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# Increased Ear Infections of *candida Ciferrii* and *candida Guilliermondii* compared to *C. Albicans* and other Non-Albcanis Candida Speciesin AL-Najaf Governorate.

**Hawraaghalebidrees AL-yassree[1], AlrufaeMohamed M.[2]**

[1]Branch of Basic Science / Faculty of Nursing/ University of Kufa
[2]Biology Department/Faculty of Science/ University of Kufa

## Abstract

*C.albicans*is known to be the main cause of ear infections but in this study found that there are two kinds of yeasts considered rare PathogenistyC*andida ciferrii*and*Candidaguilliermondii*.Thesewere diagnosed and its antifungal susceptibility was measured using the Vitek2.It was resistance to fluconazole, Amphotericin B and Caspofungin.also thesespecies has a high capacity to configure biofilm .The presence of gene MDR1 and ERG9 was also detected in the isolates using PCR technology.

## Introduction

External ear infection is 5 to 20% of Ear visits to hospitals and external clinics, most of these infections are caused by bacteria and only from9 to 25% caused by fungi.Be more common in tropical countries.Infections may be either semi-sharp or sharp and characterized by inflammation, pruritus, scaling, and severe discomfort(Kaur *et al*., 2000).

*Candida Ciferrii* is a kind of unusual yeast, and was first detected in 1965.it is an anamorphic image of *Stephanoascusciferrii*, and was described as the causes of fungal diseases in superficial and rarely cause systemic disease(De Gentile *et al*., 1991).

In humans, It was isolated from the toenails of elderly patients those who suffer from trophic disorders in the legs(Gentile *et al*., 1995).

Knowledge of natural habitats for *Candida ciferrii* are very limited. Systemic mycosis it causes significant morbidity and mortality in immunocompromised patients .Fluconazole are increasingly used to blocking the fungal infection or is used as a first line of treatment of mucocutaneous candidiasis (Wingard*et al*., 1993).

*Candida Guilliermondii*has been isolated from environmental surfaces, skin and nails working in health care.It has been proven to cause HaematologicalCandidiasis(Medeiros et al., 2007).In this study was diagnosed and a pharmaceutical sensitivity test was conducted for these two types using the Vitek 2 as well as investigate resistance genes(MDR1-ERG9) using PCR technology**.**

## Materials and Methods

### Collection of specimens

54 specimens were collected from patients with ear infection from the hospitals and the outpatient medical clinics in Najaf Governorate during the period from January 2021 to March 2021,swabs from the ear were transferred to the advanced mycology laboratory /faculty of science –university of Kufa , for diagnosis and study. Samples taken from the ear using the sterilized cotton swabs and Move circularly inside the patient's permission,and the

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cotton swabs were sterilized into sterile culture tubes containing sterilebrian heart Infusion(BHI) with Chloramphenicol and transferred as soon as possible to the laboratory for incubation. At 37 ° C for 24 hours. The next day, they were sub-cultured on both SDA and ChromagarCandida and incubated at 37 ° C for 1-3 days for the visible growth of Candida colonies (Loveday *et al*., 2014).

**Identification of *Candida* Species**

*Candida SPP*. Isolation is diagnosed depending on cultural characteristics, microscopic, biochemical and molecular characteristics.

**Morphological and cultural characteristics**

The shape, size, edge, colorand appearance of the yeast colonies were noted on Sabouraud dextrose agar (SDA) medium after 24-48 hr incubation.While the CHROM agar Candida assay used to aid in the pinpointing of *Candida* species relay on the color, one cell was captured from yeast colony on SDA and the isolation documented and accepted for 24-48 hours at 37 ° C (Horvath *et al*., 2003).

**A/ Microscopic examination**

A glass slide of the colonies was prepared with a drop of normal saline, covered with the cover slide and examined by light microscopy after staining it with crystal violet dye to observe the shape, size of the cell and its budding (De Hooge*et al*., 2000).

**B/ Gram stain**

Thesmears were prepared on slides that were previously cleaned using alcohol.Latter, thermostable staining is performed by immersing the smears in a crystal violet solution for one minute and then with iodine-gram for one minute. After being washed, the swabs were decolorized with 95% ethanol and the meter strained with aqueous basic fuchsin. Check the slide to note of *Candida* morphology under the objective lens of oil immersion (100 ×) for a bright field microscope(Smith and Marise., 2005).

