

# Molecular Characterization of Yeasts Isolated from the Urinary Tract of Pregnant Women in Kirkuk City

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## Abstract

The current study aimed to the molecular diagnosis of yeasts isolated during the study. This study was conducted in the Graduate Studies Laboratory / College of Science - Kirkuk University, and included the isolation and diagnosis of yeasts that cause urinary tract infections in pregnant women, and they were diagnosed by routine laboratory methods, and the diagnosis of yeasts was confirmed using polymerase chain reaction (PCR) technology. 200 urine samples were collected from pregnant women with urinary tract infection who attended the maternity, gynecological and children's hospital in Kirkuk governorate and their ages ranged between 17-43 years, in the period between November 2021 and until February 2022. The results showed that the sequencing was performed for the selected isolates and entered into the NCBI BLAST program to identify the similarity between them and the results in the global gene bank after obtaining the nucleotide sequence of the DNA package of the local isolates and comparing it with the sequences of the same region for some global and local *Candida* spp isolates registered in NCBI BLAST, where *Candida albicans* showed a match between 99.80 to 99.39 percent with the results in the gene bank, and *Candida glabrata* showed a match with the gene bank with a percentage of 99.85% to 98.82%, and for the type *Pichia kudriavzevii* (*Candida krusei*) it was 100%.

**Keywords:** Polymerase Chain Reaction, *Candida Albicans*, Yeasts.

DOI: 10.47750/pnr.2022.13.S03.135

## INTRODUCTION

Urinary tract infections are one of the most common types of yeast infections, affecting approximately 150 million people worldwide each year. Urinary tract infections are considered a serious public health burden and greatly disturb the lives of infected people. This infection can affect men and women of all ages. However, women are more likely to contract this infection than men and this is due to the shorter urethra in females compared to the urethra in males, and its proximity to the outlet opening [1]. Urinary tract infection is the most common infection during pregnancy, although all women are susceptible to infection, but pregnant women in pregnancy are at increased risk due to anatomical and hormonal changes [2]. It is estimated that about (10-20) % of women suffer from urinary tract infection during childhood and this case continued to contract this infection during pregnancy [3]. Urinary tract infection occurs in pregnant women at all ages and in different stages, and the ages of (21-25) years are among the most susceptible to urinary tract infection, and the third stage of pregnancy is one of the most frequent stages of pregnancy with urinary tract infection [4-5]. Fungi are part of the microbes that may contribute as fungal pathogens to urinary tract diseases. In the past two decades, fungal urinary tract infections due to

the *Candida* genus have increased significantly. Healthy urinary tracts are usually sterile, so the presence of *Candida* yeasts in urine indicates a variety of clinical conditions. Fungal UTIs can be symptomatic or without symptoms, and lower urinary tract infections caused by yeasts are four times more common in women than in men [6]. *Candida* species usually live in the reproductive tract, digestive tract, and skin, and cause UTIs either through the bloodstreams or ascending routes from the focus of *Candida* colonization near the urethra. Most *Candida* infections occur as a result of overgrowth and subsequent invasion by *Candida* species that are inherent in the gastrointestinal tract of the host, as well as nosocomial infections from an external source [7]. *Candida albicans* is one of the most common types of *Candida* isolated from urinary tract infections, in addition to other types such as *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*, where these types appeared as an important factor in the causes of infection [8]. Therefore, the current study aimed to the molecular diagnosis of yeasts isolated during the study.

## MATERIALS & METHODS

### Collection of urine samples

200 samples of midstream urine were collected from

pregnant women suffering from symptoms of urinary tract infections, who attended the maternity, gynecological and children's hospital, for the period from the beginning of November (2021) to February (2022) and their ages ranged between (17-43) years. The patients' information, which included their name, age, date of review, stage of pregnancy, whether they had chronic diseases (diabetes and pressure), and whether an antibiotic was taken or not, was written down, according to a questionnaire form shown in Appendix No. (6).

Urine samples were collected after giving the patients the necessary instructions to collect urine by washing the external genitourinary organs from front to back, omitting the first drops of it, and collecting the urine sample in sterile plastic bottles with a wide mouth to facilitate collection and closing the container directly to avoid contamination. Then, the microscopic examination and culture of the samples were carried out within less than two hours of collection.

### Microscopical Examination

5-10 ml of urine samples were taken and transferred into a test tube, and precipitated in a centrifuge at a speed of 3000 rpm for (5) minutes. In the presence of pus cells, epithelial cells and red blood cells, *Candida* yeasts are seen microscopically as small and oval.

### Culture of Samples

Urinary samples containing pus cells were cultured at a rate greater than 10 purulent cells/microscopic field under 40X magnification. All urine samples were cultured on Sabouraud dextrose agar. The dishes were placed in the incubator at a temperature of (37) C for a period of (24-48) hours to isolate the inflammatory germs [9].

