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Sensitivity of Fungi Isolated from Patients Infected with Otitis Externa by Using Antifungal Drugs

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ABSTRACT: Otitis externa refers to an acute or sub-acute inflammatory condition affecting the skin of external ear and its uricle. Otitis externa is a common disease in all regions especially in tropical and subtropical areas and is usually caused by bacterial or fungal infections. This study aimed to study the pattern of different antifungal drugs activity by disc diffusion method on fungi isolated from patients having otitis externa attended ENT clinics of different age group of both sexes over one-year period in Mosul city/Iraq. One hundred patients identified clinically as having otitis externa and 100 control group were included. Skin scrap and swab of discharge were taken from each patient. The clinical specimens were examined macroscopically and microscopically to identify the species and genus of fungi involved in otitis externa. Among the 100 patients included, 64 (64%) showed fungal elements in their clinical specimens, 36 (56%) were male and 28 (44%) were females. The isolated fungi of genus *Aspegillus* were 38 (59%); *Aspergillus niger* 24 (37%), *Aspergillus flavus* 14 (22%). Isolates of the genus *Candida* accounted 22 (34%); 20 (31%) were *Candida albicans* and 2 (3%) were *Candida glarata. Penicillum* spp. was identified in 3 (5%), and *Rhizopus* spp. was identified in 1 (2%) sample. However, coinfection with *Aspergillus niger* and *Candida albicans* was identified in 2 (3%). On the other hand, *Candida albicans* was identified in 8 (8%) of control group. Seasonal variation was found in this study, the highest number of otitis externa cases was found in June, July and August. In this study, males were found more affected by otitis externa than females. *Aspergillus niger* and *Candida albicans* were the most commonly encountered species. Seasonal variation was reported with higher percentage in summer months. Voriconazole and ketoconazole have had the higher activity against fungi isolated in this study than other antifungal agents.

KEYWORDS: *Candida*, Fungi; Otitis externa; Sensitivity test.

1. INTRODUCTION

Recently, there has been a dramatic increase in the incidence and diversity of pathogenic fungi involve in otitis externa, which refer to a spectrum of superficial mycotic inflammatory infection affecting external auditory canal and its utricle, the unique structure of external auditory canal contribute to development of otitis externa, which is warm dark and prone to becoming moist that make it an excellent environment for bacterial and fungal growth [1,2]. Ear canal is easily traumatized, which debris, secretion and foreign bodies is impede by curve at the junction of the cartilage and bone in addition to the presence of hair especially thicker hair is common in older men, Excessive moisture and trauma, both of which impair the canal natural defenses [3].

Otitis externa infections characterized by generalized edema, erythema that associated with itching, discomfort with ear discharge and pain, although sometimes associated with a variety of non-infectious systemic or local dermatological process, the most characteristic finding on ear examination is the presence of grayish, white, thick debris called as wet blotting papers [4].

Pathology of otitis externa is common throughout the world, its frequency varies according to different geographic zone, in relation to environment factors as temperature, humidity with a higher prevalence in the hot and dusty climate of the tropical and subtropical area in addition to the time of the year [5]. The most commonly cause of otitis externa is bacterial infection, and occasionally fungi, about 1 in 8 of infection is fungal in origin 90% of fungal infection involve from genus *Aspergillus* species and the rest from genus *Candida* [6].

From genus *Aspergillus,* species *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* were the most commonly involved species, however from genus *Candida* (*Candida albicans*, and *Candida parapsillosis*) were the most commonly involved in otitis externa infection then followed by *Penicillium*, and *Rhizopus* species [7].

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Antifungal treatments chosen based on identification of fungi by direct microscopical examinations and culture, this might be due to various types of causative fungi were sensitive or resistant to specific types of antifungal agents [8].

This study aimed to study the pattern of different antifungal drug activity by disc diffusion and broth dilusion method on fungi isolated from patients having otitis externa attending ENT clinics in different age group of both sexes in one year period in Mosul city.

