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# ISOLATION AND IDENTIFICATION OF FUNGI FROM ORAL OF CANCER PATIENTS AND EVALUATE OF ITS RESISTANCE OF SOME ANTIFUNGAL AGENTS

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***Abstract***

*This study was conduct to investigate phenotypic identification of fungal species isolated from oral of cancer patients in Al-Najaf governorate and evaluate inhibition activity of some antifungal agents. During the period from January to Februry 2021, 50 isolates were collected from Middle Euphrates Cancer Center in AL_Najaf province, which included swabs from the oral cavity, only 12 isolates gave positive growth. Cultivation of all 12 isolates initially on potato dextrose agar and then diagnosed on chrom agar medium depending on the color of the colonies, finally evaluate some antifungal on growth of diagnosed fungi by using well diffusion method. After isolation and identification the results showed that all isolaled belonged to genus* candida *these are* : C. albicans *6 (50%)* , C. Parapsilosis *3( 25%),* C. kruzei *2(16.6%) and* C. glabrata *1 (8.3%), the ability of* C. albicans *and non* albicans *were tested for produce some virulence factors such as germ tube , biofilms and production of enzymes phospholipase when culturing* Candida *spp. on different media. The results of the culture show that* Candida albicans *produces all virulence factor that mentioned above .Also the results showed the antifungal activity for (Nystatin and Amphotercin B) , explaining the effects against* Candida *spp. We can consider the species of yeast are the most prevalent fungal pathogenetic cause of soul invading pathogens in cancer sufferers who are extremely immunocompromised or those who have undergone severe trauma and therapy that necessitates prolonged stays in acute care units. Collect the samples from oral cancer patients, isolation and identification of fungal species on CHROM agar* Candida *media ,Study some virulence factors of* Candida species *and Study the effects of some antifungal on growth of* Candida species .

***Keywords:*** *Fungi, Cancer, Patients, Antifungal*

## INTRODUCTION

Fungal infections are considered danger diseases with profound effect on human life because the metabolism and cellular activity of microorganisms (fungi) which similar to cells of human or animals which reason identification and methods of treatment of fungi difficulty , as well the challenge in defining the fungal species' causes (1). Candida infection or Candidiasis is a disease caused by the genus of yeasts named Candida, one of most widely known is *Candida albicans*, which causes itching, red patches or white patches, and irritation in the vaginal region , mouth, and on the skin. Candida infection affects a few people and spreads through the blood system to various parts of the host body. like heart valves, eyes,spleen and kidneys (2). *Candida* is a common flora of the upper respiratory tract's mucous membrane, but it can also be a pathogen yeast that invades the cell membrane and causing *Candida* infection (opportunistic infection) in

susceptible immunocompromised patients.(3).Acute fungal disease has increased dramatically in the last , two station disease superficial and serious systemic disease (4) .

**METHOD**

**Samples collection**

During the period from January to Februry 2021, 50 samples were collected from Middle Euphrates Cancer Center in AL_Najaf province ,take the swabs from the oral cavity of cancer patients, and transported by sterial screw vials to the microbiology lab in Faculty of Medical techinqes / Islamic University for the purpose of diagnosing the species of fungi depending on the color of the colonies .

**IDENTIFICATION OF FUNGAL ISOLATES**

All the culture characteristics of fungi colony isolates and color, edge were observed on Sabraued dextrose agar media followed by incubation for 24-48 hr.for yeast isolates and 5-7 days for molds isolates while chrom agar testing was using for diagnosed species of *Candida* depending on the color, a few cell were scooped up from the growth of yeast on PDA and culturing and incubated at 37C° for 24-48 hr. (5).

**STUDY OF VIRULENCE FACTORS**

**Germ tube examination**

The culturing of cells gained from a pure culture of yeast were interrupted in 5 µl of human serum in a sterile plastic tube , the tubes centrifugation at speed 1500 rpm for fifteen min then the serum was gained, the tubes could be incubated at 37C° for 2-3 hr., the peroid of incubation must not take more than 3 hr. because other species of yeasts may begin to forming germ tube, a drop of serum was putted on a slide, covered with a cover and observed under a microscope for the existence of a germ tube (6).

