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Isolation and diagnosis of *Candida albicans* from children in AL-Muthanna Province

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Abstract
Oral swabs from 50 patients in various age groups (1 to 60 months) and both sexes were collected, along with 40 control samples. They were gathered from Samawa Pediatric Teaching Hospital patients who had oral thrush between the years of 2023 and 2024. Yeast isolates were isolated and diagnosed using techniques based on their morphological, cultural, and biochemical traits. Additionally, differentiating between different species of Candida was done using the CHRO Magar Candida confirmation medium. The phenotypic data demonstrated that 70% of Candida albicans was isolated from other fungi. *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* were seen, although lower amounts of other species, like *Candida albicans*, were isolated. Testing for chlamydia spore development revealed both.

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Introduction

The commensal yeast Candida albicans considers an opportunistic endogenous fungal pathogen which is responsible for candidiasis (candidiosis) in human. It is inhabiting as a part of microflora of oral cavity, skin, the gastrointestinal tract, vaginal canal, and the urinary environment of the human. Over growth of these organisms, however will lead to infection that are ranging from superficial infection of the skin to life threatening systemic infections, and it usually occur in immune compromised patients, such as human immunodeficiency virus (HIV), infected victims, transplant recipients, chemotherapy patients, pregnant women, patients with chronic disease and low birth weight babies.

Candida spp. reside as a part of the lower genital tract microflora in (20%– 50%) of healthy asymptomatic women C. albicans is the most common colonizer Through the last ten years, research evidence demonstrated an increase in the repeatedly of cases caused by non-albicans species, with C. glabrata consistently being the leading species.

Morbidity and mortality rates associated with systemic infection by C. albicans remain unacceptably high, the main reasons being the difficulties in the diagnosis and treatment of this type of infection. In recent years, a number of publications have confirmed the immunogenicity and efficacy of vaccines against candidiasis in animal models, and even have tested the efficacy and safety in clinical trials. Fungal cell-wall polysaccharide, proteins, and live attenuated fungi have been investigated as vaccine targets. Even considering the capital and technical barriers, bringing protective vaccines to the clinic appears promising.

Fungal diseases

Fungal diseases Fungal diseases or fungal infections (Mycosis) are an emerging problem all over the world. They are usually difficult to treat and the incidence associated with them remains very high depending on the pathogen and the number of patients. Recent studies have estimated that fungal infections kill more than 1.5 million people annually worldwide. These fungi contribute to infection and can be primary or opportunistic pathogens in humans.

The disease is called mycosis. Advances in medical care and life-saving treatments, which may lead to compromised immune function, have increased the number of patients susceptible to fungal infections.

In severity, fungal diseases vary greatly from relatively simple infections of the skin and mucous membranes to severe, life-threatening infections affecting various organs. The symptoms of fungal diseases are similar to those of multiple infections. Others, leading to delayed diagnosis and treatment, which may lead to poor outcomes for patients, are likely to be Diagnosed largely incompletely and there is no public health surveillance for most fungal diseases. In order for fungi to successfully infect the human body, they must overcome many challenges such as high temperature, low water activity and low pH. If they penetrate the skin, few live fungal pathogens Also called (protozoal pathogens) that can infect healthy individuals, it is likely that specialized mechanisms to counter the immune defenses described have evolved in response during infection.

Oral candidiasis

Is an infection of the tongue and other mucous membranes of the mouth and may extend to the pharynx. It may appear in the form of white bugs resembling curds (false candidiasis) or red bugs, known as oral thrush. Many factors combine to affect In the appearance of candidiasis, such as age, gender, nutrition, oral hygiene, smoking, dentures, salivary pH disturbance, and dry mouth make diabetic patients more susceptible to oral candidiasis and it is also reduced in patients with immunosuppression (Dehghan *et al*., 2016) [18].

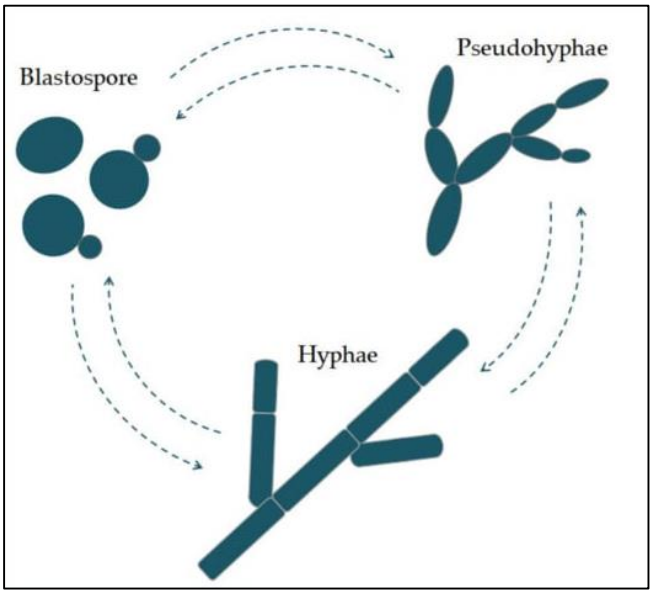

Fig 1: Morphological forms of *Candida albicans* buds (Thompson 2011)