**C/ Germ tube test**

For this assay, yeast cell obtained from a pure colony were suspended in 0.5 mL of human serum in a small tube, and the pipes were embraced at 37 ° C for 2-3 hours.Nursery must not exceed 3 hours, Because other species of yeast can begin to form germ tubes, Put a drop from incubated serum on a glass slide, covered with a lid, and examining it with a microscope to see the presence of germ tubes as we notice the presence of the germ tube emerging from one side of the cell in the form of a bud It is 3-4 times the length of the same cell (Deorukhkar*et al*., 2012).

**D/ Biofilm formation test**

Use the tube method for qualitative assessment of Biofilm formation Brian Heart Infusion (BHI )Setting up and sterilized by autoclave, After that inoculated with a loopfulof each pathogenic microorganism which has ability to making biofilm and incubated for 24 hrs at 37°C . After incubation, the tube was discharged from fungal suspension and then washed with (phosphate buffer saline (PBS)) pH 7.4 and leave to dry at room temperature.Pipes were stained with 1% crystal violet for 10 minutes and custody at room temperature,The excess

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dye was removed and washed with deionized distill water ,the tubes put upside down to drain.. The formation of positive Biofilm was observed when a visual film was lined up on the wall and bottom of the pipes, The results were recorded based on visual viewing (0 absent, 1 - weak, 2- moderate, 3-strong).

**Identification by culturing on CHROM agar Candida**

*Candida* It grows abundantly on commonly laboratory media used in isolation pathogenic fungi and bacteria (Deorukhkar*et al*., 2014). Sabouraud Dextrose Agar (SDA) Widely used in primary isolation methods for *Candida Albicans* of clinical samples (Odds, 1991).

CHROM agar Candida (CHROM agar Candida Himedia-India) .It is commercially available selective and differential media for isolation and identification of *Candida* spp.

This medium contain substrates of chromogenic (hexosaminidase) that interact with species-specific enzymes secreted by yeast cells, Which led to the development of contradictory colored colonies on Chromagar,*Candida*Spp. Such as *C. albicans*, *C. tropicalis*and *C. krusei*It can be easily distinguished on the basis of colonial morphology and color. *C. albicans*produce leaf-green colored colonies, *C. tropicalis*colonies are dark blue-grey with a purple halo and *C. krusei*forms pink colonies with whitish edge(Neppelenbroek*et al*., 2014).

On CHROMagar, NAC spp. like *C. famata*, *C. firmeteria*, *C. guilliermondii*, *C. kefyr*, *C. lusitaniae*, *C. norvegenesis*and *C. parapsilosis*The colonies of variable shades of ivory, lavender and pink can not be distinguished from each other (Neppelenbroek*et al*., 2014).*C .glabrata* colonies It is produced in a dark purple color and can be distinguished from pink and white colors produced by other species(Letscher-Bru*et al*., 2002).

**Vitek 2identification and antifungal Susceptibility**

The state-of-the-art Vitek 2is completely Automatic system designed for diagnosis and pharmaceutical sensitivity testsof microorganisms. In conjunction with Vitek ID-YST card, Vitek 2 determines the identity of the yeast is clinically important and yeast-like organisms within 15 hours due to a sensitive technology based on luminescence .The ID-YST label contain of 47 biochemical reactions. The database contains 51 categories, Includes modern species described(Graf*et al*., 2000).

**Molecular identificationand to identify isolates containing resistance genes by PCR technique**

To identify antifungal resistance genes, yeast isolates extracted from 83 samples were used, 16/54 (29.62%) from yeast isolates were considered positive samples that were used to detect the resistance genes they possess. The genomic DNA of the isolates was extracted from the small colonies using DNA extraction by a special purification kit(A specific D1900-100T Yeast genomic DNA extraction kit (Solarbio Science ,Beijing China), The amount of 5 µl has been used from the DNA solution as a template in the following PCR.

The PCR check was performed to amplify the 16S RDNA sequence to determine the presence of resistance genes (MDR1- ERG9) in *Candida* Spp.Using primers specific to these genes (Table 1).The samples were examined and quantified on 2% agarose gel and by using a spectrophotometer.

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**Table (1):Primers used in determining resistance genes in *Candidia*Spp:**

| Gene | Primer | Sequence (5 to 3) |
|---|---|---|
| *ERG9* | Forward | AAAATGGGTAATGGTATGGC |
| | Reverse | ACTTGGGGAATGGCACAAAA |
| *MDR1* | Forward | GAGTCGTAGCTACATTGCCATTAACA |
| | Reverse | GGTGATTTCTAATGGTCTCCATAATGT |

## Results and Discussion

The current study included a range of(54) sample of ear swabs from patients out of 54 only 27 gave positive growth. The overall patient samples are distributed according to the genus of patients from 54 samples of male samples 26 (31.32 %) and female samples 28 (33.73%) table (2).