2. RESULTS

The studied individuals including 100 (100%) patients, the male patients were 50 (50%), while the female patients were 50 (50%), from them there were 64 (64%) who showed the presence of fungal elements in their clinical specimens, the male were 36 (56%), and the female were 28 (44%). The presence of fungal element increases when age increase in females the highest presence of fungal element where in age group (31-35, 36-40, 41-45, 46-50), while in male the presence of fungal elements decreases when age increase, however the highest presence of fungal elements where in age group (21-25, 26-30, and 31- 35) years, the presence of fungi in external ear of control group was 8(8%), from males were 6(6%) while from females were 2 (2%) as shown in table 1.

Table 1. The isolated fungi in male and female patients and control according to age group.

Age group	Patients Male	Patients Female	Control Male	Control female	Positive Male patients	Positive Female patients	Positive Male control	Positive female control
5-10	0	0	0	0	0	0	0	0
11-15	4	0	2	0	0	0	0	0
16-20	5	4	6	6	4	0	0	0
21-25	9	4	10	6	6	2	2	1
26-30	10	6	8	10	8	4	4	0
31-35	10	8	10	10	8	6	0	1
36-40	6	10	6	6	6	4	0	0
41-45	4	8	6	8	2	6	0	0
46-50	2	10	2	4	2	6	0	0
Total	50	50	50	50	36	28	6	2

2.1. Lateral and side of otitis externa

The infection affecting the ear were unilateral in all patients involved in this study, although unilateral right ear infection 41 (64%) was higher the unilateral left ear infection 23 (36%) as shown in table 2.

Table 2. Lateral and side of ear infection.

Side of ear infection	No.	%
Unilateral right ear infection	41	64
Unilateral left ear infection	23	36
Bilateral ear infection	0	0
Total	64	100

2.2 The isolated fungi

From 64 (64%) patients showed that fungal elements in their clinical specimens the higher isolated fungi from patients with otitis externa were from genus *Aspegillus* 38 (59%) isolates from them there were *Aspergillus niger* 24 (37%) *Aspergillus flavus* 14(22%), followed by isolates from genus *Candida* 22 (34%) from them there were *Candida albicans* 20 (31%), and 2 (3%) *Candida glarata*, in addition to *Penicillum* spp. 3(5%), from genus *Rhizopus* there were 1 (2%). However, the mixed isolates were 2 (3%), these mixed isolates were *Aspergillus niger* with *Candida albicans*. In addition to 8 (8%) isolated *Candida albicans* from control group (Table 3).

Table 3. Fungal species isolated from pure and mixed culture.

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Isolated fungi	Total		pure		Mixed	
	No.	%	No.	%	No.	%
Aspergillus niger	24	37	22	34	2*	3
Aspergillus flavus	14	22	14	22	0	0
Pencillum spp.	3	5	3	5	0	0
Rhizopus spp.	1	2	1	2	0	0
Candida albicans	20	31	18	28	2	3
Candida glabrata	2	3	2	3	0	0
Total	64	100	60	94	4	6

*Mixed *Aspergillus niger* with *Candida albicans*

2.3. Seasons and otitis externa

Seasonal variation was showed figure (1) the number of cases were increased when the temperature increased, the higher isolated cases were during the following month of year (August, July, and June) then the number of cases were decreased. Seasonal variation was observed during collecting the cases.

