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ISOLATION THEN IDENTIFICATION CANDIDA SPECIES THAT CAUSING ORAL CANDIDIASIS AND TESTING THEIR SENSITIVITY TO SOME ANTIBIOTICS

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Abstract

The current study included the isolation and diagnosis of Candida yeast from various clinical cases. 125 samples were collected and distributed (25 samples from healthy people, 25 samples from patients with kidney failure, 25 samples from diabetic patients, 25 samples from patients wearing braces, and 25 samples from newborns). After diagnosis, subsequent tests, including phenotypic, microscopic, and genetic tests of the isolated samples, confirmed that 80 (60%) of the isolates belonged to the yeast *Candida spp*., and showed that the *Candida albicans* isolate was the most frequent (50%). The results of the vitek2 test showed that the probability rate was between very good and excellent (91-98%). The study showed that the disinfectants used are very effective using the poisoned food technique, With regard to the results of the drug sensitivity test using the disc diffusion method, the antibiotic fluconazole proved effective in inhibiting the growth of the yeast *C. albicans* with a diameter of 40mm, and the yeast was the highest resistant to the antibiotic Nystatin.

Keywords: (*candida albicans , candida spp* , antibiotic)

1. Introduction

Candida yeasts grow at a temperature of 37 degrees. They are dimorphic fungi and are considered part of the body flora. Normal human flora, formerly called Monilia. They coexist in a natural form on the body[1], and are usually non-pathogenic and are found on the skin and mucous membranes. [2]Opportunistic infections can cause changes in the body's immune status, or in diabetics, or those with cancer, or tissue and organ transplantation, to change the internal environment and transform into parasitic pathogenic fungi. This condition is called candidiasis [3] Candidiasis. Studies indicate that Candida yeast colonies are naturally present in the oral cavity at a rate of 20-40% in normal people, the respiratory tract, the intestinal tract, and the vaginal tract. Oral Candidasis, or thrush, is an infection that affects both the oral cavity and the mucous membrane of the pharynx. It is a widespread disease in the world. It appears in the form of milky spots covering the cerebral membrane It extends to the oral cavity, and the infection may spread to the pharynx, bronchioles, and esophagus, and cause angular cheilitis. Angular Chelitis: Oral thrush occurs as a result of the increased growth of fungal hyphae of *Candida spp*. It affects about 2% of newborns.

[4]. The infection appears on the child's tongue, gums, inside the cheeks, and the roof of the throat. Severe pain and difficulty swallowing occurs when eating or breastfeeding. It is also followed by the appearance of red spots or severe ulcers on the membranes of the mouth, which may cause slight bleeding when touching the site of the infection or trying to rub it.

Some studies have reported that antiseptics prevent the growth of fungal spores in the mouth. Among the antiseptics (alum, mouthwash), alum is considered a powerful substance because it contains compounds such as potassium and aluminum sulfate and is in the form of transparent granules. It has benefits for the mouth and is used as a mouthwash when dissolved with water. It is considered an effective mouthwash and antiseptic. It is excellent and helps in treating gums, which are resistant to *Candida albicans* [5]. Mouthwash contains compounds that eliminate germs in the mouth and removes bad breath from the mouth caused by fungi and harmful bacteria, which are (Chlorhexidine, Cetylpyridinium chloride). chloride, menthol, thymol, alcohol (chole). Mouthwash removes food waste, relieves pain in the affected gums, and aids healing and healing [6].

Azoles and polyenes are chemical compounds that inhibit or kill fungi by interfering with the metabolism of the cell membrane, specifically with (Ergosterol), which leads to deformation of the cell membrane of the fungal cell and causes it to lose its contents and die. These antifungals were used with opportunistic fungi and gave mixed results, as studies indicated sensitivity of opportunistic fungi to these antibiotics[8][7], while other sources indicated that opportunistic fungi are resistant to these antifungals, and this is due to to mutations that occur in the genome [10,9]

2.Materials and Methods

2.1 Samples collection: 125 oral swab samples were collected from healthy people, diabetics, and kidney failure patients hospitalized at the General Teaching Hospital in Al-Diwaniyah, and people wearing braces from the Specialized Dental Center. Also isolated from the mouth area of children aged one day to one year hospitalized in the Maternity and Children's Hospital.

The samples were distributed as follows, according to the sources of collection: 25 samples from the mouths of healthy people, 25 samples from diabetics, 25 from patients with kidney failure, 25 samples from infants and newborns, and 25 samples from people wearing braces.