**Table (2): Numbers and percentages of total samples divided by sample type and gender.**

| Type of samples | Male | | Female | |
|---|---|---|---|---|
| | No. of samples | Percentage of samples (%) | No. of samples | Percentage of samples (%) |
| Ear | 26 | 31.32 % | 28 | 33.73% |

Positive samples (16) were distributed according to the sample type and patient sex, male samples 5 (31.25 %) and females 11 (68.75%%) table (3).

**Table (3): Numbers and percentages of total positive samples divided by sample type and gender.**

| Type of samples | Male | | Female | |
|---|---|---|---|---|
| | No. of samples | Percentage of samples (%) | No. of samples | Percentage of samples (%) |
| Ear | 5 | 31.25 % | 11 | 68.75% |

It is clear from the above table that the ear infeaction are the highest proportion. Earwax (Cerumen) protects the ear lining of fungi, so anything reduces the amount of wax (such as spraying marine water to the ear and over-use cotton sprouts) will allow the occurrence of fungal infection. The eczema of the leather inside the ear can be another risk factor.

External temperature plays a major role. Fungi grows faster in heat, so it is more common in warm climates (Knott., 2017).

The samples were collected during the summer, and in this season the injuries are given due to the availability of conditions for fungi growth of heat and moisture.

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The *Candida* Yeast included (7) species and was as follows*C.ciferrii* 5(31.25%)*C.glabrata*5(31.25%), *C.* ,*C.guilliermondii*2(12.5%), *C. parapsilosis*1(6.25%), *C. tropicals*1(6.25%) ,*C. krusi*1(6.25%) ,*C. albicans*1(6.25%)As shown in table (4).

**Table (4): Numbers and percentageof*Candida Spp.*isolated from ear.**

| *Candida Spp.* | No. | percentage |
|---|---|---|
| *C. ciferrii* | 5 | 31.25% |
| *C.glabrata* | 5 | 31.25% |
| *C. guilliermondii* | 2 | 12.5% |
| *C. parapsilosis* | 1 | 6.25% |
| *C. tropicals* | 1 | 6.25% |
| *C. krusi* | 1 | 6.25% |
| *C. albicans* | 1 | 6.25% |
| Total | 16 | 100% |

The following results were recorded in the current study:It was found that both*C.glabrata* and *C. ciferrii*at the top frequency compared to other with the other species of *Candida*in ear which were 5(31.25%), for two spescies while *C. guilliermondii*appeared in second rank in ear , which were 2(12.5%).

All samples collected on SDA has been developed, and the Candida Spp. Colonies were white or creamy colors, and quickly grow in 3 days at (35-37Cº), a soft colony or shiny or dry, depending on the species.These results were agreed with (Lynch.,1994).(Figure 1).

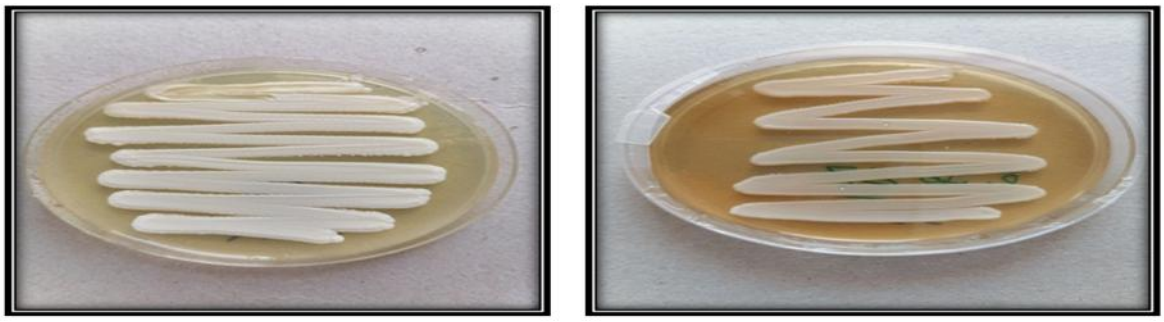

**Fig (1):Micrography show *Candida spp*. Colonies growing on SDA ,2Days ,37Cº.**

The direct microscopy examination was used to determine pseudohyphae and blastoconidia, true hyphae, buds and germ tube of some *Candida* species. (Koneman and Roberts 1990).All species produce blastoconidia, which were round or extended shape, Most of them produce pseudohyphae that are long, branch, or curved. In addition, true hyphae and chlamydospores are produced by some *Candida* species, (Figure.2).

