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Original Research Article

Isolating and Diagnosing Some Types of *Candida spp* Yeast and Studying Their Sensitivity to Some Antifungals

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Abstract: The present study was aimed to isolation and identification Candida species from different clinical cases and to determine antifungal resistance. A total of 116 samples were collected from various clinical cases, including 41 oral swabs from children with oral thrush attending Tikrit Maternity and Paediatrics Teaching Hospital in Tikrit, 32 vaginal swabs from women with vaginal candidiasis, and 43 urine samples from individuals with urinary tract infection. The morphological and biochemical testing revealed that 16 (45.7%) isolates of Candida spp. were found in the oral samples and were determined to be pathogenic, 10 (31.1%) from vaginal swabs, 14 isolates (36%) from urine samples, C.*tropicalis* pathogenic samples that 6(17.3%) isolate from oral samples, 7 (21.8 %) from vaginal swabs, 10 isolates (26%) from urine samples, C.*parapsilosis* pathogenic samples that 5 (14.2%) isolate from oral samples, 8 (25.3%) from vaginal swabs, 13 isolates (33%) from urine samples. While C.*krusei* pathogenic samples that 8 (22.8%) isolate from oral samples, 7 (21.8%) from vaginal swabs, and 2 isolates (5%) from urine samples. Agar well diffusion method results revealed that the *Rezafungin* was the most effective antifngal which inhibited 87.9%.

Keywords: Candida spp., C.*albicans*, C. *tropicalis*, C. *parapsilosis*, C. *krusei*, rezafungin, Ketoconazole, itraconazole, Grisofulvin.

INTRODUCTION

Fungi are classified as eukaryotic organisms. There are around 50,000 species of fungi found in nature, including 80 kinds of moulds and yeasts that can cause various diseases in both people and animals (Jawetz *et al*., 2004). Mycosis refers to fungal infections, which typically manifest as chronic conditions due to the slow growth of fungi. These infections can be categorized as superficial, systemic, or opportunistic, and they primarily afflict individuals with impaired immune systems (Tortora *et al*., 2020). Candida colonies are naturally found in the oral cavity of many healthy individuals, although in small numbers, these colonies are present in 20% to 40% of healthy people, as well as in other areas like the vagina and respiratory tract (Lewis, 2000). However, Candida can become a pathogen opportunistically when certain virulence factors are present. These factors include the ability to adhere to epithelial cell surfaces, the production of enzymes that break down fats and proteins, and the formation of germination tubes (Panagod *et al*., 2001). Opportunistic fungi exploit the compromised immune system of the body to become harmful. They are also referred to as saprophytic fungi. These fungi have a wide distribution and are not restricted to a single geographic area (Rippon, 1988). These microorganisms have low virulence, and their ability to cause infection is contingent upon the host's immune status (Robert, 1990). Candida is responsible for various pathological conditions, including mucocutaneous infections, vaginitis, oral thrush, and some persistent infections affecting the mucous membranes and skin. In addition, Candida is also responsible for systemic infections, which can affect several parts of the body, such as the blood, heart, brain, lungs, bones, and others (Rex *et al*., 2000). Several antifungals have been employed to mitigate fungal infections in infected individuals. However, the unselective and repetitive utilization of antifungals has resulted in the development of resistant strains to these antibiotics (Paranhos *et al*., 2000). The resistance of pathogenic strains to antibiotics is related to their possession of several mechanisms, including genetic ones that encode the mechanisms of antibiotic resistance. Pathogens employ many strategies to evade the effects of antibiotics, including modifying the permeability barrier and shifting the target. The genetic

components present in fungi encode these systems (Chelsea and Theodore, 2010). Our current study aimed to do the following: Isolation and diagnosis of some types of Candida spp. yeast from different clinical samples and conducting a drug sensitivity test for fungal isolates against some antifungals using the agar-well-diffusion method.

MATERIALS AND METHODS

Samples Collection:

A total of 107 clinical samples were gathered from the Women and Children's Teaching Hospital for the study. The samples consisted of 36 oral swabs from children with oral thrush who visited the Women's and Children's Hospital in Tikrit, 32 vaginal swabs from women with vaginal candidiasis who visited private clinics in Tikrit, and 39 urologists among patients with urinary tract infections who visited the Women and Children's Hospital in Tikrit between October 2012 and March 2013.

