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Isolating and identifying yeasts in women with vaginal candidiasis and studying their ability to form biofilms and haemolysin

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Abstract

One hundred clinical samples were collected from married women aged between 22 and 53 years during the period from October 2022 to January 2023. Several diagnostic techniques were used, such as growing samples on Sabroid Dextrose Agar medium and doing direct microscopic examinations. Together with the VITEK2 Compact diagnostic instrument, biochemical assays such as urease and the differential medium Chrom Agar were used to diagnose four different species of yeast. By microscopic inspection, the results revealed that 55% of the samples tested positive and 45% tested negative. Following sample culture, 52% of the samples tested negative and 48% of the samples tested positive. Four yeast species were detected by the urease test and the VITEK2 Compact device: C. albicans was found in 75% of isolates, followed by C. glabrata at 19%, C. parapsilosis at 4%, and C. kefyr at 2%. Additionally, the study looked into virulence factors, such as the formation of biofilms and the production of hemolysin. The findings showed that C. albicans and C. glabrata were both capable of producing biofilms and hemolysin, whereas C. parapsilosis and C. kefyr were not.

Introduction:

Candida spp. It belongs to the biggest group of fungi found on Earth's surface, the phylum Ascomycota, and is a single-celled eukaryotic fungal microbe [1]. *Candida spp*. One of the world's most common opportunistic fungus [2] is vulvovaginal candidiasis, sometimes referred to as thrush, an inflammation of the vagina brought on by the overgrowth of some opportunistic yeasts from the genus Candida that are normally present in the vaginal flora. After bacterial infections, this infection is the second most typical kind of vaginal infection [3]. *Candida spp*. Yeasts are known for having a variety of virulence factors that aid in their ability to spread and infect different parts of the human body [4]. A pathogen's virulence factors serve as an indicator of its level of pathogenicity, since a pathogen cannot cause infection unless it possesses virulence. These elements can take the shape of enzymes, cellular structures, or mycotoxins, which speed up the process of infection and shield the pathogen from the body's defenses and environmental factors, The virulence factors in yeasts of the Candida genus are summarized in the property of adhesion, which is the first step in causing infection, followed by polymorphism, phenotypic change, and the formation of chlamydial spores, Chlamydospores, hemplysin, the formation of the germ tube, which occurs under

certain conditions, and the production of secreted hydrolytic enzymes that facilitate the invasion process , And penetration by contributing to the analysis of peptide bonds that connect the plasma membrane proteins of host cells [5] , The Biofilm is defined as a structure that allows a complex accumulation of microscopic organisms. It has a three-dimensional shape. In the case of yeasts of the genus Candida spp, it consists of yeast cells, yeasts, real fungal filaments and false Hyphae and Pseudohyphae. This membrane can stick to the flatness of living epithetic cells as well as the solid surfaces of the catheter and surgical instruments. The biomembrane of the candida provides a stable site of infection as well as being a place from which yeast-shaped cells spread to new sites for infection and protection from antifungals, so strains resistant to these antibiotics have emerged in recent years [6] , Hemolysis is a group of enzymes that have a role in the pathogenesis of the candidiasis, as the effectiveness of erythrocyte analysis contributes to the cause of infection with candidiasis and also facilitates the penetration of the fungal thread [7].

To achieve the goal, the following steps were followed:

1- Isolating the yeasts that cause vaginal candidiasis in women, diagnosing them using biochemical tests, and confirming the diagnosis using the Vitek2 device.

2- Detection of some virulence factors of yeasts and their ability to produce hemolysin and biofilms.

Materials and method

During the period October 2022 to January 2023 , 100 clinical samples were collected from married female patients. They were collected from Salah al-Din General Hospital and some private clinics. Samples were collected for women with vaginal candidiasis whose ages ranged from (22-53). The initial diagnosis and examination for follow-up examinations were conducted with the assistance of the gynecologist. Samples were collected from married women with vaginal candidiasis using sterile cotton swabs from the vagina. Samples were kept in special sterile containers until they were transported to the laboratory to conduct the necessary tests.