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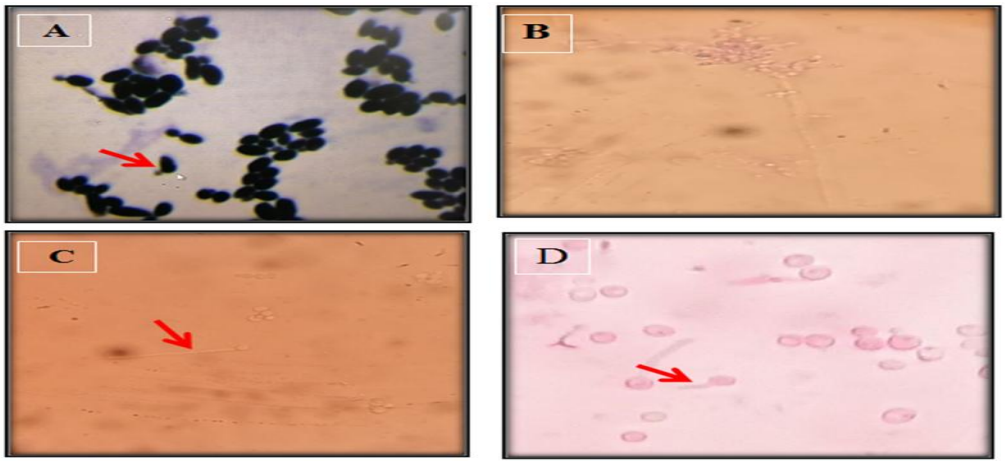


**Fig (2):Micrographs showing Candida yeast stained with crystal violet , A:*C.albicans* ( yeast and bud) B:true hyphae C: pseudohyphe D: germ tube (all with magnification power 40x).**

This study has proved using CHROMagar *Candida* which is considered a differential agar the colonies appear *C.glabrata* Dark pink,*C.krusei* forms pink colonies with whitish border, *C.parapsilosis* white or pale pink , *C.albicans* characterized by Light green color or apple green smooth colonies and dark blue-grey with a purple halo of *C.tropicalis*(Neppelenbroek*et al*., 2014) . Mauve with white peripheral (smooth) of *C. ciferrii.* color, *C. guilliermondii* appeared on CHROMagar transparent green or Mauve with white periphera shown in table (5).

**Table (5):Colony color of *Candida* isolates on CHROMagar Candida medium.**

| No. isolates | *Candida* species | Color of Colony |
|---|---|---|
| **1** | *C. ciferrii* | Mauve with white peripheral |
| **2** | *C.glabrata* | Pink |
| **3** | *C. guilliermondii* | Transparent green |
| **4** | *C.albicans* | Light green |
| **5** | *C.parapsilosiscerevisiae* | Whiet |
| **6** | *C.krusei* | pink with whitish border |
| **7** | *C.tropicalis* | blue-grey with a purple halo |

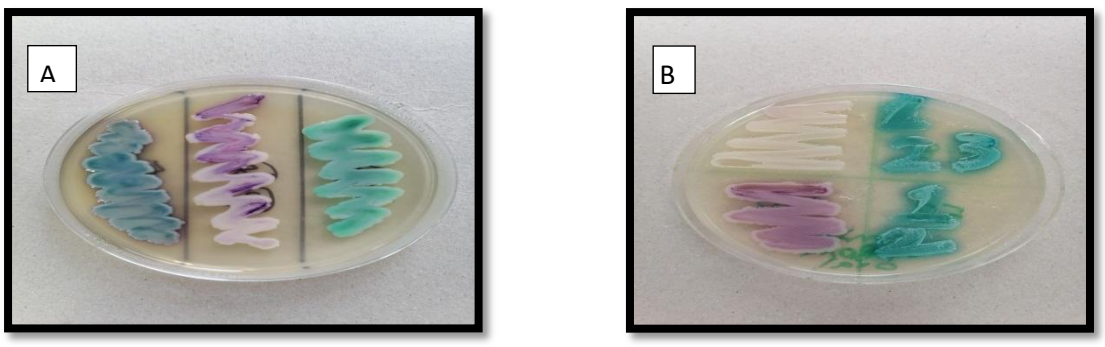

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**Fig (3):Micrography showing *Candida* species colonies growing on CHROMagar 2 days 37 Cº, A:***C.tropicalis*(blue*), C.ciferrii*(mauve)**,***C.albicans* (green)**B :***C.krusie*(pink),*C.parapsilosis*(whiet) ,*C.albicans* (green) **C:***C. guilliermondii.*

**Test the configuration of the Germ tube for *Candida* spp.**

The results have proven to be isolates of *C. albicans* produced germ tube when incubated in human serum at 37 ° C for 2.5-3 hours while the other species from non *C.albicans* shown a negative result except *C.dublinesis*.These results are in agreement with (Deorukhkar*et al*., 2012). The germination tube is the characteristics of the distinctive appearance in *C.albicans*, confirm the germ tube available as a quick way to determine *c.albicans* (Figure 4.A).