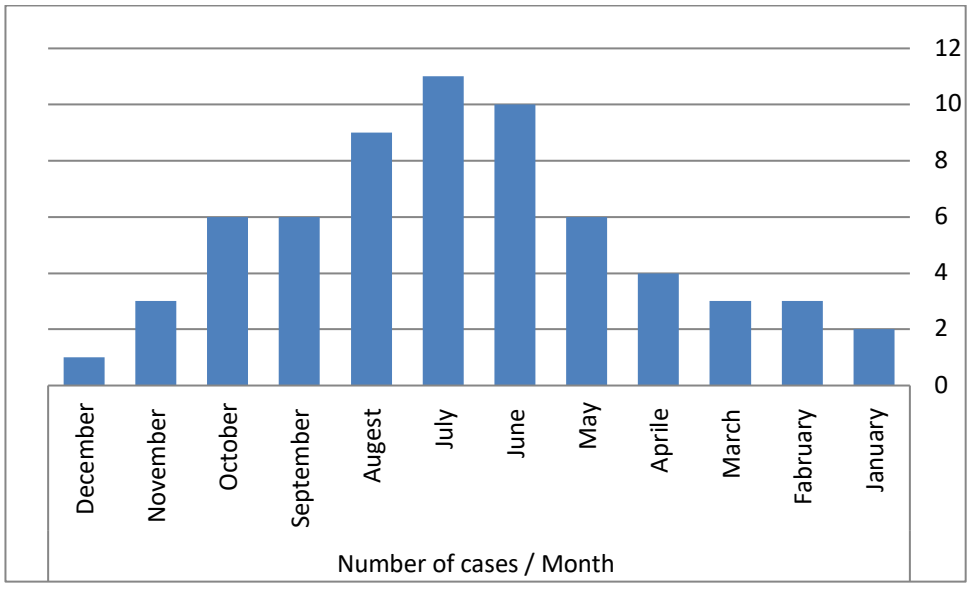


Figure 1. Monthly number of otitis externa cases during one year period duration.

2.4 Risk factors and otitis externa

Among the risk factor present in patients during the study, swimming was the most commonly risk factor associated with presence of otitis externa in 25(39%), followed by the use of topical antibiotics 7(10%),

while wearing hear aids 7 (10%), in addition to trauma in ear canal 5 (8), chronic dermatological disease 5 (8), and excessive ear wax 5 (8%) (Table 4).

Table 4. The isolated fungi from patients with otitis externa according to risk factors.

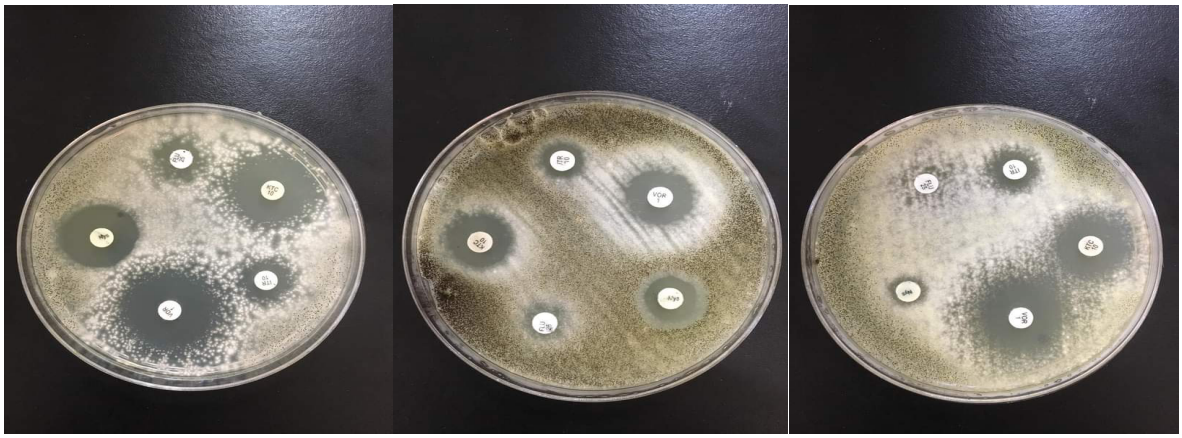
Risk factors	Isolated fungi	
	No.	%
Swimming	25	39
Topical antibiotics	7	10
Wearing hear aids	7	10
trauma to ear canal	5	8
Chronic dermatological disease	5	8
Excessive ear wax	5	8
Total	64	100