Laboratory examination of samples

Direct Microscopic Examination

A portion of the sample taken by swab cotton was placed on a glass slide, and a drop of 10% KOH potassium hydroxide solution was added to it, then it was covered with a cover slide and then passed over the flame two or three times, after which it was examined with a microscope. Under 10X power and then 40X power in order to confirm the presence of yeast [8].

Macroscopic examination

The external appearance of the colonies growing on sabroid dextrose agar (SDA) medium was examined, in terms of colour, texture, odor, and the shape of the colony from both the plate and diameter sides [9].

Yeast diagnosis test using HiCrome™ Candida Differential Agar Base

Chrom agar medium was used to distinguish the types of Candida spp., based on the color of the colony, according to the manufacturer of the medium. The isolates taken were grown on the above medium, for a period of 48 hours at a temperature of 37°C, after which the results were recorded for Candida yeasts according to the type and color of the colony [10].

Biochemical tests

Urease test

This test was performed by taking a portion of the colony growing on SDA medium and stimulated at 24 hours of age. It inoculated tubes containing urea agar medium at an angle, in a planing manner, and incubated the tubes at a temperature of 37°C, for 24 hours, when a change in the color of the medium occurred. Yellow to dark pink color, this indicates a positive test (complete decomposition of urea), but when the color of the medium remains yellow and no change occurs, it indicates the yeast's inability to decompose urea [11].

Diagnosis using the VITEK2 device

The diagnosis was performed using the Vitek device in the Al-Raya laboratory located in Salah al-Din Governorate/Tikrit city, with the aim of final and conclusive confirmation of the diagnostic results obtained from the previous biochemical tests for vaginal isolates using the YST Card, according to the instructions of the French processing company Biomr Ieux.

Virulence Factors

Biofilm formation test

This test was conducted by transferring a portion of the 24-hour-old growing colony using a loop carrier to test tubes containing liquid potato dextrose medium (PDB) supplemented with 1% glucose. The tubes were incubated at a temperature of 37°C for 24 hours. After completing the incubation period, the samples were poured. The tubes were washed with Phosphate Buffer Saline, then the tubes were dried and dyed with Crystal Violt dye at a concentration of 1% for 3 minutes, then the excess dye was poured out and washed with distilled water free of hardness ions, then the tubes were left to dry upside down to observe the biofilms on the inner walls. The bottom of the tubes has a purple layer [12].

Hemolysin test

This test was conducted according [13], to detect the ability of yeasts to analyze blood, sterile sabroid dextrose agar SDA medium was used, and 7% sheep blood was added to it. Part of the colony was transferred to the dish using the planning method. The dishes were incubated at a temperature of 37°C for 24 hours. The results were recorded, Based on observing the decomposition area around the colony [14].

Results and discussion

Samples of yeasts isolated during the study

The results of the microscopic examination of the total number of samples (100) showed that there were (55) positive samples, at a rate of 55%, while the number of negative samples was 45, at a rate of 45%. As for the results of laboratory culture after culturing the samples on sabroid dextrose agar (SDA) medium, they showed (48) of the swabs were positive, at a rate of 48%, while (52) of the swabs were negative, showing no growth, at a rate of 52%, as in Table (1). The results of the statistical analysis showed that there were no significant differences, P-Value = 0.322.

Table 1: Numbers and percentages for direct microscopic examination and laboratory culture of samples

%	Negative samples	%	Positive samples	Total	Type of examination
45%	45	55%	55	100	Direct microscopic examination
%52	52	%48	48	100	Laboratory culture
P-Value = 0.322		Chi-Square = 0.981 ns			

The reason for the appearance of negative results and the difference in results is due to the similarity in symptoms between candidiasis and bacterial or viral infections, the random use of antibiotics without consulting a doctor, or the inadequacy of the sample collected. It can also be attributed to inappropriate development conditions on the agricultural medium (SAD) [15]. The increase in the prevalence of vaginal candidiasis among women is due to lack of attention to personal hygiene, lack of awareness, poor nutritional habits, wearing tight underwear for some women, and lack of effective treatment [16]. Therefore, clinical diagnosis does not provide sufficient accuracy for diagnosis, so it must be supported by other types of diagnosis, such as direct microscopic examination, which gave more accurate results than clinical examination, because microscopic examination depends on the presence of yeast cells, fungal hyphae, or any part of the pathogen, so it is considered more accurate [17].