2.2 Culture and identification yeast

The samples were cultured on Sabouraud Dextrose-Agar medium, which was marked with cotton swabs on the surface of the nutrient medium. Three replicates of cultivation were performed on the above-mentioned medium to ensure that the fungal growth resulted in pure, single colonies. The plates were incubated at a temperature of 37°C for 24 hours. [11]. Person genus Candida. *Candida Spp*. The growth of Candida was examined and the shape, color and size of the developing colonies were observed based on a set of phenotypic and biochemical specifications, as stated in [12], which included:

2.3 Direct microscopic examination of samples: Use the Gram stain, which is considered one of the most important dyes that distinguish the dye-positive and stain-negative types. The examination was conducted based on the method [14][13].

2.4 chrom candida different ager

This test was carried out by plotting Candida yeast on chromium candida ager medium, and the dishes were incubated at a temperature of 37°C for 48 hours, in order to distinguish between the types of *Candida* in the examination based on the type of colony, based on the method [15].

2.5 Identification by Vitek2 system

This test was conducted using a Vitek device equipped by the French company Biomerieux. This test was conducted at the Al-Amin Center for Research and Biotechnology in the Najaf Governorate. It is a fully automated diagnostic system used in diagnosing pathogenic yeasts using the Vitek 2YST card. This diagnosis was performed for four isolates by following Next steps

1. Candida isolates were grown on Saprod Dextrose Agar medium and incubated at 37°C for 24 hours.
2. Prepare the bacterial culture suspension and transfer four to five colonies to glass test tubes containing 3 ml of a standard sterile physiological solution accompanying the Vitic 2 device. The turbidity of the growth suspension was controlled, and it should range between 1.8-2.2, using the special Denise device. With the Vitek 2 device
3. The tubes containing the suspension were placed in the special holder and the YST cards equipped with carrier tubes were installed to insert them manually in their designated place in the device.
4. The tubes were incubated for a period of 12 to 18 hours, and the results were automatically read from the device and given in the form of a report for each isolate. Each isolate is given a probability ratio and a confidence level. If the probability ratio is 96-99, the confidence level is excellent, 93-95 is a very good confidence level. If the confidence level is good, 89-92 is good. If the confidence level is 85-88, the confidence level is acceptable and less than this percentage, the confidence level is weak. [16].

2.6The poisoned dish method

In this case, I followed the poisoned food technique [17] by placing 1 milligram of the disinfectant (alum) and 1 ml of the disinfectant (rinsing mouthwash) in a clean, sterile Petri dish, adding 15 ml of sterilized (SDA) to it. I moved the dishes in a circular way to ensure that the disinfectant was evenly distributed in the medium and left the dishes to solidify. Using a loop, the dishes were coated with the most susceptible yeast *C. albicans* in an even manner and incubated in the incubator for 24-48 hours at a temperature of 37°C, with three replicates for each disinfectant [18]

2.7Testing the sensitivity of yeasts to antifungals

The drug sensitivity of the *C. albicans* isolate under study was tested using 3 types of antifungal tablets, and the results were determined by measuring the Zone's of Inhibition as shown in (Table3) based on [19].

3.Results and Discussions

3.1Isolation and Diagnosis

This study included the collection of 125 oral swabs from healthy people, people with diabetes, patients with kidney failure, people wearing braces, and children aged between one day and one year. It was found, after culturing the samples and studying the agricultural and microscopic characteristics, that the number of positive samples was 80, with a percentage of 60%, and the remaining samples. 45 samples recorded negative results at a rate of 36%

(Table 1) shows the number of samples, the percentage of infection with candidiasis, and the age groups isolated.

Age groups isolated from	Number of samples	Percentage
newborn babies	10	40%

healthy people	15	60%
people who wear braces	16	64%
diabetic patients	19	76%
patients suffering from kidney failure	20	80%

(Table 2) shows the types of yeast isolated from different age groups and percentages.

Species	**Number of patients**	**Percentage**
C.albicans	Isolated from (40 patients)	50%
C.glabrata	Isolated from (16 patients)	20%
C.krusei	Isolated from (12 patients)	15%
C.tropicalis	Isolated from (8 patients)	10%
C.parapsilosis	Isolated from (4 patients)	5%

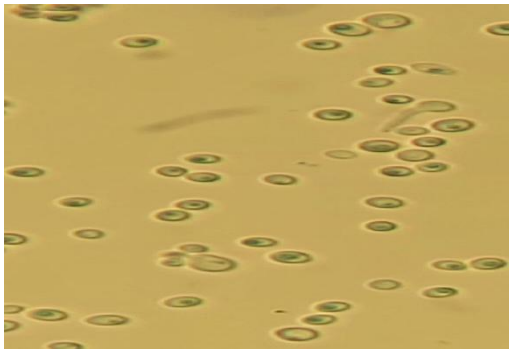
3.2Colony morphology examination

Colonies growing on SDA medium appeared in the form of white to cream-colored colonies, and the colonies were smooth and in the form of circular colonies. [20] indicated that colonies of *Candida spp*. possess such phenotypic characteristics when grown on the aforementioned medium. The clustering of cells in this order and shape is attributed to genetic factors in the cells themselves that ensure their clustering in this order under normal conditions. This result is consistent with what [21] mentioned, with the appearance of shiny, cream-colored, smooth, and circular colonies, as in the (fig.1).