Isolation and Identification:

The specimens were cultivated on Oxoid Sabouraud's Dextrose-Agar by streaking cotton swabs on the surface of the nutrient medium. Regarding the urine samples, a sterile culture carrier (Loop) was submerged in them and thereafter spread onto the previously prepared nutrient medium. Three instances of culture were conducted on the previously described medium. To prevent contamination of the fungal development during cultivation, the plates were incubated at a temperature of 37°C for 24 hours (Atlas, 1995). The individual belongs to the Candida genus. Candida Spp and its related species were classified according to a precise set of morphological and biochemical characteristics, as described in (Baron *et al*., 1994; Murray *et al*., 1999). These characteristics were used to differentiate between different species.

Direct Microscopic Examination of Yeast Samples:

The examination was conducted using the method described by Sood (1994) and Morello *et al*., (2003). CHROMagar medi and Germ tube formation was assessed using the methodology described by Baker (2002). The production of chlamydospores was examined using the approach described by Ellis *et al*., (2017).

Biochemical Tests: Biochemical Tests

Sugars Fermentation Test: The test was conducted following the methodology outlined by Robert (1990).

Sugars Assimilation Test: The test was conducted using the methodology outlined by Bukly (1989).

The Agar-Well-Diffusion Method: The method was conducted based on (McGinnis, 1980).

Statistical Analysis:

The chi-square test and the LSD test were used to statistically analyse all results, with a significance level set at $P<0.05$ (Al-Rawi, 2000).

DISCUSION & RESULT

Isolation and Diagnosis

The current study proved the traceability of 107 isolates to Candida yeasts through studying some cultural and microscopic characteristics and biochemical tests, as follows:

Cultural Characteristics

Colonies cultivated on SD medium had a white to cream-colored, smooth appearance, and were round in shape (Figure 1). According to Ellis *et al*., (2017), colonies of Candida spp. exhibit certain phenotypic traits when cultivated on the medium. The aforementioned outcome aligns with the findings reported by Singh *et al.*, (2013), where the colonies exhibited a glossy, cream-colored appearance that was both smooth and circular, creating optimal circumstances for planting.

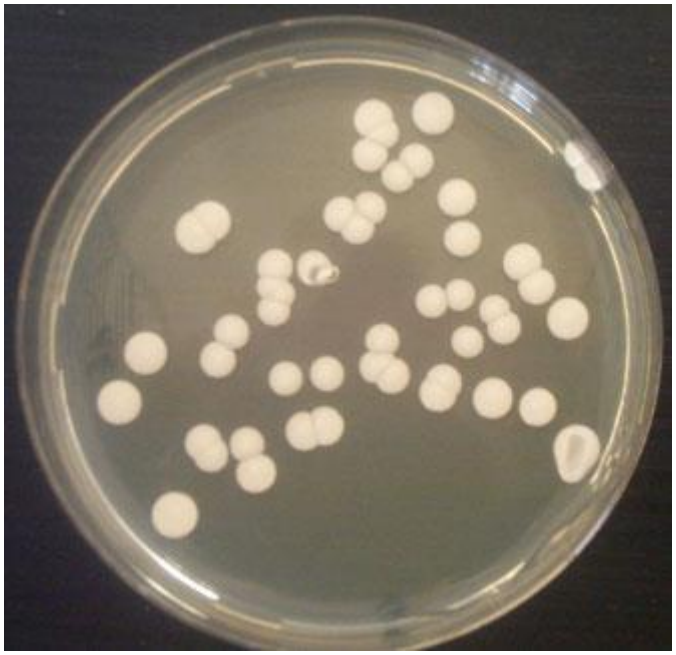

Figure 1: Growth of C.albicans on SDA medium at 37°C for 7 days

Characteristics Microscopic

The isolated species exhibited positive findings when subjected to Gramme staining, as the cells displayed an oval to spherical, oval to elongated, or cylindrical form resembling yeast-like fungi. This finding was in line with the findings of Boon *et al*., (2013), as seen in Figure 2. The blue hue of Candida cells is due to the retention of the dye by the peptidoglycan layer in the cell wall (Sudbery *et al*., 2004).