Diagnosis of the types of isolated yeasts:

Phenotypic diagnosis

The results of culturing on Sabroid dextrose agar (SDA) medium showed colonies of a creamy white color, shiny and smooth, with an oval or spherical shape and a sticky consistency. These results were in agreement with [18] who stated that Candida spp. It has shiny, cream-colored colonies with a smooth texture and convex surfaces. SDA medium is used to grow yeasts for several reasons, including the low pH of this medium, which reaches (5.6), which enhances the growth of yeasts and prevents the growth of bacteria. To ensure that bacteria do not grow, a bacterial antibiotic is added [19] , As mentioned in the following Figure.

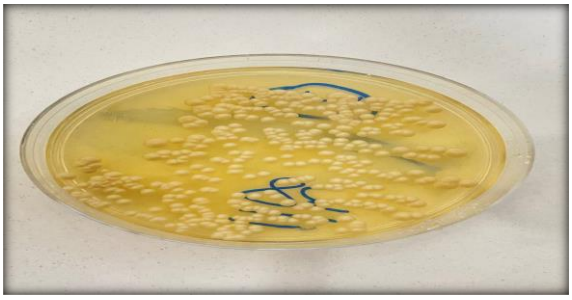


Fig. 1 shows the growth of yeast *C.albicans* on sabroid dextrose agar (SDA) medium.

Microscopic diagnosis

The results of the yeast cells showed a positive reaction after staining them with lactophenol blue dye, as the yeast cells showed a spherical to oval shape or long, single and budded. These results were in agreement with [20]. Who showed that yeast cells appeared in the form of oval or spherical buds. The appearance of yeast cells in blue is due to the peptidoglycan layer in the cell wall of the yeast retaining this dye. [21]. In addition, cotton blue dye (lactophenol) has an important role in observing chlamydial sporangia and fungal hyphae [22].

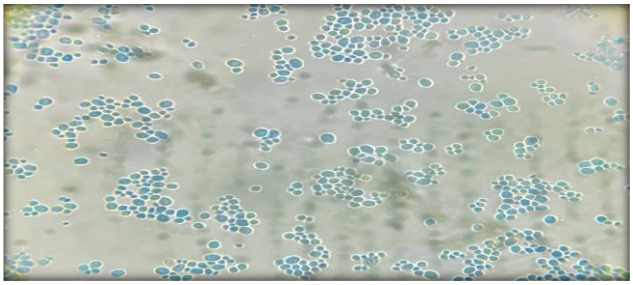


Fig. 2 shows the growth of yeast *C. albicans* dyed with lactophenol cotton blue (X40)

Diagnosis using differential medium (Chrom Agar)

The test results showed that colonies appeared in different colors on the medium (Chrom Agar), where the *C.albicans* colony appeared in a light green color and the *C.glabrata* yeast appeared in a purple color. Yeast. *C.parapsilosis* is a creamy white color and *C.kefyr* yeast is a light pink color. The growth test on chromium agar medium is an easy way to identify the most common *Candida* species based on color changes in the medium. The differential medium can be a primary isolation and differentiation method for clinical samples that are likely to contain *Candida* [23]. The work of Chrom Agar medium depends mainly on the presence of the chromogenic substance, which works to identify the types of Candida through the appearance of special colors given to them by special enzymes in *Candida* when the medium reacts with it, and then each species becomes a specific color [24].