(Fig.1): *Candida albicans* colonies on SDA medium

3.3 Microscopic examination

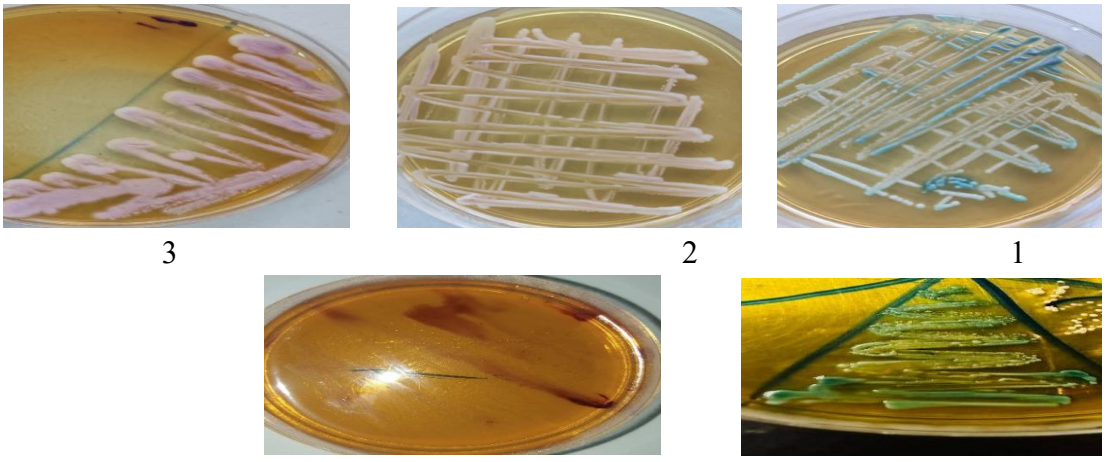
The isolated species gave positive results by interacting with the Gram stain, as the cells appeared oval to spherical, elongated, or cylindrical in the yeast form of yeast, and were single or in the form of chains as a result of the cells budding several times without separating from the mother cell. This is known as pseudohyphae, and the appearance of Candida cells is stained purple. As a result of the accumulation of the peptidoglycan layer present in the cell wall with the Gram stain, this result is consistent with [22] as in (Fig.2).



(Fig.2) shows a microscopic image of *C.albicans* yeast

3.4 Candida Crome Differential Agar

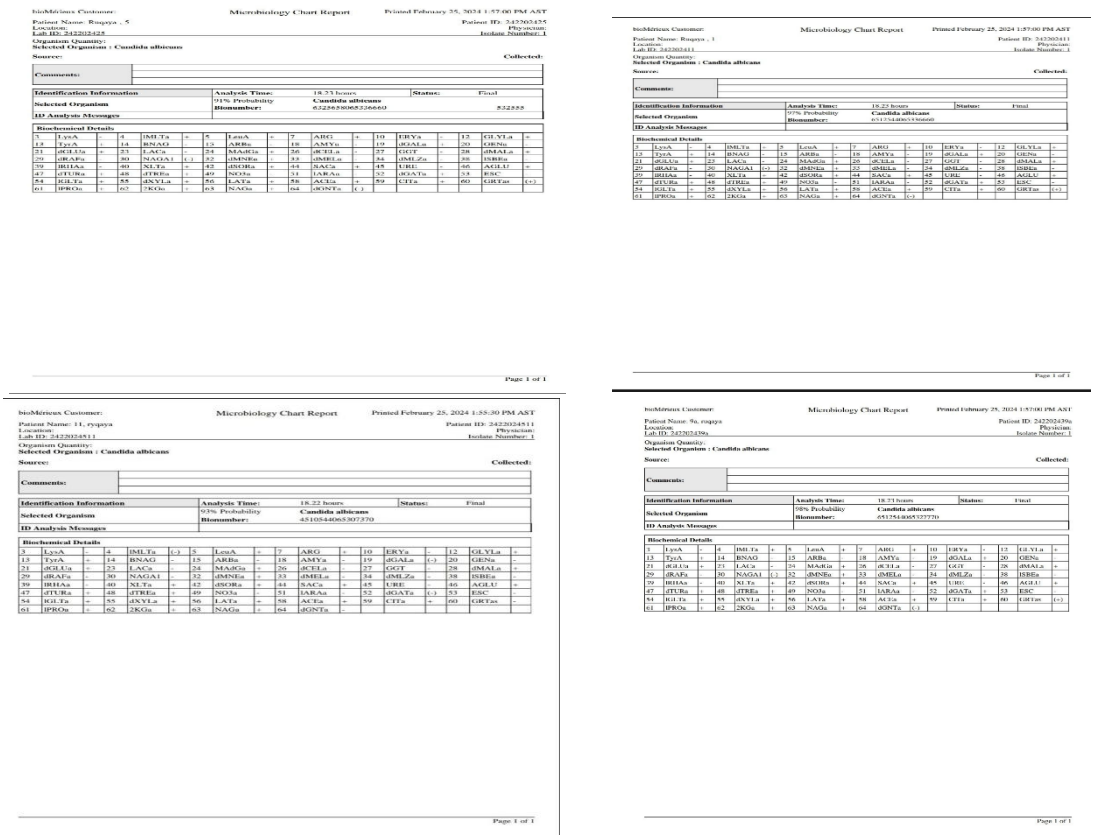
When the isolated Candida species were grown on Candida Crome Agar for 24-48 hours at 37°C, the Candida yeasts showed different colours, each species having its own color. The diagnostic results showed that the *C.albicans* species had a bright green colour, while the *C.tropicalis* species grew in a blue colour. *C. glabrata* is purple, *C. krusei* is pink, and *C. parapsilosis* is white. This medium was used as a differential medium with accurate results in diagnosing Candida species, and this result was identical to a study by [23] on the isolation and rapid diagnosis of Candida species isolated from the mouth. It is one of the most important media used in the field of fungal diagnosis, as the diagnosis relies on staining in the medium.



