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Diagnostic study of otomycosis in Hilla city/Iraq

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Abstract : The aim of the study was to identify the fungi in the external ear canal of patients with otomycosis admitted to the hospital in Hillacity , Iraq (during 2015). This study include 126 patients with otomycosis in different ages (<1 - >61 years)including males and females. After removal of ear infectious samples, was plated on the potatoes dextrose agar with chloramphenicol for fungal growth. Conventional methods were performed to determine fungal colonies. Results showed that males were more affected than females (51.6% males, 48.4% females). There are many isolates founded as *Aspergillus niger*, A.*terreus*, A. *flavus*, *Penecillium digitatium*,, *Candida albicans*, C. *glabrata*,*Candida Krusie*.and *C.parapsilosis* was more appearance (31.959%), then *Candida Albicans* (30.55%), *Aspergillusniger* (31.27%), while the lowest isolates were *Candida krusie* (14.58%). More agegroup affected was 21-30 years (18.9%) while the lower age group affected was <1-10 years (9.5%).

Introduction:-

Otomycosis is a fungal infection often affecting the external auditory canal with infrequent complications involving the middle ear[1-2]. Fungalexternal otitis (otomycosis) isa common pathology throughout the world. Itsfrequency varies according to differentgeographic zones, in relation to environmental factors (temperature, relative humidity) and thetime of year[3] ,and dusty climate of the tropical and subtropical region[4] . Otomycosis can occur as acute, subacute or chronic, the main signs and symptoms of infection areitchingness, pain, otorrhea[5-7].There are several factors have been proposed for otomycosis including a humid climate, presence of cerumen, immunocompromised host and use of antibiotic [8].Other factors like moisture, humidity, swimming, and using of hearing aids can lead people to infect by this disease[9] .Otomycosis found in all ages especially in middle ages, and also in both genders [10].Identification of this disease is based on direct examination, macroscopic and microscopic of fungi colonies[11]. Aspergillus and Candida are the most genera found causing otomycosis[12,13]. Many authors have focused their attention on the bacterial flora of otitis, while very little study the mycological flora of this disease, so this study was aimed to study fungi that responsible for otomycosis in Hilla city, Iraq.*Candida albicans* is generally considered the major pathogen among the Candida species. An increase in the prevalence ofnon-albicans species has been noted during the last decades. There is growing evidence of the increasing use of azoles causing this epidemiological shift. Characterization to species level helps to identify those strains which might be intrinsically resistant to some of the antifungal agents[14] .Speciation of Candida isolates is conventionally done by germ tube test, sugar assimilation and sugar fermentation tests. Newer methods include CHROM agar[15] .

Materials and methods:-

Collection of samples

This experimental study was taken on 185 patients suffering from otitis referred to the surgical Hillahospital inHillacity , Iraq, during 2015 (April to august) . The samples were taken by using fertile swab sticks which were labelled indicating the source, date, age and sex of patients, then put in translate media ,and sent to the fungal laboratory studies in Babylon university/ college of science, department of biology.

Filamentous fungi isolation and identification

In the laboratory, the samples were inoculated on Sabouraud's dextrose agar and potatoes dextrose agar, fungal colonies were identified according macroscopic and microscopic appearances such as mycelia and fruiting bodies using lacto phenol staining technique [16].

Yeastsisolation and identification

Yeast identification was done by sugar fermentation test, germ tube test, chlamydospore formation on corn meal agar[17] .Simultaneouslythe *Candida* spp. were inoculated on CHROM agar and incubated at 37°C for 24 hrs and the species identified by type and color of the colonies on CHROM agar media (table 3) according to the color key [18].DNA was extracted was done according to Promega Kit and then PCR technique was done [19].

DNA extraction and sequence analyses:

After 2 days, growth of yeasts colonies on PDA broth was counted by centrifugation and DNA was extracted from isolates using the genomic DNA purification Kit (Pro- mega, Madison, WI, USA). Small subunit ribosomal RNA (mtSSUrRNA) and β-tubulin were then amplified by PCRusing primer pairs based on the genomic sequences of DNA topoisomerase II of *C. albicans* , *C. krusie*designed[19].Primer of *C. albicans*include Forward: TTGAACATCTCCAGTTTCAAAGGT and Reverse: GTTGGCGTTGGCAATAGCTCTG. While *C. krusie* include Forward: GAGCCACGGTAAATACACA and Reverse: TTTAAAGTGACCCGGATACC .The conditions described diagnosis. For detection of DNA, gel electrophoresis method was used by using Agarose gel (1% concentration). This method consisted of three steps: preparation of agarose gel, preparation of casting agarose and the addition of samples.