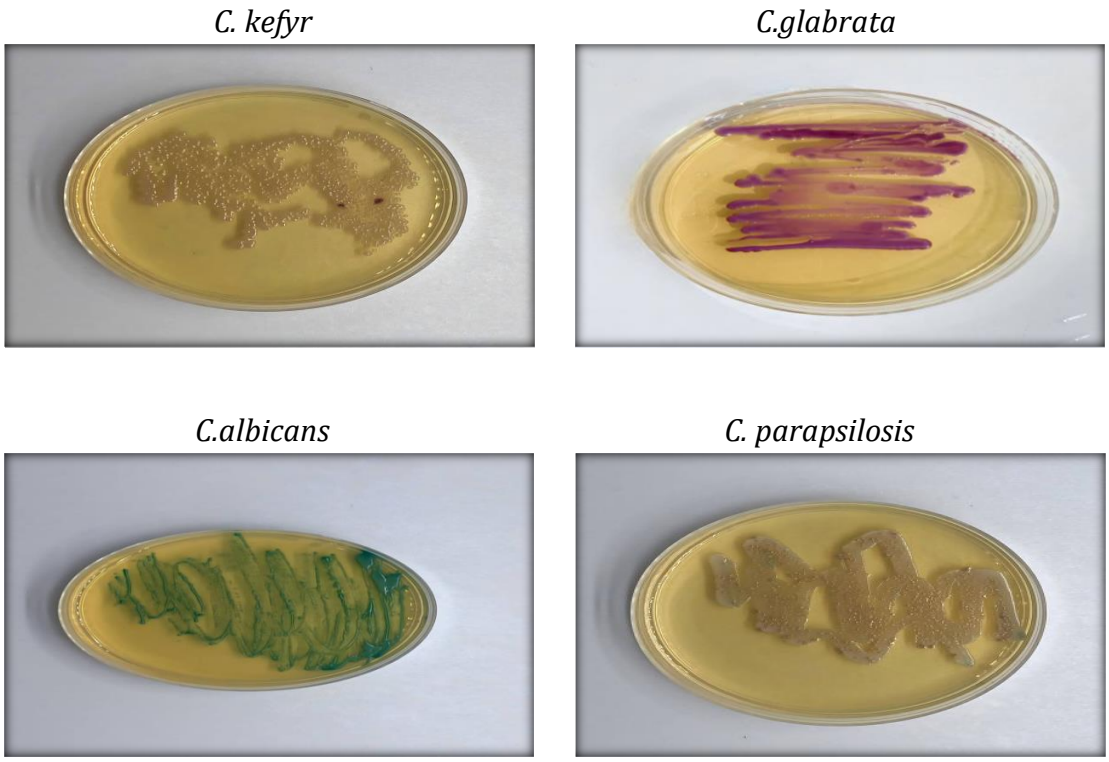


Fig. 3 Diagnosis with chromium agar differential medium

Biochemical tests for yeasts.

Urease test

This test was conducted to detect the susceptibility of *Candidia spp*. on the production of the urease enzyme (Urease), as all isolates belonging to the species *C. albicans*, *C. glabrata, C. parapsilosis* and *C. kefyr* showed that they do not have the ability to produce the urease enzyme. These results were in agreement with [25,26,27]. The urea test is an important diagnostic characteristic for distinguishing between yeast species, as it was performed using urea medium.

Table 2 : Urease test

Candidia spp	Urease test
C.albicans	-
C.glabrata	-
C.parapsilosis	-
C.kefyr	-

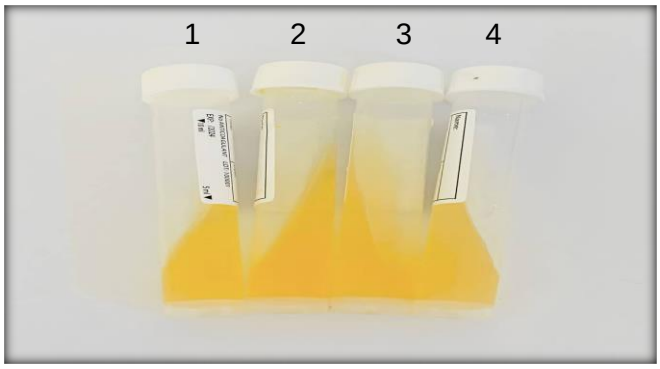


Fig. 4 Urea test (1- C.albicans 2- C.glabrata 3- C.parapsilosis 4- C.kefyr)

Diagnosis using the VITEK2 Compact diagnosis

Using the Vitek2 device for the purpose of confirming the diagnosis of the fungal species under study, Researchers in the field of medical mycology resort to biochemical diagnosis, such as diagnosis using the Vitek device technology, which is considered a rapid and commercially available biochemical system [28].