2.5. Antifungal susceptibility test

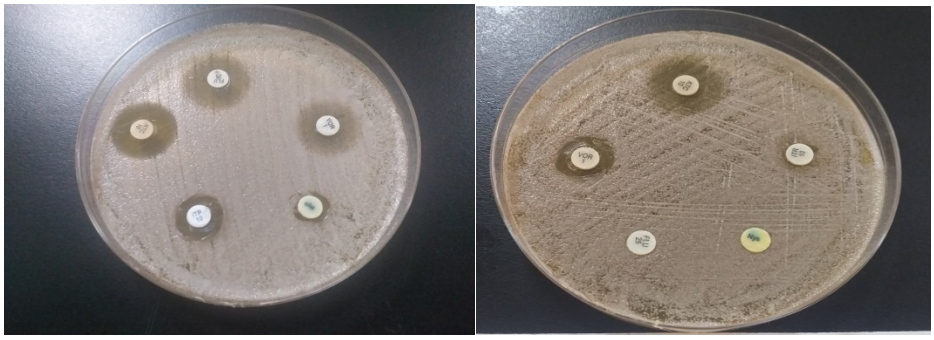
From the result which showed that ketoconazole, and voriconazole have the same antifungal activity on 46(75 %) fungal isolates, while nystain have antifungal activity on 22(23%) fungal isolates, then followed by fluconazole 14(14%), however itraconazole show a lowest antifungal activity on 11(13%) fungal isolates (Table 5, Figure 2).

Table 5. Number and percentage of sensitive isolated fungi to different antifungal agents by disc diffusion method.

Isolates	Total isolates	Number and percentage of sensitive species									
		Polyene group		Azole group							
		Nystatin		Fluconazole		Ketoconazole		Itraconazole		Voriconazole	
		No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus niger	24	10	42	2	9	17	70	3	13	19	79
Aspergillus flavus	14	6	43	2	14	7	50	0	0	7	50
Penicillum spp.	3	1	33	0	0	3	100	1	33	3	100
Rhizopus spp.	1	0	0	0	0	1	100	0	0	1	100
C.albicans	20	5	25	12	60	17	85	7	35	15	75
C,glabrata	2	0	0	0	0	1	50	0	0	1	50
Mean sensitivity (%)	64	22	23	14	14	46	75	11	13	46	75



Pencillium spp. *Aspergillus flavus* *Aspergillus niger*



Candida albicans *Candida glabrata*

Figure 2. The antifungal susceptibility test.

3.DISCUSSION

Otitis externa refer to an acute or subacute inflammatory conditions affected the skin of external ear and it utricle, otitis externa was a common disease in all regions especially in the tropics and subtropical areas and usually caused by bacterial and fungal infections. [1].

In this study the numbers of males were 50 (50%) to females 50 (50%) ratio in both patients and control group were equal by using the same number of patients of both sexes, from them there were 64 (64%) have otitis externa the male was 36 vs. 28 females as shown in Table 1. Study, conducted in Turkey by Ozcan and Coworkers 2003 [9], who reported that 65% of their patients were positive for otitis externa, this could be due to a fact that otitis externa may appeared in any age groups of both sexes in varying degrees, therefore no age is immune against otitis externa, the relationship between the presence of otitis externa in male and female varied according to the ratio of male to female in the studied sample. Studies showed that the males were more susceptible to otitis externa [10,4].

In female the number of isolated fungi increased when the age was increased this could be due to frequent exposure to dust containing fungal spores mixed with the air of the atmosphere from clean and sweep the floor of the houses, wearing turban or other clothes on head for long time especially during summer which associated with excessive sweating has been reported as a risk factors for otitis externa [2]

While in male the presence of fungal elements increase with age, however the highest presence of fungal elements where in age group (21-25, 26-30, and 31- 35) years, then decreased when age increase, infections in this age group mainly due poor personal hygiene, there works of low socioeconomic status, and to swimming as a risk factors for otitis externa approximately from 36 patients there were 16 of the patients in this study had a history of swimming in pool prior to development of otitis externa, which may be due to inhabitance of some fungi in these pools, but the most common is excessive moisture that elevates the pH and removes the cerumen [2].