**4.1.2.5 Gram Stain of *Candida* species**

All kinds of yeast summarized positive results gave to gram stain and these results were consistent with (Iehab, 2014) (Figure 4. A-B).

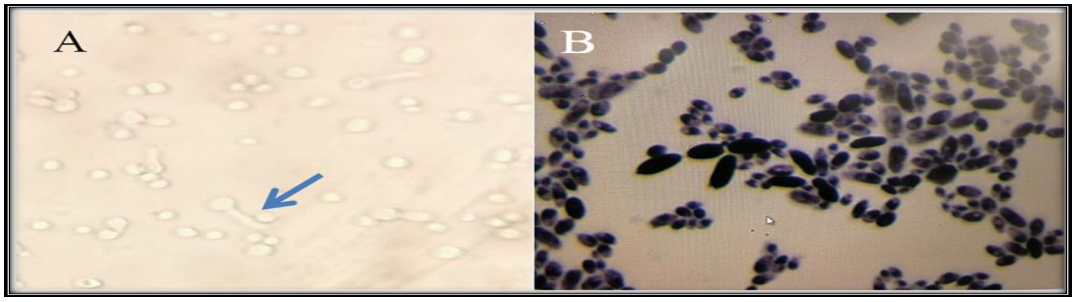


**Figure (4): A: Germ tube formation of *C. albicans* (40X). B: Gram stain of *Candida spp*.(40X) .**

**Vitek 2 identification and antifungal Susceptibility**

Using this device has been diagnosed with confidence*Cryptococcus laurentii*(90%),*C.ciferrii* (89%), *C. guilliermondii*(87%),*C. famata* (86%) and *C.glabrate*(85%).Sensitivity was tested for six types of anti-fungi(Amphotericin B - Caspofungin - Fluconazole – Flucytosine – Micafungin - Voriconazole ).these anti-fungal FDA Indications for use *C. dubliniensis, C. albicans, C. parapsilosis, C. tropicalis, C.glabrate ,C. guilliermondii, C. lusitaniae*table (6).

**Table(6):antifungal Susceptibility for some yeast isolated in this study.**

| Yeast | Antifungal | MIC | Interpretation |
|---|---|---|---|

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| | | | |
|---|---|---|---|
| ***C.glabrata*** | Amphotericin B | 0.5 | S |
| | Caspofungin | 0.25 | I |
| | Fluconazole | 8 | R |
| | Flucytosine | ≤ 1 | S |
| | Micafungin | ≤ 0.06 | S |
| | Voriconazole | ≤ 0.12 | S |
| ***C.ciferrii*** | Amphotericin B | 0.5 | S |
| | Caspofungin | ≤ 0.12 | S |
| | Fluconazole | 8 | R |
| | Flucytosine | 16 | I |
| | Micafungin | ≤ 0.06 | S |
| | Voriconazole | ≤ 0.12 | S |
| ***C. guilliermondii*** | Amphotericin B | 8 | R |
| | Caspofungin | ≥8 | R |
| | Fluconazole | - | - |
| | Flucytosine | ≤ 1 | S |
| | Micafungin | - | - |
| | Voriconazole | ≤ 0.12 | S |

***R: Resistance. *S: Sensitive. **I:Intermediate.

*C.ciferrii* and*C.glabrata*resistance to Fluconazole (MIC≥8) but*C.ciferrii* revealed sensitivity to amphotericin- B (MIC 0.5) These results were compatible with(Gunsilius*et al*., 2001).

*C. guilliermondii* more resistance to Amphotericin B and Caspofungin(MIC≥8)These results were incompatibly with(Desnos-Ollivier*et al*.,2008).

**Biofilim formation test.**

Biofilim examination has been tested for pathological isolation. Using the tube method biofilm formation was observed as a positive when a visible film was lined up on the wall and bottom of the tubes, the results were scored visually as ( 0-absent,1- weak, 2-moderate,3-strong ) (Figure 5).

Fungal species are different in its production for Biofilim Depending on the place of injury shown in table (7).