Epidemiology

Different Candida species are colonize the skin and mucosal surfaces of humans as a commensal. Fatally ill or otherwise immunocompromised patients are more tend to extend both superficial and life-threatening candidiasis. Also, Candida infections constitute the most common fungal infections in AIDS patients. These patients generally develop oropharyngeal candidiasis, that able to develop to malnutrition and interfere with the intake of medication. C. albicans is the main cause of the invasive fungal infections and act a serious general health challenge with increasing economic and medical importance because of the high mortality rates and increased prices of care and period of hospitalization. Though C. albicans is the most common species implicated in invasive fungal infections, the extent of infections because of non-albicans species is increasing. This change in epidemiology could be accompanied with illness or acute immunosuppression, prematurity, older patients and usage of broadspectrum antibiotics. In European countries, an analysis showed that more than half of the cases of candidaemia were caused by C. albicans followed by other species.

Materials and methods

Patients group

This study enrolled 50 patients who attended Samawah Teaching Hospital for pediatrics and Gynecology in AL-Muthanna governorates from October 2023 to March 2024 and labelled before being brought to the laboratory for processing according to the standard methods. All patients were (1 -20) months of both sex and clinically diagnosed with suspected oral candidiasis. A total of 50 oral swabs are collected.

Control group: The control group consists of 20 apparently healthy volunteers. Their ages are between
1-20 months.

Oral swabs

A total of oral swabs were obtained from children suffering from oral candidiasis and septicemia with oral thrush. Specimens were taken using sterile swabs and then transported to the laboratory for diagnosis. All the collection of patients and control, age, gender and disease, type of feeding and pacifier use were recorded in a questioner sheet (App. 2).

The materials used in this study are explained as following

Gram stain: According to Collee *et al*. This stain was used for mounting and staining the yeast isolates.

Isolation and Identification

Culture

One-half ml of the collected specimens are inoculated onto sabouraud's dextroseagar. The inoculates are evenly spread with a sterilized L- shape glass rod and incubated as duplicate culture and incubated separately at 25 C◦ and 37 C◦ to reveal dimorphism.

While the swabs are streaked onto sabouraud's dextroseagar and incubated. The cultures are examined microscopically, they were not considered negative for growth until after 4 weeks of incubation.

Yeast colonies are diagnosed depending on the cultural characteristics of sabouraud's dextroseagar.

Microscopic examination

The isolates were examined by swabbing the sample taken after mixing it with physiological saline solution on a glass slide, covering the slide, passing it over a flame twice, and examining it under a 40X and 100X microscope to observe the yeasts.

Cultivating the samples on special agricultural media

Samples that were collected on special yeast growth media (SDA) in petri dishes were grown for a period between 24 - 48 hours and after they were ready and sterilized, the samples were incubated at 37 degrees Celsius. A plate of the formal characteristics of colonies, which includes the shape of the colony, and color its position on both sides of the plate.

Growth test on the differential medium for Candida HiChrome yeast Candida

Differential Agar. Dilute the medium according to the company's instructions. Dissolve 42.7 m of the medium in 1 liter of sterile distilled water in a bowl and place it on the microscope device. Grind the heat until it reaches the point of boiling and complete dissolution of the solution, then distribute it in dishes, after which it is placed in a thermos to cool at a temperature of 45 °C, sterilized and prepared, and then in a thermos to harden.

This media is distinguished by the fact that it does not need to be placed in the sterilizer device according to what is mentioned in the instructions of the company that provided it. This method was used to identify Candida species and differentiate them from other species. A highlighting board showing the most numerous types of albicans. C. This type grows in a light green color. C. tropicalis and the purple type grow in the color C. krusei. The light green type grows in blue, and the type C. glabrata grows in a cream color.

Results

Isolation and diagnosis

This study included the isolation and diagnosis of yeasts from the mouths of children infected with Candida. Oral, which we obtained from 50 children from newborn to five years, who took samples from children suffering from various diseases, including simple diseases such as mouth infections, as well as children with premature birth.