PCR amplification condition:

This method is used to amplify DNA by using the specific primer. These methods are dependent on the volumes that are found in Master Mix provided from Bioneer (The time and vol. which is used for electrophoresis methods: To obtain clear band after PCR running , that used different time and volume during electrophoresis.

Table 1. Program of PCR technique for *C. albicans*

No. steps	Steps	Temperature	Time	No. of cycles
1	Initialdenaturation	**96 C**	**2 min**	**1**
2	Denaturation	**96 C**	**30s**	**30**
3	Annealing	**57 C**	**30s**	
4	Elongation	**72 C**	**1 min**	
5	Final extension	**72 C**	**5min**	**1**

Table 2. Program of PCR technique for *C. krusie*

No. steps	Steps	Temperature	Time	No. of cycles
1	Initialdenaturation	**94C**	**5 min**	**1**
2	Denaturation	**94C**	**30s**	**30**
3	Annealing	**51 C**	**30s**	
4	Elongation	**72 C**	**30s**	
5	Final extension	**72C**	**5 min**	**1**

Results:-

In the study, there were 185 samples were collected, 162 samples were positive for the presence of fungal elements. There were total 65(51.6%) male and 61 (48.4) female as shown in table 1. The age range was from <10 year to >61 years include male and female,most of patients were of age group 11 – 20 years (19.8%), followed by age group < 10 years (18.3%), least patients were in age group 31-40 (11.1) and age group >61 (11.1%), there were significant difference among them.Percent duration of yeast isolates includedC *.parapsilosis* (31.95), C. *albicans*(30.55), C. *glabrata*(23.26), C. *Krusie*.(14.58), there were no significant difference among them.Filamentous fungi included *Aspergillusniger* (31.27), A. *flavus* (27.71), A. *terreuss*(17.26), *Penicilleumdigitatium* (23.29), also appear no significant difference among them. As whole yeast are more frequent than filamentous fungi (53.63 , 46.36) respectively (table2). Candida species identify according to the color key [18] ,as shown in table 3.

Table 3:number of affected male and female according to age group

age / gender	**<1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**>61**	**Total number And percentage**
Male	12	17	13	6	5	6	6	65 51.6%
Female	11	8	4	8	13	9	8	61 48.4%
Total number	23	25	17	14	18	15	14	126 48.41%
Percentage	18.3%	19.8%	13.6%	11.1%	14.3%	11.9%	11.1%	100%

Pv=0.018

Table 4: percentage of duration of fungi according to age groups.

Fungi	**<1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**>61**	**Total percentage %**
C. *parapsilosis*	17.58	19.78	16.48	10.98	13.18	12.08	20.87	31.95
C. *albicans*	14.77	19. 31	17.04	14.77	14.77	10.22	9.09	30.55
C. *glabrata*	14.92	14.92	19.40	16.41	13.43	13.43	7.46	23.26
C. *krusie*	0.0	11.90	23.80	21.42	19.04	14.28	9.52	14.58
total	**13.54**	**17.36**	**18.40**	**14.93**	**14.58**	**12.15**	**9.02**	**53.63**
A. *niger*	15.18	22.78	16.45	15.18	13.92	10.12	6.32	31.72
A.*flavus*	11.59	26.08	17.39	18.84	11.59	2.89	11.59	27.71
A.*terreus*	0.0	18.60	27.90	20.93	20.93	6.97	4.65	17.26
P. *digitatium*	13.79	13.79	15.51	10.34	17.24	12.06	17.24	23.29
total	**11.24**	**20.88**	**18.47**	**16.06**	**15.26**	**8.03**	**20.88**	**46.36**

Pv (yeast)= 0.095 Pv (filamentous fungi)= 0.246

Table 5 : appearance of Candida species grown on CHROMagar Candida

Species Specifcity	Recovery	Color
C. *parapsilosis*	Small to medium, smooth to wrinkled	Ivory to pink to lavender
C. *albicans*	Fair to heavy growth	Green
C. *glabrata*	Small to medium, smooth, convex	Dark violetc
C. *krusei*	Fair to heavy growth	Pink, pale borders

