College of Basic Education Research Journal

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Isolation and identification of *Candida* species from oral cavity of children with candidiasis in city of Mosul

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Article Information

Article history:

Received: Abril 10.2023

Reviewer: May 21.2023

Accepted: May 25.2023

Key words : ***Candida*, Diagnosis, Oral, Isolation**

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Abstract

The study was conducted in laboratories of postgraduate studies at College of Education for Girls / University of Mosul. This study involved collecting 92 mouth samples from children hospitalized in Mosul (Al-Salam Education and Ibn Al-Atheer) whose infection was diagnosed by specialist doctors from 1/6/2022 to 5/10/2022. Samples were examined microscopically after being treated with potassium hydroxide solution, then cultured on Sabourin dextrose agar SDA medium then diagnosed microscopically and visually. Isolation results showed that infection rate of candidiasis was 70.65% positive for transplantation, and age groups studied from (0-12) months were the most infected ones with a rate of 35.87%. Through our study, we concluded that yeast isolates that were positive for germ tube formation belonged to Candida albicans species without further diagnostic tests. Furthermore, chlamydospore formation assay for both Candida albicans and Candida dubliniensis determined that HI Chrome differential medium distinguished between Candida species based on colony color, with C. albicans colored bright green, Candida tropicalis colonies showed metallic blue, while Candida krusei colonies was purple in color, and colonies of Candida glabrata were creamy to light pink.

ISSN: 1992 – 7452

Introduction:

Oral Candidiasis is an infection of the tongue and other mucous membranes of the mouth that can extend to the pharynx. It can appear as white spots resembling thrush (pseudo membrane) or red spots known as oral thrush. Several factors such as age, gender, nutrition, oral hygiene, smoking, dentures, salivary pH imbalance, and dry mouth can contribute to the development of Candidiasis. Diabetic patients and those with immune deficiencies are also more susceptible to oral Candidiasis [1] the Clinical Cases of Oral Candidiasis is Acute oral candidiasis It is a slightly elevated white to yellowish soft layer on the mucous membrane of the mouth resulting from excessive growth of yeast cells mixed with inflammatory cells. This type of infection is rapidly occurring and can cause severe damage to the lining of the mouth, and can extend to the esophagus and the rest of the digestive tract [2 , 3].

Cheilitis Angular It is a red lesion occurring at the corners of the mouth resulting from the spread of the infection. It can sometimes be painful [4].

Chronic stomatitis It is mouth inflammation with red or white sores usually located between the jaws. Symptoms can persist for a month or more and occur in individuals with weakened immune systems or the elderly. Smoking is a known risk factor for this type of infection [5] Classification of *Candida* is the genus *Candida* was first classified by a mycologist named Christine Marie Berkhout in 1923 in her doctoral thesis. According to the latest classification mentioned by [6]

Kingdom: Fungi

Phylum: Ascomycota

Class: Saccharomycetes

Order: Saccharomycetales

Family: Saccharomycetaceae

Genus: *Candida*

The Types of yeasts that cause oral candidiasis is *Candida albicans* the main cause of candidiasis in most clinical cases. It is the third most common isolated microorganism from bloodstream infections in hospital patients and is one of the opportunistic pathogens that exist in the mouth. When the host is

immunocompromised it can cause superficial infections and may also cause sepsis. [7]

Candida albicans is a polymorphic fungus called Dimorphic, meaning that it has the ability to change from yeast to hyphal form and thus invade the host's body. [8] *Candida glabrata* It is a yeast that causes opportunistic infections and is common in immunocompromised individuals such as cancer patients, septicemia patients, renal failure patients, elderly people, and is known as *Turolopsis glabrata* . It appears as a smooth, glossy, creamy-colored colony on SDA medium, and microscopically its cells are oval and budding, but not pseudo-hyphal in cornmeal agar medium. [9] The glabrata type of *Candida* comes second to the albicans type of yeast and is non-dimorphic. [10]. Candida *krusei*

constitutes almost 10-35% of serious infections caused by other non-*albicans.* It has the ability to form long, varied and dense false threads, so it has a tree-like shape. [11] Its color is yellowish-gray and it has a distinctive yeast smell when incubated at 25-37°C for 48-72 hours on SAD medium. [12] *Candida tropicalis* It is widely spread in nature and is found extensively on human skin and in the digestive tract and oral cavity. [13]

Materials and methods

Collecting pathological samples

92 mouth swab samples were collected from children staying at Al-Salam Teaching Hospital, Al-Khansaa Teaching Hospital, and Ibn Al-Atheer Hospital in Mosul City. The samples included male and female children from newborns up to five years old for the period (1/6/2022 - 5/10/2022). The sample was taken using a pre-sterilized swab, then transferred to the laboratory on the same day and cultured on the appropriate medium.

Biochemical and culture tests for the diagnosis of *Candida* spp.