Isolation results showed that 35 samples out of a total of 50 samples were culture positive, i.e. a percentage 70% of these samples showed yeast colonies on SDA medium. As for negative samples it was 15 by 30% and there was no growth in dishes containing SDA media. As shown in Figure (1).

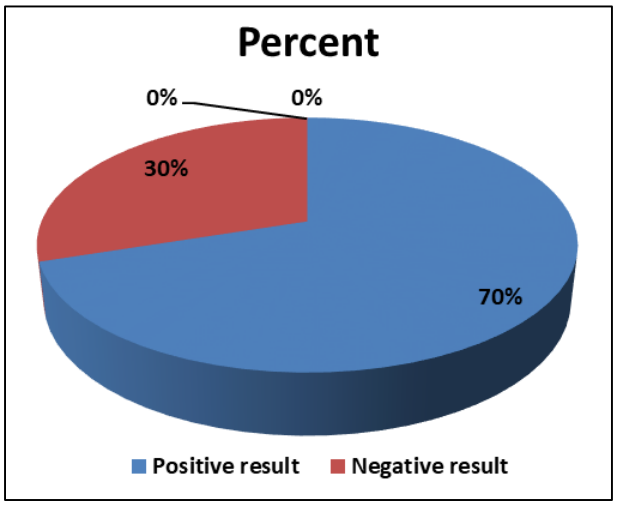

Fig 1: Percentages of positive and negative isolates in children infected with *Candida.albicans*

The study also showed that 135 samples of children who between the ages of one day and one year, of both sexes, 90 isolates were Candida infections, and it was proven that. These results agreed with who stated that children are more vulnerable to candidiasis, especially newborns it was found that among 286 children under the study, 161 children were confirmed infected, i.e. (%56.29) clinically and microscopically Oral candidiasis. This includes the results of morphological and biochemical tests of the samples.

The type of feeding affects the presence of Candida among children. Children who are fed formula their mothers for 6 months or more had lower average oral candidiasis (Taha and Haidar Al, 2019 among five-year-old children due to the low immunity of children in this age period.

Table 1: Percentage distribution of the number of infections with *C.albicans* by age groups

Age group (months)	Number of infections among children	Percentage (%)
1-12	18	(51.42 %)
12-24	7	(20%)
24-36	5	(14.2 %)
36-48	3	(8.5 %)
48-60	2	(5.7%)

It has been shown that the highest incidence of candidiasis occurs in newborns up to the age of one year this is consistent with the study of The most common infections were to the cheek In newborns and surgery in children. Intravenous nutrition and staying in the intensive care unit intensive ventilation and mechanical ventilation are major risk factors.

Distribution of Candida albicanes infections according to demographic characteristic

The rate of Candida albicanes infections among females was (54 %) which is higher than males (45 %). The age of the patients ranged from 1 day to 60 months. The positive results 8 (72.73%) in age group (1-12) months and are 3 (27.27%) their age was 13-24 months, while no positive cases among other ages are recorded. Regarding the residence the patients who attended to Hospital for Maternity and Pediatric from five districts of Samawa city.

Table 2: Distribution of *Candida albicans* infections according to demographic characteristic

Variable factors		No. (%)
Gender type	Females	19 (54.28 %)
	Male	16(45.71%)
Age groups (Months)	1-12	18 (51.42 %)
	12-24	7 (20%)
	24-36	5 (14.2 %)
	36-48	3 (8.5 %)
	48-60	2 (5.7%)
Residence	Samawa district (100%)	**12 (34.3%)**
	Others	**23 (65.7%)**

In children find that children of adults aged four to five years who are infected with candidiasis, 87% of them suffer from. On the body mass index, 5.72% of children had poor nutritional habits and did not lose weight Such as excessive obesity or constantly eating unhealthy foods.

Distribution of positive Candida albicanes cases according to type of feeding.

Of the 50 subjects with candidacies were enrolled. The distribution of positive Candida albicanes infection regarding type of feeding showed that 20 (57.14%) were used artificial milk and 13 (37.14 %) were mixed feeding that drinking artificial and breast feeding, while there was positive results recorded among children with breast feeding (5.7%) as shown in Table (4-2).

Table 3: Distribution of positive Candida albicanes cases according to type of feeding

Type of feeding	No. (%)
Breast feeding	2 (5.7%)
Artificial feeding	20 (57.14)
Mixed feeding	13(37.14)
Total	100%

Diagnostic tests

Agricultural traits

The isolates appeared when they were grown on SDA medium when incubated for a period of 24-48 hours. Colonies appears white to cream in color and has a butter-like texture is slightly higher than the middle and may become convex and smooth at first. They appear and may become wrinkled when the incubation period increases to more than 7 days. Their diameters range from the colony is 1-3 mm, as in Figure (3). These are the characteristics of the yeasts described by (Arya *et al*., 2021) [12].