(Fig.3) shows the growth of colony types (1*) C.tropicalis* (2) *C.parapsilosis* (3) *C.krusei* (4) *C.albicans* (5) *C.glabrata*

3.5 Identification by Vitek2 system

The diagnosis of *C. albicans* isolates was confirmed using the Vitek2 system, and the probability of identification rates were between very good and excellent (91-98%). This result is consistent with many studies [24] and [25] that indicated excellent identification. on *C. albicans* using the Vitek2 system. The Biomerieux Vitek2 system includes diagnostic identification cards from Vitek2, and this system is based on diagnosing species by evaluating biochemical tests with approved identification cards in addition to the Vitek2 system, and this led to reducing the time required for diagnosis and improving the percentage of yeast diagnosis at the species level [26] as shown in (Fig.4).



(Fig.4) shows the results of Vitek2 diagnosis

3.6The poisoned plate method

The results proved the success of the two disinfectants (alum and mouthwash) used in inhibiting the yeast *C. albicans*, and the experiment showed that growth was inhibited or inhibited under the influence of disinfectants because disinfectants weaken the cell wall and plasma membrane and enter the cytoplasm, which leads to the leakage of cell contents and leads to its death [27] and these results are consistent with [28] as shown in (Fig.5).

a b c

a Before adding disinfectants
b After adding the mouthwash
c after adding alum

(Fig.5) shows before and after adding disinfectants

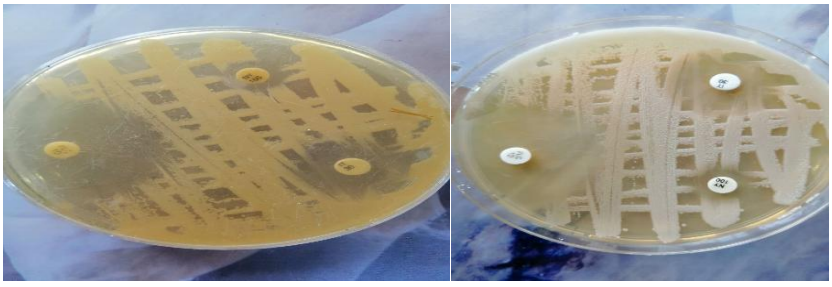
3.7Susceptibility testing of yeasts to antifungals

The drug sensitivity of the most frequent *C.albicans* isolate under study was tested using 3 types of antifungals using the tablet method. The results were shown by measuring the Zone's of Inhibition as

shown in (Table3) based on [29]. The results showed There is a discrepancy in the sensitivity of the isolate under study to the antibiotics used, as the *C.albicans* isolate showed more sensitivity to the antibiotics fluconazole and itraconazole, and these two antibiotics belong to the azole group, which work by interfering with the fungal cell membrane and affecting the fatty compound Ergosterol, which causes openings or holes in the membrane. The cell makes it completely permeable, which leads to the disruption of osmotic pressure, so the cell contents come out, leading to cell death [30]. As for the least sensitive and highest resistance antibiotic, Nystatin, this result agrees with [31] and due to the frequent use of the antibiotic Nystatin, and also the development of the type of resistance that this isolate possesses against this antibiotic [32], according to (Table3) and (Fig.6).

Antifungal	**Inhibition (mm)**
Fluconazole	40
Itraconazole	31
Nystatin	4

(Table3) shows the drug sensitivity test for *C.albicans* yeast



(Fig.6) shows drug sensitivity to antifungal agents

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