who: usually had humidity in the ear, used previous antibiotic, had malnutrition status, usually used to swim in pools, had chronic diseases and had low personal hygiene.

4- The most commonly isolated filamentous fungi were *Aspergillus sp* and *penicillium sp* and *candida sp* were the second most isolated fungi.

5- Itraconazole and ketoconazole were the most effective antifungals against most filamentous fungi isolates, while Fluconazole, Itraconazole and Clotrimazole were more effective against *Candida sp.*

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