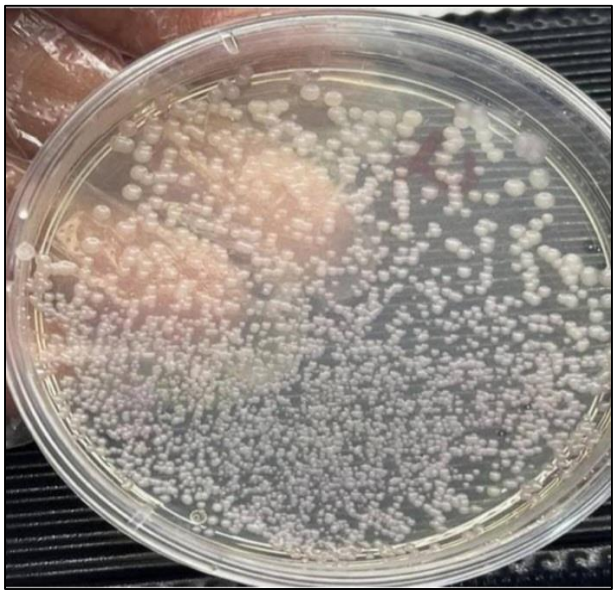

Fig 3: Candida albicans isolates on Sabouraud's dextrose agar showing round, smooth and creamy colonies

Diagnosing Candida spp species requires growing them in laboratory media suitable, and solid saprodextrose (SDA) media is one of the media generally used. In large scale, the primary isolation of Candida species from clinical samples specimens.

Microscopic examination

Microscopic examination of yeast cells for 50 isolates grown on yeast extract agar showed that 35 isolates appeared mostly spherical to oval or elongated oval or cylindrical and positive for Gram's stain as shown in figure (4). The presence of clusters is relatively sensitive and highly specific for C. albicans, and application of these results could provide useful preliminary information for guiding diagnosis as shown in figure (4).

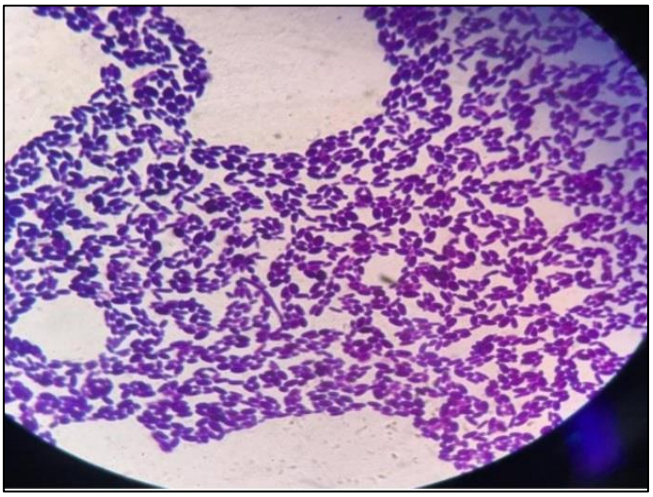

Fig 4: Gram's stain smear of Candida albicans (100X) showing spherical to ovel yeast cell with budding

Diagnosis of isolates by differential plastering

HiChrome Candida Differential: Agar r esults showed the identification of Candida species using HIChrom agar for Candida, a yeast colony appears colonies of different colors appear, and this is considered an intermediate condition Candida albicans appeared bright green, and yeast colonies appeared. Candida tropicalis is blue metallic, as for yeast colonies Candida krusei was purple in color and sometimes had rough edges. Rough, as for Candida glabrata colonies, they were creamy to light pink in color. Pink light, and as in Figure (4-10).

Fig (5): Candida species isolates on CHRO Magar Candida showing color colonies (green = *C.albicans* or, *C. krusei*=pink colonies, *C. tropicalis*=metallic blue and *C. glabrata*=white to mauve)

Stated that the isolation and secretive identification of Candida species isolated from the mouth, using differential media, one of the most important media used in the field of diagnosing fungi, as identification depends on the coloring in the media.

The identification of dependent isolates for Candida, it depends mainly on the different pigmentation colors of the colonies of each species, and this determines because of the enzymes secreted by each type of these candida and their interaction with the basic substance in the medium. Therefore, colors differ between species.

Conclusions

The results of the current study showed that out of a total of 50 samples, there were 35 confirmed cases of the disease. Candida in the mouth of infected children, i.e. 65-70%, and the age was 0-12 months is the highest incidence rate is.

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