Types of yeasts isolated during the study

The results of the aforementioned tests revealed the diagnosis of four types out of a total of 48 isolates belonging to the genus *Candida spp*. Which were determined based on cultural and microscopic characteristics and biochemical tests. As shown in the table, it noticed (4) that most of the isolated species belong to the *C. albicans* type, as the number of isolates reached 36 isolates, at a rate of 75%, followed by the *C. glabrata* type, as the number of isolates reached 9 isolates, at a rate of 19%, while the two types came *as C. parapsilosis and C.kefyr*, at a rate of 2 and 1, and at a rate of 4% and 2%, respectively. The results of the statistical analysis showed that there were significant differences between the types of yeast isolated during the study, P-Value = 0.00002.

Table 4: Types of yeasts isolated during the study

Candida spp.	n.	%
C.albicans	36	%75
C.glabrata	9	%19
C.parapsilosis	2	%4
C.kefyr	1	%2
Total	48	%100
Chi-Square = 49.556		P-Value = 0.00002

The dominance of the *C.albicans* species is an expected result of the presence of *Candida* species as natural flora in the human body in 80% of cases. It is also found in the pathogenic form in 5%-20% of cases, but without symptoms , It is believed that the stimulation of the fungus' transformation into the pathogenic form and the appearance of symptoms is due to several factors. Factors that cause changes in the host vaginal environment, weak immunity, and the high ability of *C.albicans* to adhere to epithelial cells and its ability to form germination tubes in infected tissues [15].

Detection of some virulence factors

Detecting the ability of *Candidia spp*. On the production of biofilms

Table (5) shows the ability of isolated yeasts to produce biofilms, if 32 *C.albicans* isolates showed their ability to form a biofilm out of a total of 36 isolates, at a rate of 88%, and 4 *C.glabrata* isolates showed the ability to form a biofilm. Biotechnology out of a total of 9 isolates, at a rate of 12%. As for the two types, *C. parapsilosis* and *C. kefyr*, they were not able to form biofilms. The results of the statistical analysis showed that there were significant differences, p-value = 0.0007, regarding the ability of *Candidia spp*. On the production of biofilms.

Table 5: Detection of the ability of *Candidia spp.* On the production of biofilms

Candida spp.	the total number	production of biofilms	%	non production of biofilms	%
C.albicans	36	32	%88	4	%33
C.glabrata	9	4	%12	5	%42
C.parapsilosis	2	0	%0	2	%17
C.kefyr	1	0	%0	1	%8
Total	48	36	%100	12	%100
Chi-Square = 17.185 **			**P-Value = 0.0007**		

Our results agreed with [29] regarding the production of biofilm for the species *C. albicans* and *C. glabrata*, while with regard to the species *C. parapsilosis and C. kefyr*, they were not able to form a biofilm, which agreed with [30].

The ability of yeasts to form biofilms varies according to strains as well as species, and is affected by environmental factors. Biofilm is one of the most important virulence factors for *Candida spp*. Through resistance to the process of phagocytosis by autophagic cells, in addition to resistance to antibiotics [31].

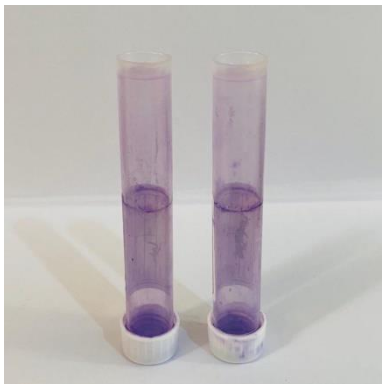


Fig. 5 : Candida ssp. Biofilm Producer.