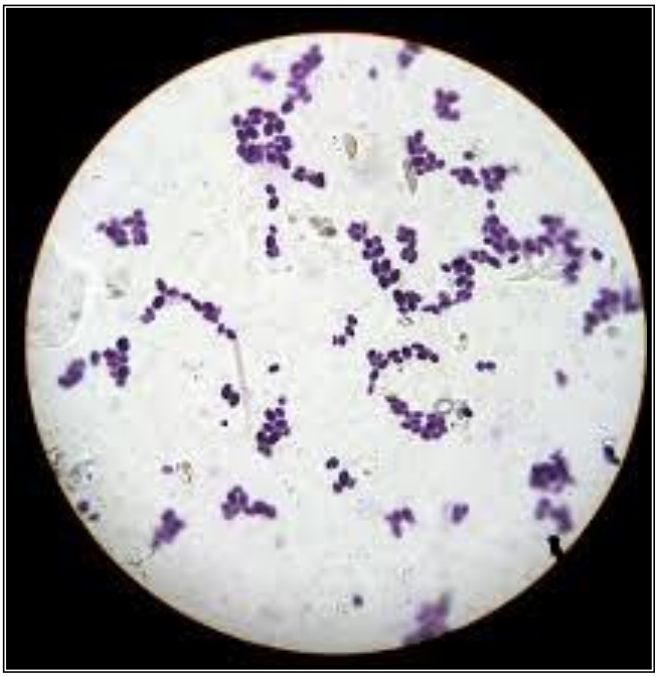

Figure 2: C.albicans cells stained with Gram stain (40x magnification)

Growth on CHROMagar Candida Medium

Cultures of Candida species were incubated on medium at a temperature of 37°C for a period of 24-48 hours. The C. albicans species exhibited a green hue, whereas the C. tropicalis species displayed a blue hue. C.parapsilosis exhibits a purple hue, while C.krusei displays a pink hue. This medium served as a differential medium, yielding precise outcomes in the diagnosis of Candida species. This finding aligns with the research conducted by Raut and Varaiya (2009) and is also in line with the study conducted by Manikandan and Asmath (2013) on the identification and prompt detection of

Candida species. Candida, which is obtained from the oral cavity, is a crucial medium utilised in fungal detection. The diagnosis relies on staining inside the medium.

Germ Tube Formation

All C. albicans isolates formed a germ tube after 2-3 hours of incubation at 37°C in 0.5 ml of human blood serum. C. tropicalis, C. parapsilosis, and C. krusei did not produce the tube under identical conditions (Figure 3). Akortha and colleagues (2009) found that only C. albicans can make germ tubes. Similar findings were reported by Boon *et al*., (2013). Only C. albicans can produce the germ tube in this experiment. The germ tube encircling the yeast cell forms when serum is present. The germ tube is essential for penetrating the body's epithelial cells and reaching the circulation. Most people agree that yeast feeding is necessary (Sudbery, 2001).