**Test for the production of biofilm**

Colonies of *Candida* were inoculated into a saline tube then incubation at 37 C for 24 hr. followed by added 5µl from it , to a circular polyethylene tube containing 5 ml of SDB enhanced with glucose ultimate concentration of 8% ,finally tubes incubation for 48 hr. at 35 C°, remove the broth of the tubes after incubation with a Pipette, wash the tubes with D.W usually twice and stain the tubes with dye (Safranin 2%) , pour the remaining stain , after 15 min then rinse the tubes with D.W to remove the excrescent stain. Biofilm production was suggested by the presence of transparent adhering film at the tube's bottom and the tube's walls. (7).

**Phospholipase production test**

Egg-yolk agar medium was used to detection of phospholipase producing by *Candida* after inoculation of the medium agar, then plates incubation for 2 days at 37 C° , the positive results which represented by appearance of precipitation zones around the colonies (8) .

**RESULTS AND DISCUSSION**

**Isolation and identification of fungi species**

Out of 55 isolates only 12 isolates of the genus Candida appeared and they were diagnosed Morphological identification as follows :

**Identification on PDA medium**

All collected isolates were grown on PDA, *Candida* spp. colonies appeared cream to yellow in color, Figure (1), formed quickly to maturation in (1-2) days at 37°C, and the colony texture was smoother, glossy or dry. These results are similar with other results of Bhavan (9).

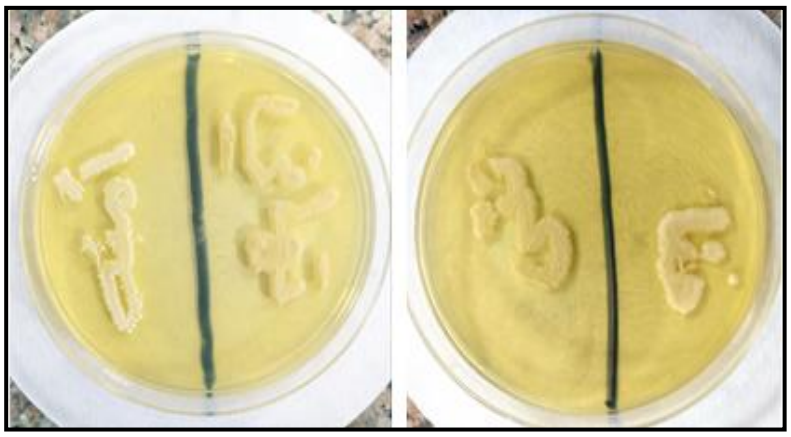
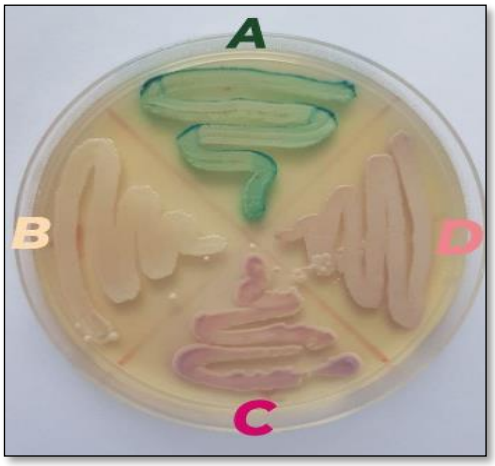


Figure (1): *Candida* spp. growing on PDA, at 37Cº for 2 days

**IDENTIFICATION OF CANDIDA SPP. ON CHROMAGAR MEDIUM**

The chromagar of *Candida* which utilized as a differential agar between the species of *Candida,C.glabrata* colonies appear dark pink while *C.krusei* appear pink with white to fuzzy in peripheral of colonies , *C.parapsilosis* characterized by white to pale pink and *C.albicans* appeared smooth colonies with light green color (10) .Figure (2), table (1).These results consistent with (11,12) we found the similar features of colonies for *Candida* spp. Chrom agar media are rapid and effectiveness for diagnosis of *Candida* species the color appear after incubation compared with other traditional culture methods, The medium substantially improves the diagnosed of specimens having mixtures of species by changing color as a result of reactions of special enzymes with an unique chromogenic substrate. (13). After incubation for 48 hr. at 37°C, on chromagar *Candida* ,the isolates of yeast occur and the special of yeasts had grow well .