Detection of the ability of *Candidia spp*. On the production of hemolysin

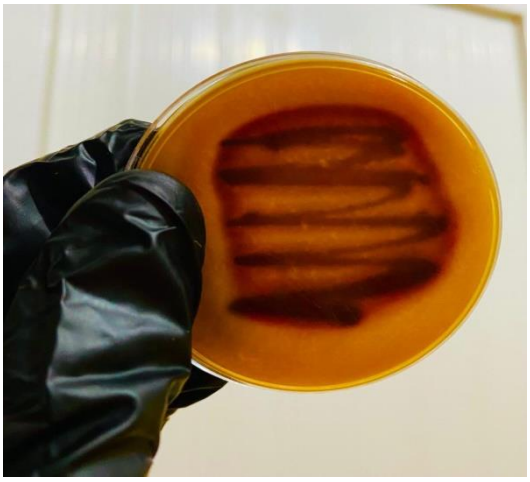
The results of the study, shown in Table (6), showed that the ability of Candida to decompose blood. The yeast *C.albicans* had the ability to decompose blood at a rate of 85%, with a rate of 34 out of 36, while the yeast *C. glabrata* had the ability to degrade blood at a rate of (15%) and the number of samples reached 6 out of 9, while all isolates belonging to *C. parapsilosis* and *C. kefyr* did not have the ability to lyse blood. The results of the statistical analysis showed that there were significant differences, p-value=0.0007, regarding the ability of *Candidia spp*. on the production of hemolysin.

Table 6 : Detection of the ability of *Candidia spp.* on the production of hemolysin.

Candida spp.	the total number	production of haemolysin	%	non production of haemolysin	%
C.albicans	36	34	%85	2	%25

C.glabrata	9	6	%15	3	%38
C.parapsilosis	2	0	%0	2	%25
C.kefyr	1	0	%0	1	%12
Total	48	40	100 %	8	%100
Chi-Square = 20.10 **			**P-Value =0.0007**		

Our results agreed with [32]. with regard to the two species *C. albicans* and *C. glabrata*, and with regard to the species *C. parapsilosis*, our results agreed with the results of the researcher [33], Who showed that *C.albicans* and *C.glabrata* produce hemolysin while *C.parapsilosis* does not produce hemolysin.



Conclusions:

1. Isolation of four types of *Candida spp*. The *C.albicans* species ranked first among the other isolated species.

2. Both species, *C.albicans* and *C.glabrata*, were shown to be capable of producing hemolysin and biofilms, with the exception of *C.parapsilosis* and *C. kefyr*.

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عزل وتشخيص الخمائر عند النساء المصابات بداء المبيضات المهبلي ودراسة قدرتها على تكوين الاغشية الحيوية والهيمولايسين

راوية محمود حميد*، هوازن احمد عبد
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تأريخ القبــول: 2023/11/03
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الكلمات المفتاحية:
C. albicans, C. glabrata, C.parapsilosis, C.kefyr.
داء المبيضات المهبلي

معلومات المؤلف
الايميل:
الموبايل:

الخلاصة:
خلال الفترة من تشرين الأول 2022 إلى كانون الثاني 2023.تم جمع 100 عينة سريرية من النساء المتزوجات (22-53 سنة). استخدمت طرق تشخيصية متعددة بما في ذلك الفحص المجهري المباشر وزراعة العينات على وسط Sabroid Dextrose Agar. تم تشخيص أربعة أنواع من الخمائر باستخدام الوسط التفريقي كروم اكار كذلك الاختبارات الكيموحيوية منها اليوريا وجهاز التشخيص VITEK2 Compact. أظهرت النتائج وجود 55% من العينات إيجابية و45% سلبية بواسطة الفحص المجهري المباشر. بينما أظهرت زراعة العينات أن 48% منها إيجابية و52% سلبية على وسط SDA. أما اختبار اليورياز وجهاز التشخيص VITEK2 Compact، فأظهرا وجود أربعة أنواع من الخمائر، حيث تم تحديد نوع C. albicans بنسبة 75%، يليه C. glabrata بنسبة 19%، وكل من C. parapsilosis و C. kefyr بنسبة 4% و2% على التوالي. تم دراسة عوامل الضراوة وهي كل من الاغشية الحيوية والهيمولايسين فقد اظهرت النتائج قدرة كل من C. albicans, C. glabrata, على انتاج الأغشية الحيوية والهيمولايسين , أما فيما يخص النوعين C. parapsilosis, C. kefyr فلم تكن قادرة على انتاج الاغشية الحيوية والهيمولايسين.