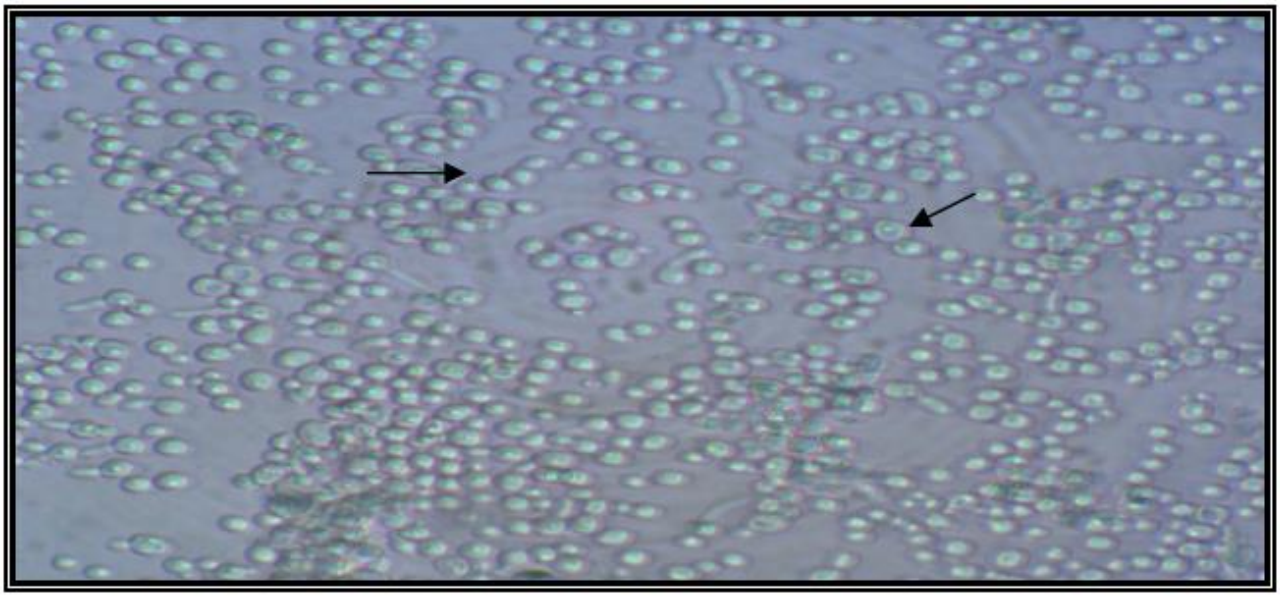

Figure 3: The bacterial tube indicated by an arrow for the type C.albicans (100x magnification)

Chlamydospores Formation

The results of this test showed that all isolates belonging to the type C. albicans formed chlamydial spores, while the other diagnostic species, C. tropicalis, C. parapsilosis, and C. krusei, did not form them under the same conditions (25°C and for 24 hours). The result is identical to the result of Saroj and his group (2013), as the results of the study showed that the species C. albicans formed chlamydial spores on Corn meal agar medium, as this medium is considered a diagnostic characteristic of the species C. albicans. It also agreed with Bose *et al*., (2021), that spores with thick, circular walls were formed. The formation of the shape at the end of the fungal hyphae (Hypha), whether singular or clustered, on CMA medium is a consequence of yeast starvation caused by a scarcity of food sources. These shapes emerge when conditions are unfavourable for yeast growth. This medium is characterised as a nutrient-deficient environment for the yeasts.

Biochemical Tests

The isolates were diagnosed based on biochemical tests, where the results of the sugar fermentation test for the isolates under study showed that the type C. albicans has the ability to ferment the sugars glucose, maltose, galactose, and trehalose. As for sucrose, the isolates belonging to this type were unable to ferment it, while the type C. tropicalis was able to ferment all the isolates belonging to this type. The type ferments the sugars maltose, glucose, sucrose, calactose, and trehalose. As for the C. parapsilosis type, all isolates belonging to this type have fermented the glucose sugar, but the other sugars did not ferment maltose, calctose, trehalose, and sucrose. As for the C. Krusei type, all isolates belonging to this type. It fermented glucose and did not ferment the sugars galactose, trehalose, and sucrose, while there were isolates that fermented galactose and other isolates that did not ferment it. The results showed that glucose is one of the simple sugars that most isolated species tend to use as a simple and energy-rich food source, and also the results showed that all species fermented glucose. These results are consistent with (Nassir, 2010), which showed that the species C. albicans, C. tropicalis, and C. krusei fermented glucose, and differ from the results of (Al-Shibli, 2006), which showed that the species C. albicans C.krusei, C.parapsilosis fermented glucose, but C.tropicalis did not ferment it. While the results of the sugar assimilation test showed that all isolates belonging to the type C. albicans represented all the tested sugars, while the isolates belonging to the type C. tropicalis represented the sugars glucose, trehalose, galactose and maltose, while there were isolates that represented sucrose and other isolates that did not, as for type C. parapsilosis, all isolates belonging to this type did not represent the sugars glucose, maltose, and lactose, and represented trehalose sugar, while they represented sucrose after a longer period of time than the rest of the sugars. As for the C.krusei type, all isolates belonging to this type represented

glucose late and did not represent the rest of the sugars. As for the type C. glabrata, all isolates belonging to this type represented glucose and did not represent the rest of the types of sugars. C. albicans and C. tropicalis have a high ability to exploit most sugars, and this explains their presence in high percentages among the isolated types. This result is consistent with. This result is consistent with Ellis and his group (2017), who showed that the two species, C.albicans and C.tropicalis, have high efficiency in assimilation of most types of sugars, which explains one of the reasons for their frequent isolation from tissues infected with Candidasis.

Numbers and Percentages of Isolates of Pathogenic Candida Spp.

Pathogeic 107 isolates were identified (an isolation rate of 92.2% out of a total of 116 samples), and four species belonging to the genus Candida spp were isolated and diagnosed. Candida in the current study, based on biochemical characteristics, was isolated from different clinical samples, including the vagina, urine, and mouth, in different percentages (88%, 100%, and 90.6%), respectively, as shown in Table (1).

