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ISOLATION AND DIAGNOSIS OF SOME *CANDIDA* SPECIES FROM SOME BAGHDAD CITY HOSPITALS WITH PCR TECHNIQUE AND EVALUATION OF THE EFFECTIVENESS OF SOME ANTIFUNGALS

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Abstract

The current study aimed to isolate and diagnose Candida spp yeasts that cause candidiasis with a PCR device from patients reviewed for some hospitals in Baghdad city and by 190 samples, the study recorded 123 isolates and the total percentage of infection was 64.7% .Samples were taken from different clinical cases of the vagina, blood and mouth and the Candida spp were (70.37%, 41.26%, 86.95%) respectively. Five types of yeasts were isolated and diagnosed, namely *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C.glabarta*. They were confirmed by PCR device and the most notable were yeast C. albicans, where 91 isolates were found, 73.98%, while the lowest infection was recorded. *C.glabarta*with 3 isolates, at 2.43%, significant differences at $P \leq 0.001$.The *C. albicans* showed the ability to form a Grem tube and Chlamydospore formation. Cultivation on the differential medium, chromo agar, showed that the yeast of *C. albicans* in a light green color, *C.tropicalis* in a metallic blue and *C.parapsilosis* in a creamy white color as the *C .krusei* was light pink in color, while *C.glabrata* was pinkish-purple in color. Isolation and diagnosis of these species have been confirmed by the Vitek2 Compact System. Four types of antifungal agents, Nystatin, Amphotericin, Clotrimazole and Ketoconazole, were used, The results showed a different effect of antifungals on Candida spp. The results of the PCR using the fungi starter pair (ITS1, ITS4) showed that they produced different molecular sizes ranging from (510-870) bp where the type of *C. albicans* was 535 bp and *C. krusie* was bp 510 and *C.tropicalis* was bp 524 and *C. parapsilosis* was bp520 and *C.glabrata* were bp 870.

Keywords: Candida spp, antifungals, PCR diagnosis of candida spp.

Introduction

The genus Candida includes species of clinical importance (Höfs *et al*., 2016). *C. albicans* are the most isolated species responsible for the appearance of various symptoms and affect different parts of the body (Chouhan *et al*., 2019). During the past decades, other types of Candida species have been detected, such as *Candida glabrata*, *C.tropicalis*, *C.parapsilosis*, *C.krusei* , *C.dubliniensis* and *C.kefyr* (Saunte *et al*., 2017; Muhsen *et al*., 2020). Candida spp is one of the most widespread yeasts and has the ability to cause an infection that is usually superficial and may be limited to infection to the mouth and mucous membranes and possible penetration and entry into the blood stream, which leads to systemic infectionsand inflammation of the tissue (Dadar *et al*., 2018). *Candida* spp is the fourth most common cause in many developed countries (Pappas *et al*., 2018). Candidiasis is a term for fungal infections caused by *Candida* spp that are frequent and common due to their natural presence in the mucous membranes of the vagina and mouth and Intestine (Mahmoudabadi *et al*., 2013). The Candida spp has virulent factors such as biofilm formation, adherences and extracellular hydrolysis enzymes causing tissue damage (Sardi *et al*., 2013). PCR Polymerase Chain Reaction is used to diagnose fungi, whether the fungal colony is missing its diagnostic properties, is newly developed, or dead (Faggi *et al*., 2002). This technology is known as the process of duplicating a piece of DNA with a specific sequence as part of the individual's entire genome, and it is outside the body of the living entity In vetro using a specialized polymerase enzyme and in the presence of primers that correlate with a complement sequence on the DNA

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template Template (Mullis, 1990).

Materials and Methods

190 different samples (63 blood samples, 81 vaginal samples, 46 oral samples) were collected in different ages (one day - 65 years) from some Baghdad city hospitals, for the period from October 2019 to February 2020. Blood samples were collected by tube containing EDTA and oral and vaginal samples with Swabs. After that, the following tests were performed.

Morphological and microscopic examination

The Sabourand Dextrase Agar culture media was used for the initial isolation of *Candida* spp and incubated at 73°C. For 48 hours, One of the colonies that was developing was taken on the SDA culture media and add a drop of blue Lactophenol dye.

Formation Test Germ tube

Take part of the colony and put it in a sterile test tube containing 0.5 ml of serum Serum, and incubated at 37^0C for 2-4 hours. (Forbes *et al*., 2007).

Chlamydospore Production Test

Cornmeal Agar was inoculated with a colony of Candida spp and incubated at 25^0C and monitored for 4-6 days (Gupta *et al*., 2016).

Diagnosis by Chromogenic agar culture media

Incubated at 37^0C for 48-72 hours, Chromogenic agar culture media determines the species of *candida* by color (Hospenthal *et al*., 2006).

