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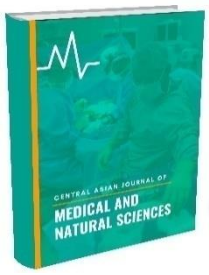

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PHENOTYPIC DIAGNOSIS OF LABORATORY CULTURE OF FUNGI ISOLATED FROM THE EXTERNAL EAR CANAL EXTERNAL AUDITORY CANAL CAUSING OTITIS EXTERNA

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Abstract: 69 isolates of fungi causing fungal external otitis EAC Otomycosis were obtained from patients with fungal external otitis 76.66% of the total number of 90 otological smears, 17 isolates of which belong to the genus Candida spp, 27 isolates belong to the genus Aspergillus spp, 4 co-infections of the said sexes, 5 isolates belong to the genus Alternaria, and 9 isolates belong to the genus penicillium, 6 isolates belonging to the genus Fusarium, and 5 isolates belonging to the genus Rhizopus all fungi were diagnosed by traditional methods through phenotypic examinations on a petri dish as well as the study of microscopic traits, so candida albicans and Aspergillus niger is the most visible fungus species in this study.

Key words: EAC Otomycosis, Candida spp, Aspergillus spp, Penicillium, Rhizopus.

1. Introduction;

The fungi that cause infection of the outer ear are saprophytic fungi, which are widespread in nature and part of the naturally occurring organisms in the ear, and the genera Aspergills and Candida are the most common (Prahan, et al., 2003) and mentioned (paulose et al.,1989) that the genus Penicillium is sometimes the cause of fungal external otitis and (Kennedy,1998) showed that dermatophytes dermatophytes infection probably occurs. Fungal infection of the ear was described by Mayer in 2006 describing otitis externa, the main issue of discussion among scientists was that the fungus causes an initial injury to the auditory canal or it invades the skin only after an increased percentage of bacterial toxins.

Haley stated in 1950 that Aspergillus, Penicillium are the two most responsible genera for the infection. While Latonch and Gregsoson in 1961 isolated sexual Aspergillus, candida from 83 cases of infection. Four genera with Aspergillus were isolated from 37 cases of infection (Shehab,2005) and Ismail indicated in 1995 that the causative agent of all cases of infection is A.niger and Jones in 1974 believed that patients suffering from repeated infection with otitis externa are mainly infected with one of the fungi with the presence of an acute bacterial infection and this was evident after bacterial treatment, as people remain suffering from infection due to the presence of fungi that cause relapse. Kennedy (1998) stated that the

genera Penicillum, Mucor, Actinomyces, Dermatophyte, infection probably occurs rarely. Noted by Linstrom et al., 1988)) to the probability of recurrence of infection in the absence of control of the infection.

2. Materials and Methods;

2.1. Collection Samples;

90 clinical samples were collected from people with otitis externa from the dermatological consultation at Al-Sharqat General Hospital and some private clinics in Salah al-Din governorate for the period between 1/11/2022 and until 1/3/2023, as the study included collecting samples from people with otitis externa under the direct supervision of a specialist doctor, for different ages and for both sexes.The samples were taken using soap and brought to laboratories at the Faculty of education for pure sciences /Tikrit University for examination and cultivation.

2.2. preparation of planting media;

All planting media have been prepared according to the manufacturer's instructions. All transplant media were sterilized with the autoclave steam sterilizer and under a temperature of 121 m and a pressure of 15 psi /Ang2 for 15 minutes. The glassware and instruments used in the experiments were sterilized by electric oven at a temperature of 180 m for 120 minutes (Harley and Prescott,1996).

Dextrose Agar;

2.2.1. Sabourauds

This medium was prepared according to the recommendations of the manufacturer Himedia by dissolving 65 g of SDA powder in 1000 ml of distilled water and then sterilized, then 0.05 g of ChIoramphenicoI antibiotic was added to it to prevent the growth of opportunistic fungi and after sterilization poured into sterile plastic dishes with a diameter of 9 mm, use this medium to isolate dermatophytes (Emmons et al.,1974).

2.2.2. Urea medium agar:

This medium was prepared according to the manufacturer's instructions by dissolving the medium with distilled water, sterilized by the sterilizer, and then left to cool, then a urea solution with a concentration of 40% was added to it under sterile conditions.,1994).

2.2.3. Yeast extract agar:

8g of the medium was weighed and dissolved in 1000 ml of distilled water in a glass flask.this medium was used to differentiate the genera of dermatophytes (Emmons et al.,1974).

2.2.4. Milkagar:

Prepare by dissolving 28 g in 1000 distilled water and sterilize the medium with a sterilizer and after cooling to a temperature of 50 m. Add to it 10% skimmed milk,sterilized in advance by rooting for 10 minutes, shake well to homogeneity, then pour into plates and use this medium for testing fungi on the production of protease enzyme that decomposes protein (Larone, 1995).