Table 1: Numbers and percentages of Candida isolates isolated from different clinical samples

Clinical samples	Number of samples tested	Number of positive isolates +ve	%	Number of positive isolates -ve	%	Total Number	
						%	No.
Oral samples	41	36	85	5	12	92.2	107
Vaginal samples	32	32	100	0	0		
Urine samples	43	39	90.6	4	9.4		
Total Number	116						
$X^2_{tab} = 0.266$ $X^2_{cal} = 2.64$ There is a significant difference at the probability level of $p<0.05$*							

The results also showed that the percentage of c.albicans isolation represented the largest percentage of the species isolated from the various clinical samples mentioned above, and this agrees with 72 (Al-Obady, 2017), as five species belonging to the genus Candida were isolated from the oral cavity, vagina, and urine, and the C type came. Albicans in its introduction and also agrees with Hussain, 2011. He isolated five species belonging to the genus Candida from the oral cavity C. albicans is at the forefront, followed by the following species: C. tropicalis, C. parapsilosis, krusei..C as in Table (2).

Table 2: Shows the percentage of Candida species isolated from clinical samples

Isolated species	Oral samples	%	Vaginal samples	%	Urine samples	%
C.*albicans*	16	45.7	10	31.1	14	36
C.*tropicalis*	6	17.3	7	21.8	10	26
C.*parapsilosis*	5	14.2	8	25.3	31	33
C.*krusei*	8	22.8	7	21.8	2	5
Total	35	100	32	100	39	100
LSD = 7.4 among isolates per sample LSD = 6.34 between samples for each isolate LSD overlap = There is a significant difference at the probability level of $p < 0.05$.						

Antifungal Susceptibilit Test

The drug sensitivity of all Candida isolates under study was tested using 5 types of antifungal solutions, and the results were determined by measuring the Zone's of Inhibition as shown in Table (3) based on Prize *et al*., 1990), as the results showed that there is variation. In the sensitivity of the isolates under study to the antibiotics used, the yeast isolates showed high resistance (87.9) to the antibiotics Rezafungin, and to Ketoconazole, the resistance rate reached (74.6), while it reached 15.6% towards the antibiotic Iitraconazole, or the sensitivity to the anti-Grisofulvin reached 0%.

Table 3: Average diameters of zones of inhibition for Candida species resulting from the use of antifungals

Antibodies	Damping diameter rate (mm)	Resistance		Sensitive	
		No.	%	No.	%
Rezafungin	17-30	10	12	73	87.9
Ketoconazole	16-22	21	25.5	62	74.6
itraconazole	9-10	70	84.3	13	15.6
Grisofulvin	5-0	83	100	0	0

The antibiotic used was the antibiotic *Rezafungin*, which was one of the most effective antibiotics with the highest zones of inhibition appearing (Sheehan *et al*., 1999). The antibiotic rezafungin inhibits the synthesis of Ergosterol, a crucial component in the construction of yeast cell membranes. This is evident from the observed zone of inhibition diameters,

ranging from 17 to 30 mm. This outcome aligns with the discoveries made by Al-Shibli (2006), which demonstrated that the antibiotic rezafungin shown exceptional efficacy in suppressing the proliferation of Candida. The antibiotic Ketoconazole had an inhibitory diameter ranging from 16 to 22 mm. The evident action of the substance was seen to inhibit 62% of the isolated yeast species. Itraconazole was placed third in its capacity to hinder the growth of Candida species. The study done by Hussein (2011) elucidated that this particular antibiotic effectively hinders the proliferation of Candida species. Conversely, the antibiotic Grisofulvin had the lowest efficacy in suppressing the growth of the Candida species examined. The resistance rates are at a maximum of 100%. According to (Al-Shibli, 2006), the anti-griseofulvin did not demonstrate any impact on Candida yeast. There was no observed hindrance of growth at the tested concentrations, which aligns with the findings of Myrvik (1988) about the effectiveness of this antibiotic. The medication exhibits a limited spectrum of activity against fungus and does not affect Candida yeast. Presumably, the reason behind this is that this antibiotic specifically targets skin fungi known as Darmatophyts.

CONCLUSION

The current study aimed to isolate and diagnose various types of *Candida spp*. yeast from different clinical samples. Additionally, the study conducted a drug sensitivity test for fungal isolates against specific antifungals using the agar-well-diffusion method. The morphological and biochemical testing revealed that 45.7% of isolates of *Candida spp*. were found in the oral samples and were determined to be pathogenic, in addition to other species that were found. The agar-well diffusion method results revealed that Rezafungin was the most effective antifungal, inhibiting 87.9%.

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