Biochemical Identification

Clinically important of *candida* spp bleach have been precisely diagnosed with the Vitek2 device, according to the manufacturer's instructions Biomerieux U.S.A. (Pincus, 2006).

Antifungal Susceptibility Test

Mueller Hinton Agar medium was used for antifungal test using four Nystatin, Amphotericin, Clotrimazole and Ketoconazole antagonists. Incubated at 37^0C for 24-48 hours with growth monitoring, the results were read by means of a ruler to measure the Inhibition Zone diameters around antifungal tablets (AL-Bajilan, 2016).

Extraction of DNA from Candida spp

DNA extraction was performed from growing *candida* spp colonies in its culture media, using the method recommended by ABIOpure, which is equipped with the extraction kit.

Statistical Analysis

The Statistical Analysis System program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.There were no significant differences at $P \leq 0.001$

Results and Discussion

The results of the microscopic examination showed the presence of (123 positive isolates) from 190 isolates belonging to the Candida spp, with a percentage of 64.7% of the total isolation of the samples. The positive clinical samples included the vagina, blood and mouth in different proportions (70.37%, 41.26%, 86.95%) respectively and confirmed the results of the statistical analysis There were no significant differences at $P \leq 0.001$ as the level shown in table 1.

Table 1: Numbers and percentages for isolating the types of *Candida* from different clinical samples.

Clinical samples	Total number	Number of positive	isolates Percentage isolates	Number of negative %	Percentage% isolates
vagina	81	57	70.37%	24	29.62%
blood	63	26	41.26%	37	58.73%
mouth	46	40	86.95%	6	15.00%
total number	190	123		67	
statistical analysis		**=0.001 LSDChi-Square= 11.48			

It was noticed through the morphology examination of Candida spp colonies growing on the cultivated media in the middle of SDA, that this species appears as white colonies that are creamy, convex and have a smooth. This result is consistent with (Belan *et al*., 2018) and (Abdulla and Mustafa, 2020). *Candida* colonies possess the same characteristics as those mentioned. In addition to that the cells are spherical in shape to oval or are long single and budding with the presence of false fungal yarn Pseudohyphae sometimes and this is consistent with his mention (Wibawa and Aman, 2015). *C. albicans* showed its ability to form a bacterial tube, Grem tube, as results showed that this type produced a germ tube, which is a diagnostic characteristic of this type consistent with Alzubaidy (2019), who stated that *C. albicans* have the ability to form a germ tube. *C. albicans* showed susceptibility to chlamydospors, a diagnostic characteristic of them and no other species.This result coincides with Böttcher *et al.,* (2016), as the center of corn sorghum is among the cells starving, it prevents vegetative growth and promotes the formation of chlamydate boards to survive the fungi in inappropriate conditions. This feature is used to identify and distinguish types of ovaries.

Species of isolated *Candida* were diagnosed by growing them on chromo agar, as *C. albicans*

appeared in a light green color, *C.tropicalis* in a metallic blue color and *C.parapsilosis* in a creamy white color. As for *C.krusei* color was a light pink color. *C.glabrata* color is purple. These results are consistent with mentioned by Jogender *et al*., (2020); Salah *et al.,* (2020), Those who mentioned that the colors of yeasts differ according to the Candida spp.

Diagnosis by Vitek2 Compact System

Fig. 1 showed the isolation of 123 isolates due to the Candida spp, from 190 samples, with a percentage of 64.7%. Whereas, the highest incidence was *C. albicans* at 73.7%, followed by *C.tropicalis* at 13%, then *C.parapsilosis* by 6.5%, *C. krusie* by 4.07% and finally *C.glabrata* by 2.43% and statistical analysis showed the presence of significant differences at the probability level $P \leq 0.001$.

The reason for the emergence of *C.albicans* and their superiority over the rest of the species is due to its having many ferocity factors such as dimorphism Romo and Kumamoto (2020). Its ability to adhere to epithelial cell membranes in a high degree compared to other types, due to the presence of a number of surface receptors that affect the increased ability of Candida spp to adhere to the epithelial tissue cells of the host body, In addition to its ability to secrete enzymes digesting proteins, the most important of which is Aspartic Proteinase responsible for protein analysis, causing an increase in the speed of entry of yeast cells into the host tissue, and consequently, injury and secretion of phospholipase enzymes that analyze phospholipids, which are one of the main components of the cell membrane (Mohammed *et al*., 2020).

Isolation of sex types of *Candida* spp from the vagina

81 vaginal isolates were taken from patients with candidiasis, and Fig. 2 showed that the *C. albicans* recorded the largest percentage was 82.47%, followed by *C.parapsilosis*, *C.krusie* and *C.glabrata* with 8.78%, 5.27% and 3.50%, respectively and analysis. The statistic demonstrated significant differences at $P \leq 0.001$. This result is consistent with Khudhur *et al*., (2019).