Forming the biofilm It is a powerful virulence factor for a number of *Candida*Spp. (Tumbarello*et al*. 2007).There are many genes organizer During the different stages of the

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formation of the biofilm. Among these, Genes Pump azole Efflux CDR1 and MDR1 are organized in the early stage of the formation of the biofilm (Mukherjee *et al*., 2003).

**Table (7): The yeast product biofilms distributed by sample type.**

| Yeast | No. absent | No. weak | No. moderate | No. strong |
|---|---|---|---|---|
| *C.glabrata* | - | 1 | 2 | **2** |
| *C.ciferrii* | 1 | 1 | 2 | 1 |
| *C. guilliermondii* | - | - | 2 | - |
| *C.krusie* | - | - | 1 | - |
| *C.albicans* | - | 1 | - | - |
| *C.parapsilosis* | - | - | 1 | - |
| *C.tropicalis* | - | - | - | 1 |

Different strains showed a strong, moderate or weak direction to produce biofilm, but this feature has not been related to the severity of the disease. And the axial role In vital patients play large resistance to antifungal treatment because of restrictions Materials for hacking matrix and to protect the fungal cells from host immune responses(Trofa*et al*.,2008).

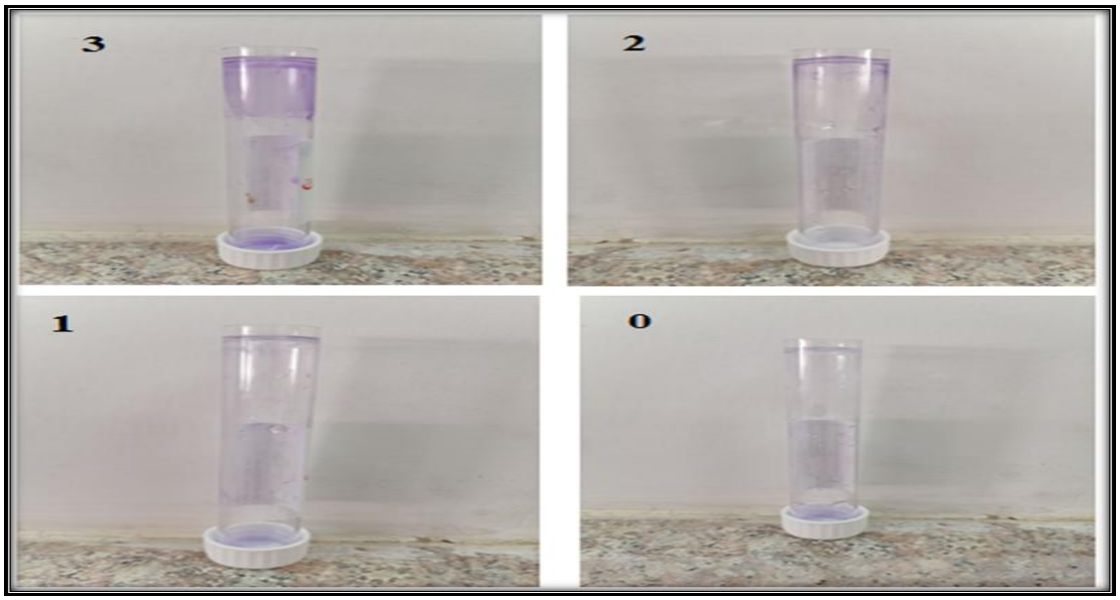


**Fig (5): Biofilim formation** 3:Strong 2: moderate 1: weak 0: absent .

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**Genetic diagnosis to determine the isolates containing resistance genes by PCR technology.**

PCR examination (16) pathologic to investigate resistance genes (MDR1- ERG9) in *Candida*spp using primers specific to these genes.

It shows that ERG9(~840-850 bp) is located in 16 isolates (100%) and MDR1(~575-585) is located at 3 isolates (18.75%). These results were compatible with(Henry *et al*., 2000).