2.2.5. Sheep blood agar:

This medium was prepared by dissolving 65 g of powder SDA medium in 1000 ml of distilled water, sterilized by the sterilizer, then left to cool to 45 M and add 70 ml of sheep's blood to it, and the medium is supplemented with the addition of 30 g of glucose sugar and used this medium to detect the susceptibility of fungi in the production of hemolytic enzyme hemolysin (Manns et al., 1994).

2.2.6. The center of the nutritious acres fortified with lecithin;

This medium was prepared according to the method described by Price and his group (1982) and modified by Aubaid (1997), the medium was prepared from the following substances: 20 g of Nutreint Agar, 1 g of NaCl, 0.05 M of CaCl2, 8% of sterilized egg yolk.

2.3. Culturtion of Specimens;

Part of the clinical samples not treated with potassium hydroxide were taken using sterile forceps and grown on the SDA culture medium

added with chloramphenicol, then the dishes were incubated at a temperature of 28 M for 4 weeks, the dishes were examined to observe the appearance of any fungal growth (2006. Kannan et al).

2.4. Examination and diagnosis of fungal colonies;

2.4.1. Purification of fungal isolates;

Pure cultures of isolated dermatophytes were prepared using container dishes on the SDA medium, part of the edge of the colony was transferred to it when it appeared in the dishes containing fungal samples using stabbing lube, and for the same purpose, cultures Slant oblique farms were prepared, they were inoculated with part of the pure colony and incubated in the incubator at a temperature of 28 M for 4 weeks depending on the type of fungus, and then kept in the refrigerator at a temperature of 4 M to use when needed.

2.4.2. Phenotypic examination of fungal structures;

To study the phenotypic qualities of the isolated fungi and accurately diagnose them, the dishes were examined after 4 weeks of incubation, the phenotypic examination of the samples was carried out depending on the external shape of the colony, the color of the colonies and the texture of the colony and the examination of the opposite side of the colony and the diameter of the colony.

2.4.3. Microscopic examination of fungal structures;

The microscopic qualities of the isolated fungi were studied by examining fungal filaments, large conids and small conids in terms of their presence, shapes, dimensions, the way they are arranged on the fungal filaments, as well as observing the formation of arthrospores arthrospores and chlamydial species Chlamydospores glass slides were made by taking a part of the colony and placing it on a glass slide containing a drop of blue lactofenol dye and examined under a light microscope under 40X Forces (Champion et al.,1998).

3. Results and discussion;

3.1. Phenotypic diagnostics of laboratory culture of isolated fungi;

10 fungal species belonging to the following genera were diagnosed, Candida, Aspergillus Alternaria Penicillium,, Fusarium Rhizopus, where, through the results, two species of the genus Candida appeared, namely Candida albicans with a frequency of 10 and glabrata Candida with a frequency of 7, where the species was diagnosed depending on the agronomic, microscopic qualities, biochemical tests and colors that it gave on the SDA medium.

Table (4-1) shows that the number of isolated samples belonging to the genus Candida spp is (17) samples, with a percentage of (18.88 %) of the total number of samples, (10) samples of which belong to the type Candida albicans and the other samples belong to the type Candida glabrata as shown in Table (4-1).

Table (4-1): percentage prevalence of otomycosis fungal otitis externa caused by the genus Candida spp. Isolated from the external ear canal EAC

yild %	Positive samples (+)	Total number of samples	Specimens samples Isolated from the EAC
11.11	10	90	*Candida albicans*
7.77	7	90	*Candida* glabrata
18.88	17		Total

As for the genus Aspergillus, 4 species belonging to it were diagnosed, the most frequent of which was the species Aspergillus niger, where the number of repetitions reached 10, the two species flavus Aspergillus and aspergillus nidulans, the number of repetitions was 6 each, and the species Aspergillus oryzae, the number of repetitions reached 5. The results of the current study, as shown in Table (4-2), showed that the number of isolated specimens of the genus Aspergillus spp amounted to 27 samples, accounting for 30% of the total number of samples, and the number of specimens belonging to the Aspergillus niger species amounted to (10) at 11.11 of the total number of samples, and the number of specimens belonging to the flavus Aspergillus species amounted to (6) at 6.66 of the total number of samples, and the number of specimens belonging to the Aspergillus nidulans it amounted to (6) by 6.66 of the total number of samples, and the number of samples belonging to the species oryzae Aspergillus amounted to (5) by 5.55 of the total number of samples.