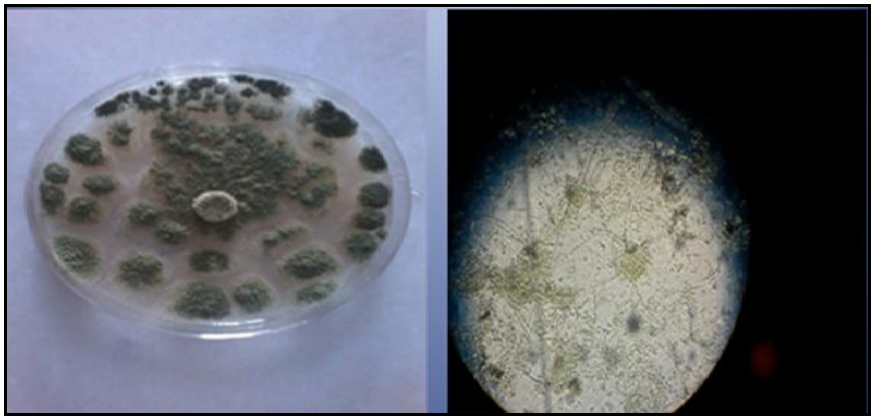

Figure 1: ***Aspergillusflavus***

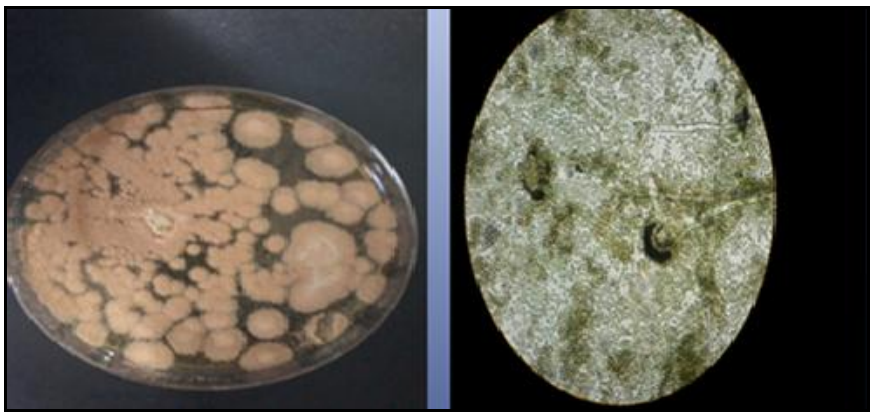

Figure 2: ***Aspergillusterreus***

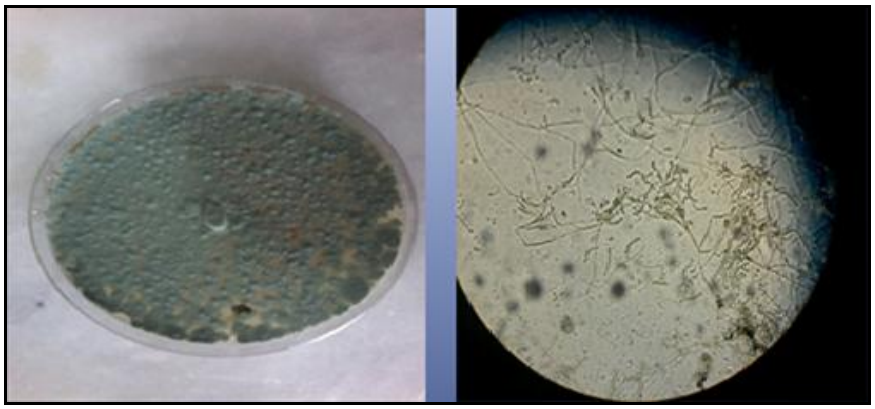

Figure 3: ***Penicilliumdigitatiam***

Figure 4: *Aspergillusniger*

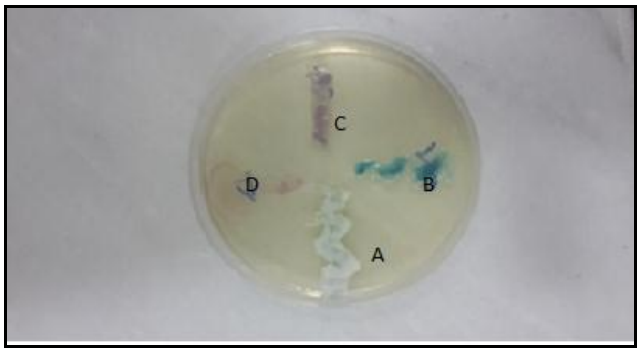

Figure 5: Candida colors on CHROMagar; A: *C. parapsilosis* B: *C. albicans* C: *C. glabrata* D: *C. krusie*