Once the protective cerumen is removed, keratin debris absorbs the water, which creates a nourishing medium for bacterial growth [11]. However, water or other foreign liquids in the ear can provide an ideal breeding ground for fungi to grow, although moist skin and tissue create a friendly environment for microorganisms and allow them to multiply that leading to infection [11]. However no isolated fungi from children in this study, but this does not mean that otitis externa does not appear in children. He and coworkers, 2006 [12] showed that otitis externa is a disease of adulthood although the children may also affected.

The infection affecting the ear were unilateral in all patients involved in this study, although unilateral right ear infection 41(64%) was higher the unilateral left ear infection 23(36%). Otitis externa in 90% of patients were a unilateral disease without any preponderance of the right or left sides [13].

In this study the higher number of otitis externa was associated with swimming then followed by topical antibiotics, and wearing hear aid, in addition to trauma to ear canal, chronic dermatological disease, and excessive ear wax. Many factors can make a person more vulnerable to have otitis externa, as swimming in addition to the habit of abuse the antibiotics without medical consultation and self-ear cleaning [14]. Prakash and Coworkers 2015 [1] showed that antibiotics have an effect on the balance of microbial flora population, which protects the body from pathogenic microbes, in addition to do not clean it periodically may lead to the accumulation of dirt in the ear and thus provide suitable conditions and nutrients for opportunistic and pathogenic microbes to grow and multiply [15,16].

This study was take a full one year period, however the highest percentage of otitis extrena was 30 (66%) of cases occur in very hot and dry summer months (June, July, and August) in Mosul city were the study has been done excessive sweating as a result of high temperature, which dilutes the wax of the ear and minimize its protection role, factors facilitate fungi growth [16, 17]. Prakash *et al* 2015 [1] reported that (81%) of cases occur during April and December, while Prasad (2014) mention that the percentage of otitis externa was high (76%) in the July, August and September. Otitis externa becomes more common when the hot and humid environment present in the area of the studies in spite of the difference in the location [15,16]. Otitis externa was found worldwide from clinical examination and laboratory diagnosis in our study showed that 64 (64%) patients have a fungal elements in their clinical specimens, the higher isolated fungi from patients with otitis externa were from genus *Aspegillus* 38 (59%) isolates from them there were *Aspergillus niger* 24 (37%) *Aspergillus flavus* 14 (22%), followed by isolates from genus *Candida* 22 (34%) from them there were *Candida albicans* 20 (31%), and 2 (3%) *Candida glarata*, in addition to *Penicillum* spp. 3(5%), *Rhizopus* spp. 1(3%). However, the mixed isolates were 2 (3%), these mixed isolates were *Aspergillus niger* with *Candida albicans*. In addition to 8 (8%) isolated *Candida albicans* from control group. Researcher mention that 80%-90% of otitis externa cases were caused by fungi from genus *Aspergillus* and *Candida*, which is the main fungal pathogens than other fungi, *Aspergillus niger* and *Candida albicans* are the commonest fungal species isolated from these genus [1]. However, Joy and Coworkers 1980 [18] showed that *Aspergillus nigar* and *Aspergillus fumigatus* were the most common species from genus *Aspergillus* in addition to *Candida albican* from genus *Candida* were the most commonly pathogenic fungi isolated from patients with otitis extena [19].

When appropriate conditions are available from temperature, humidity, dust, changes in ear wax, hearing aids, use of inappropriate tools for ear cleaning, swimming in contaminated water, represent an ideal condition for the growth fungi, that make the person susceptible to fungal otitis externa [6].