Table (4-2): percentage prevalence of otomycosis fungal otitis externa caused by Aspergillus spp isolated from the external ear canal EAC

yild %	Number of positive samples (+)	Total number of samples	Specimens specimens isolated from the EAC
11.11	10	90	*Aspergillus niger*
6.66	6	90	*Aspergillus flavus*
6.66	6	90	Aspergillus nidulans
5.55	5	90	*Aspergillus* oryzae
30	27		Total

The results of the current study shown in Table (4-3) also showed that the number of samples jointly isolated from both rot.Aspergillus spp and ovarian yeasts.Candida spp amounted to 4 samples and accounted for 4.44% of the total number of samples.

Table (4-3) the percentage of prevalence of fungal and otitis externa caused by both rot.Aspergillus spp and ovarian yeasts.Candida spp in combination and isolated from the external ear canal EAC Aspergillus-Candida Otomycosis

yild %	Positive samples (+)	Total number of samples	Specimens samples Isolated from the EAC
%4.44	4	90	*.Aspergillus spp* *.Candida spp*

The stated results differed with what he found (2009. (Pontes et al; having found the genus Candida.the number of positive samples was 9 out of a total of 20 samples, with a percentage of 45%, as for the genus spp.Aspergillus spp, the number of positive samples was 6 by 30%, distributed between 4 samples of the type Aspergillus niger by 20% and two samples of Type A. flavus increased by 10%, and stated 2022,. Bojanovic et al. when studying fungal otitis caused by filamentous fungi that the genus niger A was the main causative agent with 66.7%, followed by A.flavus increased by 33%, which is about the same as our current study.

The results of our current study converged with what he mentioned (2020,. Allam et al); finding the genus Aspergillus.spp is 52% predominant over the rest of the genera of Candida yeasts and other filamentous fungi, he stated (2020,.Gharaghani et al) when studying fungal otitis in the city of Ahvaz in Iran. Aspergillus spp is 88.3% as for candida yeasts. Candida spp had an incidence of 11.7%, and these results differed from what we found in our current study, gender supremacy. Aspergillus spp on Candida yeasts. Candida spp in some cases is due to the fact that this species has spores that are present in the air and are constantly transmitted and it is possible to spread in all environments (Gandhi et al.,2020), and the results of the current study showed the presence of combined intersex infections. Candida spp.and.

Aspergillus spp with a percentage of 4.44%, this result corresponded to what he found (.Tasic-Otaševic et al 2020); they found that the proportion of co-infections is sexual. Candida spp. And. Aspergillus spp reached 5.5% for patients with fungal otitis externa in Serbia and also corresponded, reported 2019.Aboutalebian et al.reported that the percentage of yeast / mold Mixes 11 co-infections was 13.6% when they studied patients with otitis externa in the city of Isfahan in Iran.

As for the remaining species (Alternaria alternate, Penicillium chrysogenum, Fusarium Solan, Rhizopus spp), their number of repetitions reached (5, 9, 6, 5), respectively

The results of the current study, shown in Table (4-4), also showed that the number of isolated specimens of the type Alternaria alternate amounted to 5 samples and accounted for 5.55% of the total number of samples.

Table (4-4): percentage of prevalence of otomycosis fungal otitis externa caused by the genus Alternaria lternate isolated from the external ear canal EAC

yild %	Positive samples (+)	Total number of samples	Specimens specimens isolated from the EAC
%5.55	5	90	Alternaria alternate

According to the results of the current study shown in Table (4-5), the number of isolated samples of the genus Penicillium chrysogenum amounted to 9 samples and accounted for 10% of the total number of samples

Table (4-5): percentage prevalence of otomycosis fungal otitis externa caused by Penicillium chrysogenum isolated from the external ear canal EAC

yild %	Positive samples (+)	Total number of samples	Specimens specimens isolated from the EAC
%10	9	90	Penicillium chrysogenum

The results of the current study shown in Table (4-6) showed that the number of isolated specimens of the Fusarium Solan type amounted to 6 samples and accounted for 6.66% of the total number of samples.

Table (4-6): percentage prevalence of otomycosis fungal otitis externa caused by Fusarium Fusarium Solan fungus isolated from the external ear canal EAC

yild %	Positive samples (+)	Total number of samples	Specimens specimens isolated from the EAC
%6.66	6	90	Fusarium Solan

The results of the current study shown in Table (4-7) showed that the number of isolated specimens of the genus Rhizopus spp. It amounted to 5 samples and accounted for 5.55% of the total number of samples.

Table (4-7): percentage prevalence of otomycosis fungal otitis externa caused by Rhizopus spp. Isolated from the external ear canal EAC

yild %	Positive samples (+)	Total number of samples	Specimens specimens isolated from the EAC
%5.55	5	90	Rhizopus spp.

These molds or filamentous fungi were diagnosed based on the phenotypic traits of the colony, the color of the colonies, the microscopic characteristics in terms of The Shape of the Phialids and the presence of conidiophore conidiophores.

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