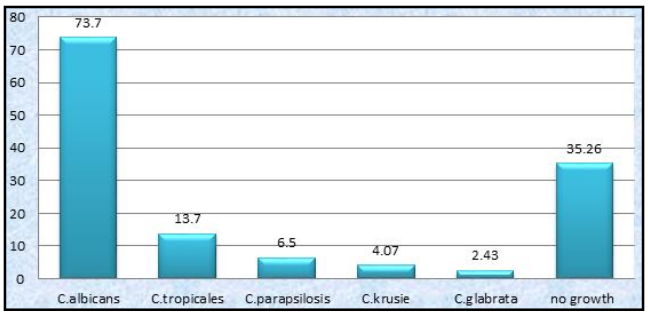

Fig. 1: Shows the percentage of species of Candida.

Isolation of species of *Candida* spp from the blood

The number of positive blood isolates reached 26 from 63 pathological isolates and it was found that the percentage of *Candida* isolated from blood was 41.26%, where the *C.tropicalis* formed the largest percentage by 50%, followed by *C.albicans* with 45.38%, then *C.parapsilosis, C.krusie*, *C.glabrata* was 3.85% for the three isolates and statistical analysis showed the presence of significant differences at the probability level $P \leq 0.001$, as shown in Fig. 3.

Our study percentage of 41.26% agreed with Tulasidas *et al*., (2018), as they indicated that the percentage of ovarian isolation from the blood reached 42%, as was also agreed by the presence of *C. tropicalis*, which was the most present and by 50%. It agreed with Tan *et al*., (2016) and Bac *et al*., (2019) Who reported that the isolation rate for Candida spp was 50.54%. Candida spp are a blood stream infection and are not confined to the mucous membranes only and have increased recently, especially from people who perform major operations and dialysis (Pappas *et al.,* 2016) and these results are consistent with Wisplinghoff *et al*., (2004) and Zaoutis *et al*., (2005) showed that candida spp are the fourth most common cause of infection in the bloodstream and cause Candidasis for hospitalized patients. Takesue *et al*., (2015) reported that Candida spp cause 25% mortality. The increased incidence is due to the presence of appropriate conditions such as long-term use of antibiotics and consistent with Schelenz, (2008) who stated that continuous antibiotic intake, intravenous catheterization, abdominal surgery and stay in the intensive care unit and invasive devices expose patients to candidiasis.

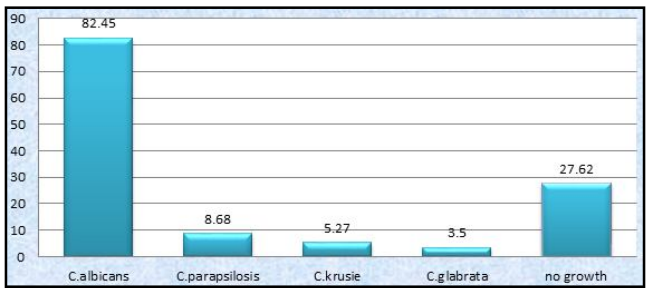

Fig. 2: Shows Candida spp isolated from the vagina.

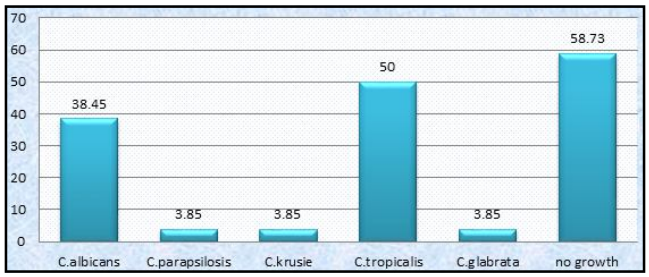

Fig. 3: Shows species of Candida spp isolated from blood.

Isolation of types of *Candida* spp from the mouth

The results showed that there were 40 positive isolates from 46 isolates, 86.95%, Fig. 4 and the *C. albicans* accounted for the largest number of 34 isolates and by 85%, followed by *C.tropicalis* by 50.7%, then *C.parapsilosis* with a percentage of 5% and the lowest percentage was *C.krusie* is 2.50% and statistical analysis showed that there were significant differences at the probability level $P \leq 0.001$, This result is consistent with the findings of Yan *et al*., (2013), which showed that the percentage of *Candida* spp isolated from the mouth was 86.1% due to the presence of Candida spp due to the lack of dental cleaning, the presence of dentures, diabetes, anemia and the use of antibiotics.