Germ tube formation test

The test was conducted using the method of [14] . 5 ml of human serum, which was separated using a centrifuge for 5 minutes, was placed in sterilized test tubes and inoculated with small parts of the fungal colonies growing on SDA medium for 48 hours. These tubes were then incubated at a temperature of 37 °C for 2-3 hours. A drop of the suspension was taken and placed on a glass slide to be examined under a microscope with 40X and 100X magnification. This test is

used to detect *Candida albicans*, *Candida dubliniensis*, and *Candida Africana* yeast and distinguish them from other yeasts.

Chlamydospores Forming Test

This test was performed after activating the yeast samples for 24 hours on SDA medium. Cornmeal agar was prepared according to the instructions of the supplier (TM Media) with a weight of 42 g in 1 liter of sterilized distilled water and stirred on a hot plate. The mixture was then poured into Petri dishes and left to solidify. After solidification, a vertical slit was made in the center of the dish using a sterilized needle heated by alcohol flame. The samples were then inoculated by taking a part of these samples with a sterilized loop and placed in the slit. A sterilized cover glass was placed over it, also heated by alcohol flame, and left in the incubator for 48-72 hours at a temperature of 25 °C. The samples were then examined under a light microscope to check for Chlamydospores formation.

Growth at 45°C test:

This test was conducted by inoculating the samples for 24-48 hours in the incubator, followed by taking a part of the colony from the active part and culturing it on Sabrouid Agar medium, then placing it in the incubator at a temperature of 45°C. The cultures were examined after 24 and 48 hours. This test is used to differentiate *C. albicans* from other species.

Surface growth test:

This test was conducted by inoculating the samples for 24 hours, followed by taking a part of the colony and culturing it in Sabrouid liquid medium (SDB) in sterilized glass tubes. The samples were then incubated at 37°C for 24-48 hours. This test is used to determine the ability of some yeast species to grow on the surface.

Test of growth on differential medium of *Candida* yeast

HiChrome *Candida* Differential Agar

The medium was prepared according to the company's instructions, which are equipped with a solution of 42 g of the medium in 1 liter of sterilized distilled water in a beaker and placed on a thermo hob device until it reached the boiling point and complete dissolution of the medium, after which it was left to cool at a temperature of 45 ° C, then it was distributed in ready-made sterilized Petri

dishes And left to solidify, this medium is characterized by the fact that it does not need to be placed in the sterilizer according to what was mentioned in the instructions of the supplied company. This medium was used to diagnose *Candida* types and differentiate them from other types.

It was noted that four types of *C.albicans* grow, the colonies of this type grow in a light green color, while *C.krusei* grows in a purple color, and the type C.tropicalis that grows in blue, and the type *C.glabrata* grows in a cream color.

Results and Discussions

Isolation and Diagnosis:

This study included the isolation and diagnosis of yeasts from the mouths of children infected with oral thrush, which we obtained from 92 newborns up to five years old. Samples were taken from children with various diseases, including simple ones like mouth infections, as well as immunological ones like diabetes, in addition to premature babies. The isolation results showed that 65 samples out of a total of 92 samples were positive for cultivation, i.e., 70.65%, and these samples showed yeast colonies on SDA medium. Negative samples were 27, with a percentage of 29.35%, and there was no growth in the plates containing SDA medium.

These results were consistent with [15] , who stated that children are more susceptible to oral thrush, especially newborns, where it was found that out of 286 children studied, 161 children were clinically and microscopically confirmed to have oral thrush, i.e., 56.29%. Likewise, [16] showed that out of 135 samples from children ranging in age from one day to one year, of both genders, 90 isolates were infections with yeasts, as confirmed by morphological and biochemical tests of the samples

Table.1: Percentage distribution of the number of infections with the pathogenic yeast *Candida spp*. By age groups

Age range (month)	The number of injuries among children	percentage (%)
١٢-٠	٣٣	% ٣٥,٨٧
٢٤-١٢	٢٥	% ٢٧,١٧
٣٦-٢٤	١١	% ١١,٩٥
٤٨-٣٦	٩	% ٩,٧٨
٦٠ – ٤٨	١٤	% ١٥,٢٢
Total	٩٢	٪١٠٠

Agricultural Traits

Isolates grown on SDA medium and incubated for 24-48 hours showed colonies that were cream to white in color, butyrous, slightly raised above the medium, and may become convex and smooth at first appearance, but wrinkled after more than 7 days of incubation. The colony diameters ranged from 1-3 mm These are the yeast traits described by [17] and [18].