Azoles and polyenes group which represent a standard classical antifungal therapy for treatment of mild to severe mycotic disease [20]. The result of antifungal sensitivity on fungi isolated in this study against 5 antifungal drugs, which showed that voriconazole and ketoconazole have the same antifungal activity on (46,75 %) fungal isolates, these good *in vitro* antifungal was higher than result obtained from other azole drugs used in this study as from fluconazole which showed antifungal activity on (14,14%) fungal isolates, and itraconazole show a lowest antifungal activity on (11,13%) fungal isolates (Table 5). Voriconazole and ketoconazole have a fungistatic and fungicidal activity on some mold, with good antifungal activity on all *Candida* species especially *Candida glabrata*, *Candida krusei*, and fluconazole resistant *Candida* species and they use as first choice for treatment of oseophageal candidiasis and invasive aspergillosis [21]. In the other hand nystain which have antifungal activity on (22,23%) fungal isolates which lower than antifungal activity obtained by voriconazole and ketoconazole and higher than fluconazole and itraconazole, however nystain have a fungistatic and a fungicidal antifungal activity against most of *Candida* species especially *Candida albicans* with lower activity against fungal mold [22]. Broth dilution test showed that 48 (70%) of fungal isolates were sensitive to ketoconazole and itraconazole, which followed by nystatin 21(18%), fluconazole 14(13%), then itraconazole 11(13 %). However, results of broth dilution sensitivity test were comparable to result obtained from disc diffusion sensitivity test. MIC values obtained from this study showed that a

maximum antifungal activity was in high concentration, with low or no antifungal activity at low concentration of antifungal drugs (Table 6 and 7) [21,22].

Table 6. Number and percentage of sensitive isolated fungi to different antifungal agents by broth dilution method

Isolates	Total isolates	Number and percentage of sensitive species									
		Polyene group		Azole group							
		Nystatin		Fluconazole		Ketoconazole		Itraconazole		Voriconazole	
		No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus niger	24	7	29	0	0	19	79	3	13	20	84
Aspergillus flavus	14	4	29	2	14	8	57	0	0	7	50
Penicillum spp.	3	0	0	0	0	3	100	1	33	3	100
Rhizopus spp.	1	0	0	0	0	1	100	0	0	1	100
C.albicans	20	10	50	12	60	17	85	7	35	17	85
C,glabrata	2	0	0	0	0	0	0	0	0	0	0
Mean sensitivity (%)	64	21	18	14	13	48	70	11	13	48	70

Table 7. Number, percentage, and minimum inhibitory concentration (MIC) of isolated fungi.

Type of antifungal drugs	MIC mg/ml	Fungal isolates	No. of isolate sensitive to antifungal drugs	%
Fluconazole	0	*Aspergillus niger*	0	0
	32	*Aspergillus flavus*	2	14
	0	*Penicillum spp.*	0	0
	0	*Rhizopus spp.*	0	0
	16	*C.albicans*	12	60
	0	*C,glabrata*	0	0
Ketoconazole	64	*Aspergillus niger*	19	79
	32	*Aspergillus flavus*	8	57
	32	*Penicillum spp.*	3	100
	64	*Rhizopus spp.*	1	100
	16	*C.albicans*	17	85
	0	*C,glabrata*	0	0
Itraconazole	0.25	*Aspergillus niger*	3	13
	0	*Aspergillus flavus*	0	0
	0.25	*Penicillum spp.*	1	33
	0	*Rhizopus spp.*	0	0
	0.25	*C.albicans*	7	35
	0	*C,glabrata*	0	0
Voriconazole	0.03	*Aspergillus niger*	20	84
	0.125	*Aspergillus flavus*	7	50
	0.125	*Penicillum spp.*	3	100
	0.25	*Rhizopus spp.*	1	100
	0.25	*C.albicans*	16	80
	0	*C,glabrata*	0	0
Nystatin	1.25	*Aspergillus niger*	7	29
	1.25	*Aspergillus flavus*	4	29
	0	*Penicillum spp.*	0	0
	0	*Rhizopus spp.*	0	0
	0.25	*C.albicans*	10	50
	0	*C,glabrata*	0	0

4.CONCLUSION

Identification of otitis externa from physical examination that often involve an otoscopy was very hard. Therefore, the most important diagnoses were by microbiological laboratory tests, for this reason the isolation and identification of fungi is very important method to identify the causative agents, in addition to know the pattern of antifungal drug sensitivity and exhibition of information about the type of predominant fungi present in Mosul city.