ERG9is located in all types of *Candida* Spp. isolated in this study while MDR1 is located in (2) *C. guilliermondii*isolates and (1) isolate of *C.parapsilosis.* As shown inFigure (6) and Figure (7)

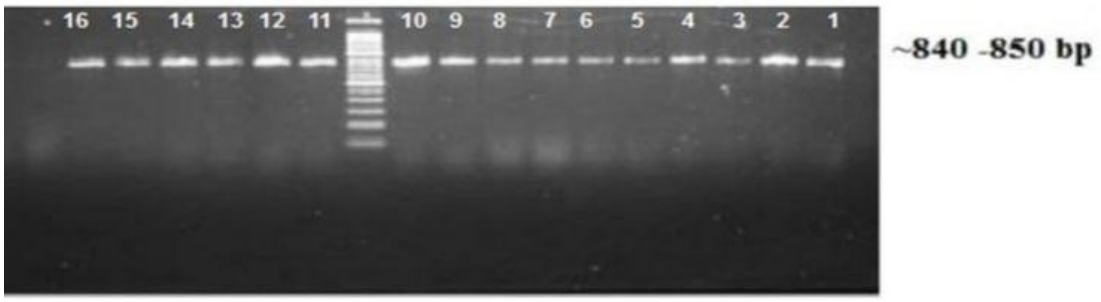


**Figure (6):**Agarose gel electrophoresis of ERG9(~840 -850 bp) regions PCR product in 18S rDNA amplified by pair primers of different yeastsp.(1.3% Agarose gel, 80 volts for 1 hour)

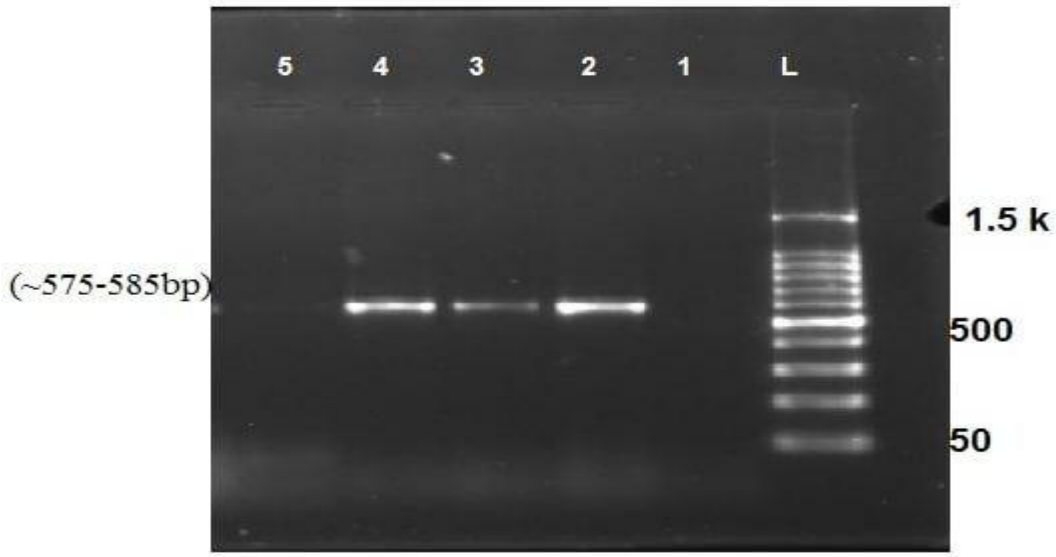


**Figure (7):**Agarose gel electrophoresis of MDR1(~575-585bp) regions PCR product in 18S rDNA amplified by pair primers of different yeastsp.(1.3% Agarose gel, 80 volts for 1 hour).

ERG9 Exist in most of the isolates and these indicate that gene is essential in yeast . This gene encodes a protein Squalene synthase .Increase observed in ERG9 expression after the use of the following treatment withlovastatin (hydroxymethylglutaryl coenzyme A reductase inhibitor),ketoconazole genetic lesions in ergosterol biosynthesis or zaragosic acid (squalene

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synthase inhibitor). The overexpression of MDR1 is a frequent cause of resistance to the widely used Antimycotic agent Fluconazole and other toxic compounds in the pathogenic yeast.

MDR1 Major facilitatorsuperfamilytransporter more existence in *C. guilliermondii*,and*C.parapsilosis.* Plasma membrane multidrug efflux pump that confers resistance to numerous chemicals including Azoles such as Benztriazoles, Fluconazole andVoriconazole as well as to Methotrexate, Benomyl, Cycloheximide,Brefeldin , 4-Nitroquinoline-N-oxide, Sulfometuron methyl and Cerulenin.