Figure(2)Macrographs showing *Candida* spp. colonies growing on chromagar 24-48 hr.37 Cº, A : *C.albicans*, B: *C.parapsilosis*,C: *C.glabrata*, D: *C.krusei*

Table (1) *Candida* species on chromagar medium

| No. | Candida spp. | Color of colony |
|---|---|---|
| 1 | *C.albicans* | Light green |
| 2 | *C.parapsilosis* | white pale pink |
| 3 | *C.krusei* | Pink with white peripheral(fuzzy) |
| 4 | *C.glabrata* | Dark pink |

**FREQUENCY OF *CANDIDA* SPECIES**

The results showed that the most isolates were returning to *C. albicans* 6 (50%), because of Candidiasis caused by opportunistic over growth of it , the isolate comes after was *C. parapsilosis* 3 (25%), then *C.kruzei* 2 ( 16.6%) and *C.glabrata* ( 8.3%) ,(Figure 3).

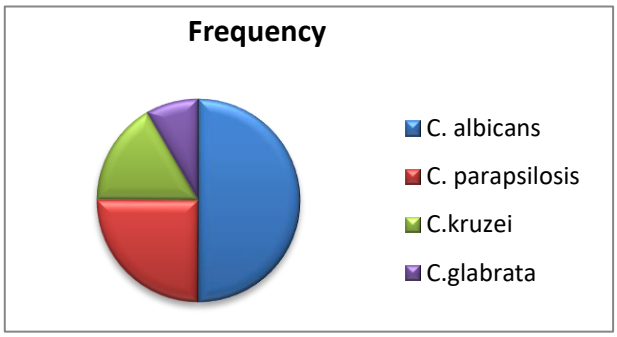



Figure 3: percentages (%) of distribution *Candida* spp.isolation from oral cancer patients

**VIRULENCE FACTORS**

**Germ tube testing**

This examination was fast method for identification some of *Candida* spp ,after incubation the testing showed many species of *Candida* spp that production germ-tube and non production germ (Figure 4).The researcher must have ability to differentiation between the pseudohyphae and germ tube . this tube referred to elongated the daughter cells from the mother cell without impediment of origin to germ tubes , but the origin constriction of the mother cells named pseudohyphae (14). *C.albicans* has a major ability to create germ tubes than other yeast (15).

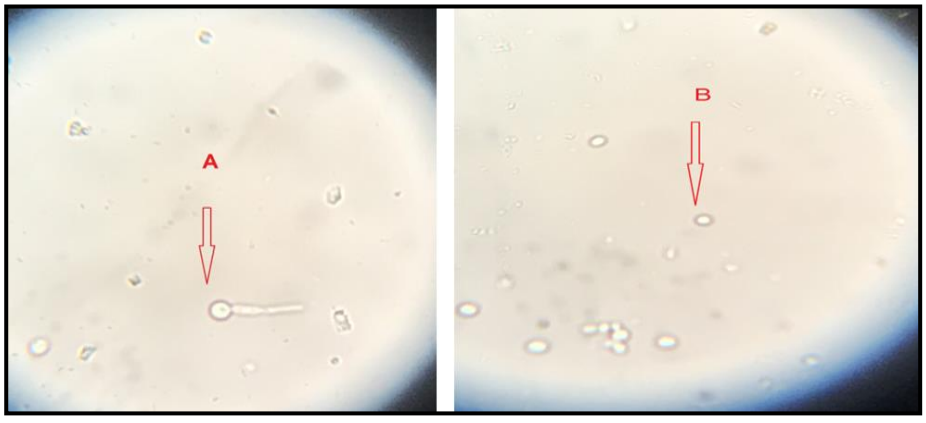



**Figure (4 ):A- *C.albicans* germ tube formation**
**B-Non germ tube formation of other *Candida* spp.**

**BIOFILM FORMATION**

The results showed that the ability of some species of *Candida* on the formation of biofilm , figure (5) .They showed a positive results in *Candida albicans* (16). Biofilms are important or major virulence factor in the pathogencity of infections disease , because biofilms correlated microorganisms showed natural resistance to disinfectants antibiotics and any mechanisms of host defense (17). The capacity of *C. albicans* to produce biofilms on surfaces or biological things is an importance virulence factor (18). The modern studies Biofilm growth has been established in the totality of *Candida* spp.-related diseases. (19).