Sensitivity of yeasts to some antifungals

Table 2 indicated that the Ketoconazole, Nystatin, and Clotrimazole antifungals recorded more inhibition on the *C. albicans* as they reached (27, 23, 22) mm respectively, while the amphotercin -B antibody recorded the highest inhibition of *C. glabrata*, Nystatin and Amphotercin -B the least inhibition on the *C. tropicalis* was (18, 17) mmrespectively, while the *C. glabrata* and *C. krusei* recorded the lowest inhibition of antifungals (12, 15) mm respectively.

The current study showed that all Candida spp were sensitive to varying degrees of the antifungals used, and these results are consistent with Bouchara *et al*., (2000); AL-Maliki *et al*., 2011 who mentioned that yeasts were less sensitive to antifungals because the random and repeated use of this antagonist leads to the emergence of resistant species of Candida spp and therefore it is natural that they differ from one species to another. Repeated use of antifungals leads to mutations that increase the resistance of Candida spp and increase the factors of their virulence and thus their resistance to fungicides. The fungal sensitivity may differ from one species to another depending on the location of the samples collection and depends on the concentration of the counter as well as the excessive use of anti-azoles randomly increases the resistance of some types of yeasts for these antifungals.

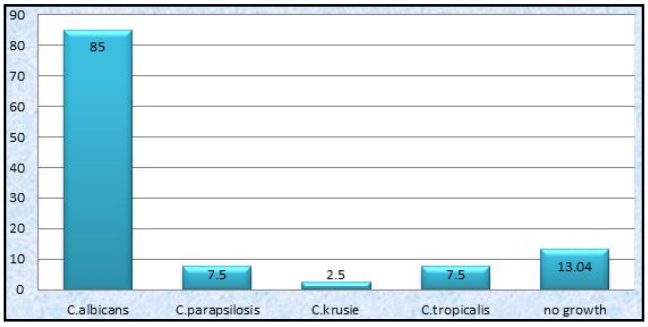

Fig. 4: Shows the species of *Candida* spp isolated from the mouth.

Table 2: Effect of antifungals on *Candida* spp and the amount of inhibition in mm.

*Candida*spp	Nystatin	Amphot-ercin-B	Clotri-mazole	Ketoco-nazole
C. albicans	23	18	22	27
C .tropicalis	18	17	20	23
C. parapsilosis	22	18	21	22
C. krusei	19	23	16	15
C. glabrata	19	26	12	17

Molecular diagnosis by Polymerase chain reaction PCRtechnique

Using the ITS1 and ITS4 pair of fungi that amplifies the internal transcribed space (ITS), which contains ITS1-5.8S-ITS2, which is a special area for testing all types of fungi, we notice that they produced different sizes ranging from (510-870) bp, This is based on the difference in the lengths between the ITS1 and ITS4 regions in the DNA of Candida spp, they produce pieces of DNA of different sizes using the PCR reaction and through the figure it was found that the type of *C. albicans* was 535 bp and *C. krusie* was bp 510 and *C. tropicalis* were bp 524 and *C. Parapsilosis* was bp520 and *C.glabrata* were bp 870 as in Fig. 5 and table 3. These results are consistent with (Abood *et al*., 2016) and (Krishnasamy *et al*., 2020).

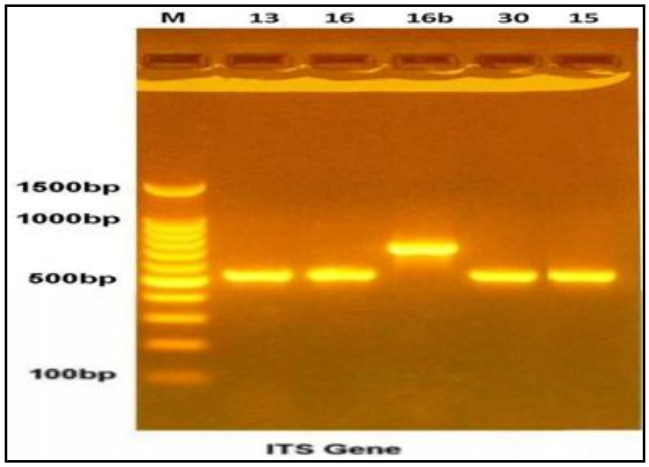

Fig. 5: Electrophoresis using a 1.5% acarose gel which shows the results of PCR for *Candida* spp isolates analysis where (1500-100)bp, M: Marker is represented.

Table 3: Species of *Candida* spp resulting from Polymerase chain reaction (PCR)technique.

Size ITS1-ITS4(bp)	No. of isolate	*Candida* spp
535	30	*Candida albicans*
524	13	*Candida tropicalis*
520	16	*Candida parapsilosis*
510	30	*Candida krusei*
870	15	*Candida glabrata*

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