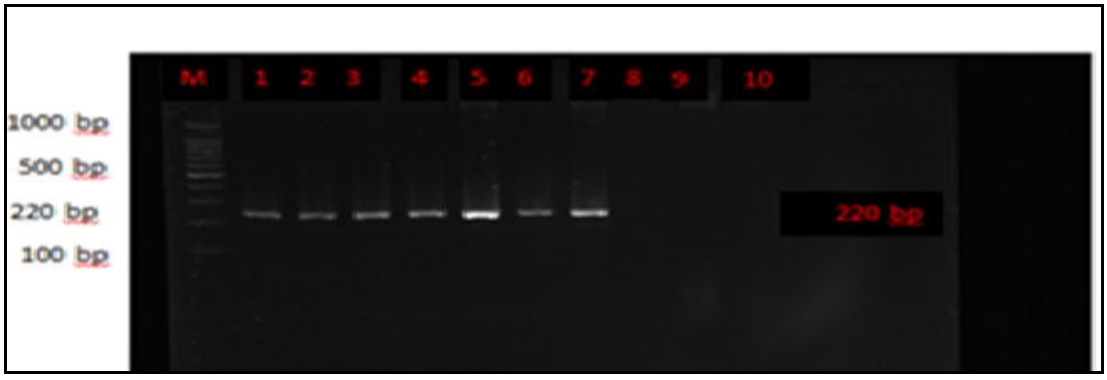

Figure 6: Gel electrophoresis of amplified PCR product , yeast species by using (CABF59F and CABR110R) for *Candida albicans* = 228 bp (7 bands).

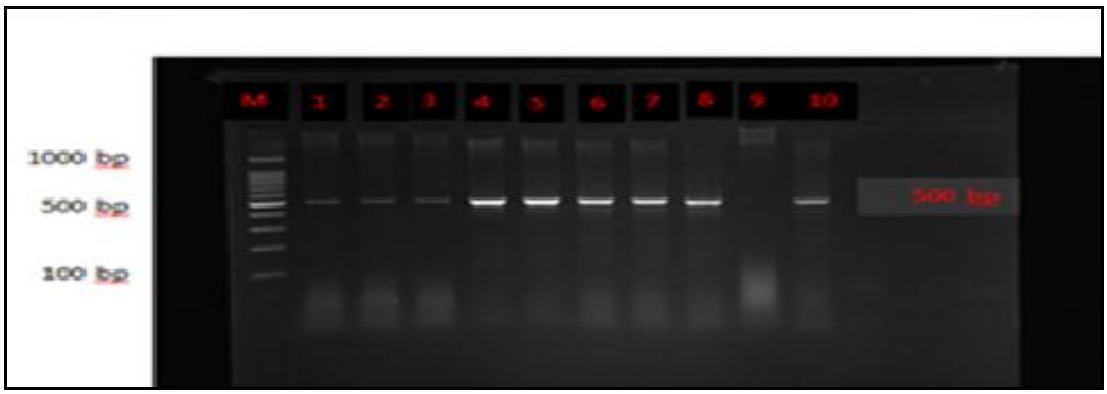

Figure 6: Gel electrophoresis of amplified PCR product , from different yeast species by using (CKSF35F and CKSR57R) for *Candida krusie*= 515 bp (9bands).

Discussion:-

Otomycosisis an entity frequentlyencountered by otolaryngologists can usually be diagnosed by clinicalexamination.HoweverChromogenic media have the advantage of rapididentification of Candida species, technically simple preparation (by boiling), rapid and cost effective compared totechnically demanding time consuming and expensive conventional method [20] .Chromogenic agar is a newer and more raped method to speciateCandidacontains enzymatic substrates that linked to chromogenic compounds when specific enzyme cleaves the substrate the chromogenic substances produce color[21] .

Otomycosis was most prevalent in the age group of children and young adult men, this result is similar to study performed by 22 and 32 , and 24, there are may be because of the Eustachian tube which is short wider in children and infants, young men generally spend more time outdoors and Aspergilli are common airborn saprophytes[25]. In our study Candida were more isolates (53.63%), then *Aspergillus* (46,36%), 14 agree with our study , which found that *Candida* species were more isolates from otomycosis (60%), while *Aspergillus*was 40%, but 26 found that *Aspergillus* yield 33.3% of the whole isolates while Candida are 22.2%. This may be attributed to the environmental effect[27-32] (hot and humid) on the cases of the otomycosis that studied in this area

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