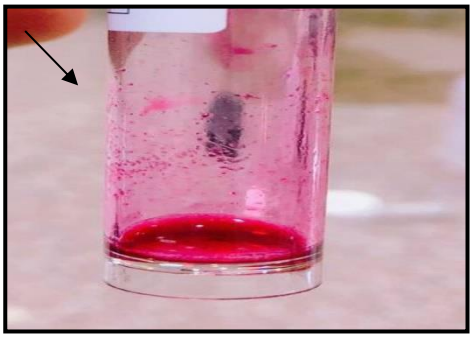


Figure (5):Biofilm formation of *C.albicans* (positive)

**PRODUCTION OF PHOSPHOLIPASE TEST**

The results illustrated the capacity of some species of *Candidia* to production phospholipase enzyme. The precipitation area round the colonies on Egg yolk agar was noticeable of the production of phospholipase enzyme,Figure (6**)** ,it was indicated the action of the enzyme of *Candida* spp from Samaranayake (20) method. Phospholipase When a precipitation region was seen across the colony on the culture plate, the results of isolate's activity was regarded positive (21). All *C.albicans* isolates was positive for phospholipase production.

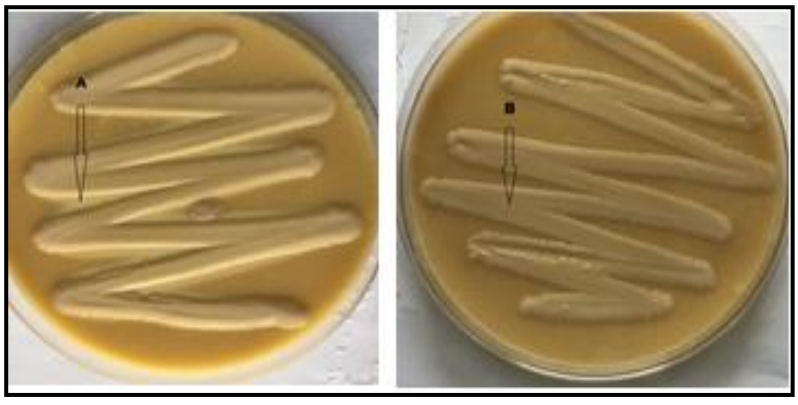


Figure (6):A- Phospholipase enzyme production for *C.albicans*.
B-Non Phospholipase enzyme production for other *Candida* spp.

**ANTIFUNGAL SUSCEPTIBILITY TEST**

There are two antibiotics belonged to antifungal drugs which are : nystatin and amphotercin B which interact with fungal membrane sterols physicochemically. All isolates of *Candida* species

showed minimum sensitivity to antibiotic ,(Figure 7, 8,9,10),it indicate that these yeasts have good virulence factor were you can counteract the entry of antibiotics through a sophisticated enzymatic system .

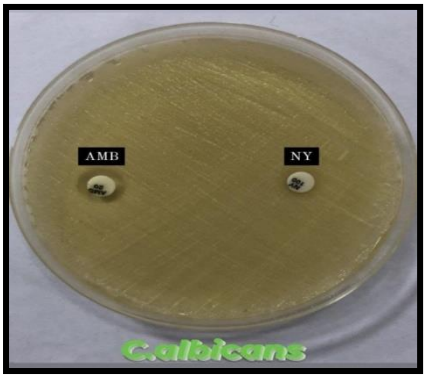



Figure 7:The susceptibility of *C.albicans* to NY and AMP


Figure 8:The susceptibility of *C.parapsilosis* to NY and AMP

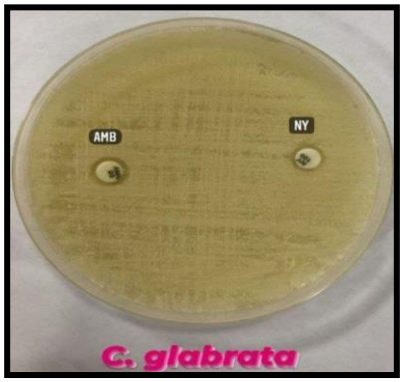



Figure 9 :The susceptibility of *C.glabrata* to NY and AMP

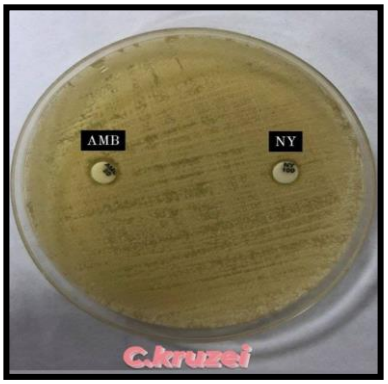



Figure 10 :The susceptibility of *C.kruzei* to NY and AM

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