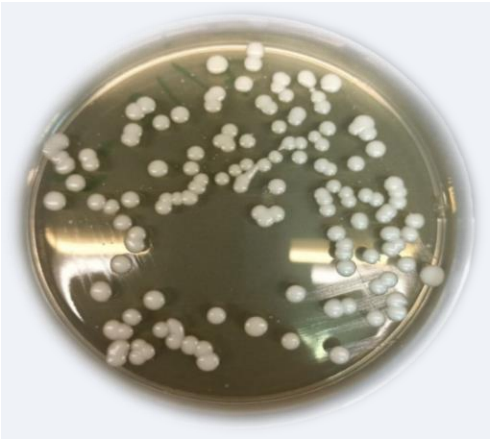

Fig.1 Yeast colonies grown on SDA medium for 48 hours at 37C°.

Microscopic examination

All yeast isolates were stained with lactophenol cotton blue stain, and the blastospores appeared oval, spherical, or elongated and budding, as shown in figure 2 . This result is consistent with [19] , who showed that blastospores appeared spherical and budding or oval.

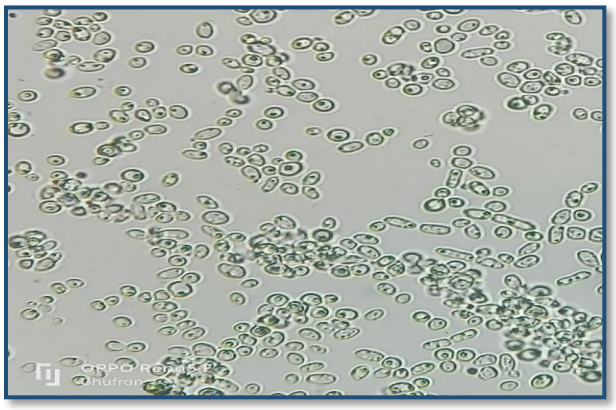

Fig. 2 Yeast cells (blastospores) under the microscope at a magnification of 100X

Germ tube formation

When examining positive isolates for this test, short filamentous structures extending from yeast cells were observed, as shown in figure 3 , which represents germ tubes [20] . These tubes have a role in penetrating the epithelial cell layer, tissues, and reaching the bloodstream, making them essential for yeast nutrition [21].

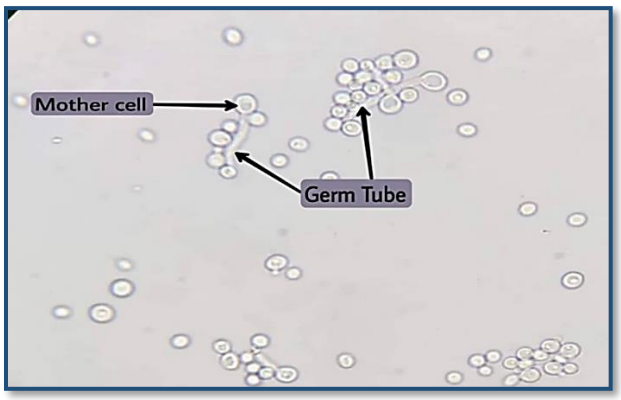

Fig.3 Germ tube formation of growing yeast cells in human serum at 37C° for 3 hours (100X)

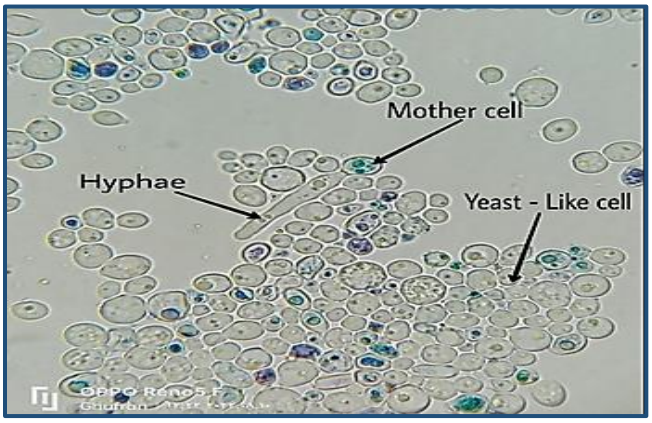

Fig.4 Germ tube formation of *C. albicans* in human serum at 37C° for 3 hours (100X).

Chlamydospore production

When examining isolates subjected to the chlamydospore production test and showed positive results, it was found that there were fungal threads with large,

spherical, thick-walled chlamydospores, usually located at the ends of the threads, [22]