## REFERENCES

1. **De Gentile**, L.; Bouchara, J.P.; Cimon, B.; Chabasse D. (1991) *Candida ciferrii*: clinical and microbiological features of an emerging pathogen. Mycoses 34: 125-128.
2. **De Hoog ,**G.S.J.;Guarro, j.;Gene,M.J. and Figueras, F. (2000) .Atlas of clinical fungi ,2nd.,centraabureeau voorschimmelculturees Utrecht ,the Netherlands. Vol.53-No.1,P: 45-49.
3. **Deorukhkar,** S and Saini, S.( 2014). Laboratory diagnosis of candidiasis through ages. Int J CurrMicrobiolAppl Sci.;4:949-5
4. **Deorukhkar,** S.; Saini, S.; Jadhav, P. (2012). Evaluation of different media for germ tube production of *Candida albicans*and *Candida dubliniensis*. Int J Biomed Adv Res.3:704-7.
5. **Desnos-Ollivier,** M.; Ragon, M, Robert, V.; Raoux, D.; Gantier, J.C.;Dromer, F. (2008). *Debaryomyceshansenii*(*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondi*i (*Candida guilliermondii*). J. Clin. Microbiol. 46:3237–3242.
6. **Gentile**, L. de.; Bouchara, J.P.; Le Clec'h,C.; Cimon, B.; Symoens, F.; Chabasse, D. (1995). Prevalence of *Candida ciferrii*in elderly patients with trophic disorders of the legs. Mycopathologia131:99–10.
7. **Gunsilius,** E.; Lass-Flörl, C.; Kähler, C.M.;Gastl, G.; Petzer, A.L. (2001). Candida ciferrii, a new fluconazole-resistant yeast causing systemic mycosis in immunocompromised patients. Ann Hematol. Mar;80(3):178-9
8. **Henry,** K. W.; Nickels, J .T.; and Thomas D. (2000). Edlind. Upregulation of *ERG* Genes in *Candida* Species by Azoles and Other Sterol Biosynthesis Inhibitors. Antimicrob. Agents Chemother., p. 2693–2700.
9. **Horvath,L.L.**; Murray, D.R.; C.K. and Doooley, D.P.(2003).Direct isolation of *Candida spp.* from blood cultures on the chromogenic medium chromagar*Candida* .J.ClinMicrobiol .41:2629-32.
10. **Iehab, Y. J.** (2014)**.**Isolation and Identification of *Candida*spp.from patients with Oral thrush in AL- Najaf Province and molecular study of some virulence factors.MSc, Faculty of Science/ University of Kufa.
11. **Kaur,** R.; Mittal, N.;Kakkar, M.;Aggarwal, A.K.; Mathur, M.D. (2000).Otomycosis a clinicomycologic study. Ear Nose Throat J;79:606-9.
12. **Knott,** L.(2017).Fungal Ear Infection.patient. Page 1 of 3.
13. **Letscher-Bru**V.; Meyer, M.; Galoisy ,A.; Waller, J.;Candolfi, E.(2002). Prospective evaluation of the new chromogenic medium Candida ID, in comparison with Candiselect, for isolation of molds and isolation and presumptive identification of yeast species. J ClinMicrobiol. 40(4):1508-10.
14. **Loveday,** H.P. ;Wilson, J.A.; Pratt, R.J.; Golsorkhi, M.; Tingle, A.; Bak, A.; Browne, J.; Prieto, J. and Wilcox, M. (2014).National evidence based guideline for preventing

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8350 - 8361
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health care associated infections in NHS hospitals in England. Journal of hospital infection 86S1 S1-S70.

15. **Lynch**, D. (1994) Oral candidiasis: History, classification and clinical presentation. Oral Surg Oral Med Oral Pathol.;78(2):189-193.
16. **Mukherjee**, P.K.; Chandra J.; Kuhn, D.M .; Ghannoum, M.A(2003) Mechanism of fluconazole resistance in Candida albicans biofilms: phase-specific role of efflux pumps and membrane sterols. Infect Immun 71: 4333–4340.
17. **Neppelenbroek ,K**.; Seo, R.; Urban, V.; Silva, S.; Dovigo, L.; Jorge, J.H.; et al.(2014). Identification of Candida species in the clinical laboratory: a review of conventional, commercial, and molecular techniques. Oral Dis.;20(4):329-44.
18. **Odds, F**.(1991). Sabouraud ('s) agar. J med Vet Mycol. 29(6):355-9.
19. **Trofa,** D.; Ga´cser, A .; Nosanchuk, J.D. (2008) Candida parapsilosis, an emerging fungal pathogen. ClinMicrobiol Rev 21: 606–625.
20. **Tumbarello,** M.;Posteraro, B.;Trecarichi , E.M.; et al. (2007) Biofilm production by Candida species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J ClinMicrobiol 45: 1843–1850.
21. **Wingard,** J.R.;Merz, W.G.; Rinaldi, M.G.; Miller, C.B.; Karp, J.E.; Saral, R. (1993) .Association of *Torulopsisglabrata*infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. Antimicrob Agents Chemother 37:1847–1849.