5.MATERIALS AND METHODS

5.1. Patients

A study included 100 patients with otitis externa attending ENT outpatients' clinics of both sexes their age ranged from (5-50) years the male were 50 (50%), while the female were 50 (50%). All the patients were diagnosed clinically as having otitis externa by ENT physician, those patients were not presented with fungal infection elsewhere in the body than ear, the clinical specimens collected for one year period from (January 2022- December 2022). Patients under chemotherapy were excluded from the study. This study has been approved by ethical committee under registration number CCMRE-phA-23-2. No. 2, date 25/1/2022. From medical research ethics committee of ethical approval, university of Mosul, which reviewed the research documents and approved the protocol of the study and gave me the ethical approval.

5.2. Control

A study included 100 control persons without otitis externa of both sexes their age ranged from (5-50) years the male was 50 (50%), while the female was 50 (50%).

5.3. Samples

The clinical specimens included 100 ear scraps of infected external ear skin, and ear swab for discharge if found. Both clinical specimens were collected under supervision of ENT (ear, nose, and throat) physician how diagnoses the patient clinically that having otitis externa, then the ear skin scraps and swab was carried to microbiology laboratory for isolation and identification as soon as possible, and then antifungal sensitivity.

5.4. Isolation

Direct microscopical examination of both ear skin and discharge by mounting the infected material with 10% potassium hydroxide (KOH) solution then cover with cover slide, then the slide heated over benzene burner just boiling, then examine under microscope to see the mycelial element, head of *Aspergillus* and budding yeast cell, the infected external ear skin and discharge culture on double plate of Sabouraud's dextrose agar with chloramphenicol antibiotic (0.05 mg/mL) to inhibit bacterial growth then the plates were incubated at 28-37 °C for 12-15 days. Plates were examined every 2-3 days to see any fungal growth, if negative growth was obtained after 15 days the culture media, then discarded [23].

5.5. Identification

5.5.1 Direct microscopical examination

If colony morphology is molding the identification firstly by macroscopical examination of colony morphology and its pigmentation, then followed by microscopical examination by mounting a small portion of colony by lactophenol to see the morphology of mold and its mycelial elements [24]. The slide culture technique was also used when a study of the morphological details of various fungi was necessary for exact identification [25].

If colony morphology is yeasting the identification firstly by macroscopical examination of colony morphology of its color and constancy, then followed by microscopical examination by staining a small portion of the colony by Gram stain to see budding yeast cell. Then germ tube test and morphology on corn meal agar with tween 80 to identify *Candida albicans*, while non *albicans Candida* species were identified by API-10 C system [24].

5.6. Antifungal susceptibility test

Disc diffusion method was used in this study to test the susceptibility of fungi to antifungal disc such as (fluconazole 25 µg, itraconazole 10µg, voriconazole 1µg, nystatin 50µg, and ketoconazole 15µg). A suspension of 0.5 McFarland were prepare from each isolate by taking a small portion of fungal colony and mix with Sabouraud's dextrose broth, then a sterile swab was soaked in fungal suspension to inoculate the fungi on Sabouraud's dextrose agar, by using a sterile forceps the antifungal discs were placed on the surface of Sabouraud's dextrose agar then the plates were incubate aerobically for 24- 72 hr. at 37 C, after that zone of inhibition was measured to determine the number of sensitive isolates [26,27,28].

5.7. Broth dilution test

Broth dilution method according to CLSI M51-A (2010) [27,29] protocol for fungi, was used for determination the susceptibility of fungi from stock dilution of fluconazole, ketoconazole, itraconazole, voriconazole, and nystatin serial dilution was prepared, then inoculated with fungi suspended with Sabouraud's dextrose broth after adjusted the density which equal to 0.5 McFarland standards, after incubation for 24- 96 h at 37 °C. MIC was calculated. *C. krusei* strain was used as a reference ATCC 2297.

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