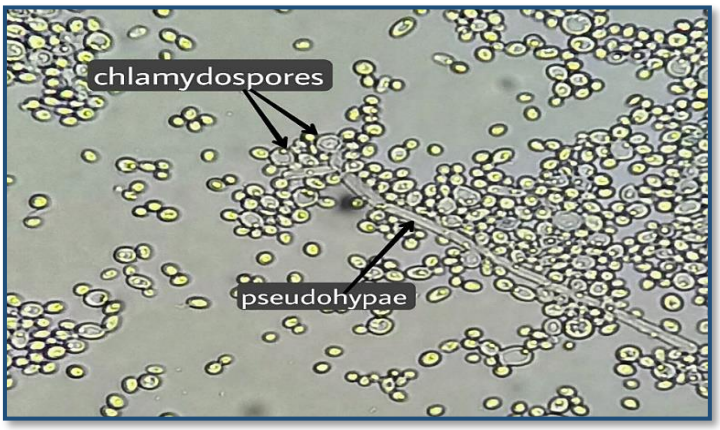

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Fig.5 Formation of chlamydial spores in *C. dubliniensis* in maize flour medium, 25°C, for 96 hours, magnification 40X

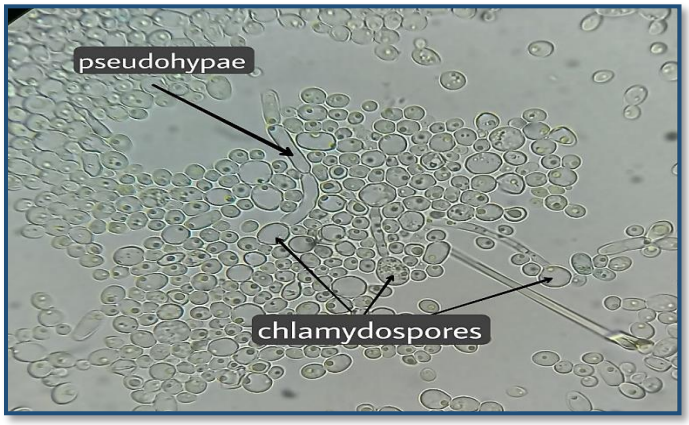

Fig.6 Formation of chlamydial spores in *C.albicans* yeast in corn flour medium, temperature 25°C, for 96 hours, magnification power 40X

Growth at a temperature of 45 ° C

This test was conducted on the isolates that exhibited positive results for the chlamydial spore formation test. The growth was observed to be well-developed in some isolates and dense in others, forming creamy or white colonies on the surface of the SDA medium. These findings suggest that all isolates displaying growth are attributed to the Candida albicans type. These results align with the findings of (23), who discovered that C. albicans was the sole species capable of thriving at a temperature of 45°C.

Fig.7 *Candida albicans* cultured on SDA medium at 45°C for 48 hours.

Diagnosis of isolates using differential medium HiChrome *Candida* Differential Agar

Diagnosis of *Candida* species using the HiChrome agar medium showed the appearance of colonies of different colors, making it a differential medium for yeasts. Colonies of *Candida albicans* appeared bright green, while colonies of *Candida tropicalis* appeared metallic blue. Colonies of *Candida krusei* were purple and sometimes had rough edges, while colonies of *Candida glabrata* were creamy to light pink.

Fig.8 shows four different types of *Candida* yeast growing on HiChrome *Candida* differential agar at 37C° for 48 hours.

Diagnosis of yeast isolates depends largely on the differences in colony pigmentation for each species, which occurs due to the enzymes secreted by each of these yeasts and their interaction with the substrate in the medium, resulting in different colors between species [24]

Surface Growth Test

Results of the surface growth test showed that both *Candida albicans* and *Candida tropicalis* had the ability to grow on the surface of the tube walls containing liquid Sabouraud dextrose broth, while *Candida glabrata* and *Candida krusei* did not exhibit surface growth ability.

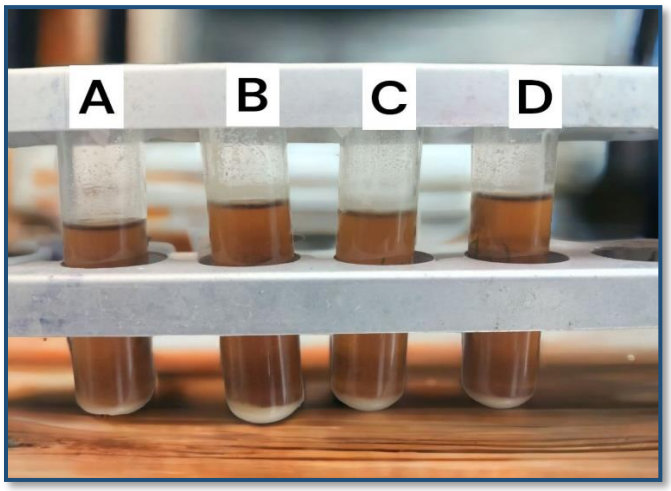

Fig.9 Surface growth of *C.tropicalis* and *C.albicans* yeast on SDB medium at 37°C and incubated for 48 hours

A: *C.glabrata* **B**: *C.albicans* **C**: C.tropicalis **D**: *C.krusei*

Declaration of competing interest

The authors have no conflicts of interest relevant to this article

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