### Molecular diagnosis

The polymerase chain reaction was carried out at the (Golden Steps) laboratory in Tikrit city to investigate the yeast *Candida* spp. Where the fungus DNA was extracted and the target region ITS was amplified using each of the primers, as in Table (1). This method was carried out according to [10], and according to the following steps:

### DNA Extraction

DNA was extracted from a single pure and active colony of *Candida* yeast using CTE 100® Chelex, BioRad® Chelex 100® solution was made in the USA, and the extraction was performed according to the manufacturer's instructions.

**Preparation of reaction mixture for PCR** A mixture of PCR reaction (25 µl) was prepared according to the manufacturer's instructions, as shown in Table (1).

Table (1) components of the PCR mix.

PCR master mix	Volume
Master mix	5 µl
Forward primer	1 µl
Reverse primer	1 µl
Free nucleas water	13 µl
Genomic DNA	5 µl
<b>Total</b>	<b>25 µl</b>

The components of the mixture mentioned in the above table were placed in tubes with a capacity of 0.2 ml, which contained the rest of the components of the PCR reaction. DNA according to the ideal conditions for thermal cycles for all genes, according to the manufacturer's method of work as in Table (2).

Table (2) Optimum Thermal Conditions for Phases of PCR for ITS gene

PCR Step	Repeat Cycle	Temperature	Time
Initial denaturation	1	95 C <sup>0</sup>	180 se
Denaturation	35	95 C <sup>0</sup>	30 se
Annealing	1	56 C <sup>0</sup>	60 se
Extension	1	72 C <sup>0</sup>	50 se
Final extension	1	72 C <sup>0</sup>	300 se

### Primers

Molecular diagnosis was performed using polymerase chain reaction (PCR) technique based on heterogeneity in the ITS gene region (Internal Transcribed Spacer) to confirm the diagnosis of *Candida* spp. In this study, the fungus DNA was extracted and the ITS target region was amplified using both ITS1 and ITS4 primers as shown in Table (3).

Table (3) DNA primers and their nitrogenous base sequences used to confirm the diagnosis of *Candida* spp.

Primer	DNA Sequence (5-3)
Forward (ITS1)	(5'-TCC GTA GGT GAA CCT GCG G-3')
Reverse (ITS4)	(5'-TCC TCC GCT TAT TGA TAT GC-3')

### Standard Sequencing

The PCR product was submitted for Sanger sequencing using the ABI3730XL, an Automated DNA Sequencer by the Korean Macrogen Corporation. The results were received by e-mail and then analyzed using genetic software.

### Statistical Analysis

The data of the experiment were statistically analyzed using the statistical analysis program (SPSS) to estimate the means and standard error Std. Error, and the differences

between the means were compared based on Duncan's multiple range test at the 0.05 probability level  $P \leq 0.05$ .

## RESULTS & DISCUSSION

### Diagnosis of yeast isolates

*Candida spp* was diagnosed based on culture, microscopic and biochemical assays. *Candida* was also diagnosed on CHROM Agar *Candida* medium, as well as confirmation of the diagnosis using polymerase chain reaction (PCR) technique.

### Morphological and Microscopic Characteristic

The phenotypic characteristics of *Candida* colonies grown on SDA medium at 37°C for 24-48 hours were white to creamy in color, convex, smooth, as in Figure (1) and had a distinctive odor [11].



Figure (1): Colonies of *Candida spp* on SDA medium at 37°C for a period of (24-48) hours

The colonies were also examined microscopically after staining them with lactophenol blue and gram dye, and the shapes of cells appeared spherical to oval or longitudinal to single to budding [12]. As in Figure (2), the yeast cells appeared more clearly after being dyed with the dye Cristal Violet compared with the dye Lactophenol Cotton Blue. The appearance of the yeast cells in blue is due to the retention of the Peptidoglycan layer in the cell wall with this dye [13]. *Candida* cells are also Gram-positive [14]. In addition, the lactophenol blue dye has an important role in the observation of fungal hyphae and chlamydial spores [15].

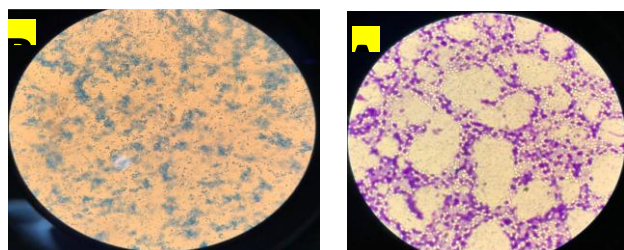


Figure (2): *Candida albicans* (A) dyed with gram dye (under 100X power) (B) dyed with lactophenol dye (40X magnification power)

### Chrom Agar Candida

The results of the diagnosis of *Candida spp* species using CHROM Agar candida medium showed the appearance of colonies in different colors on this medium, and Chrom Agar Candida medium is a differentiation medium for the species, where *Candida albicans* yeast appeared in green color, and *Candida glabrata* appeared in light pink to creamy color, and yeast appeared *Candida krusei* is in a dark pink color, as shown in Figure (4-10), as shown in Table (4-5) and as stated in the manufacturer's instructions. This finding is consistent with the findings of [16-17].

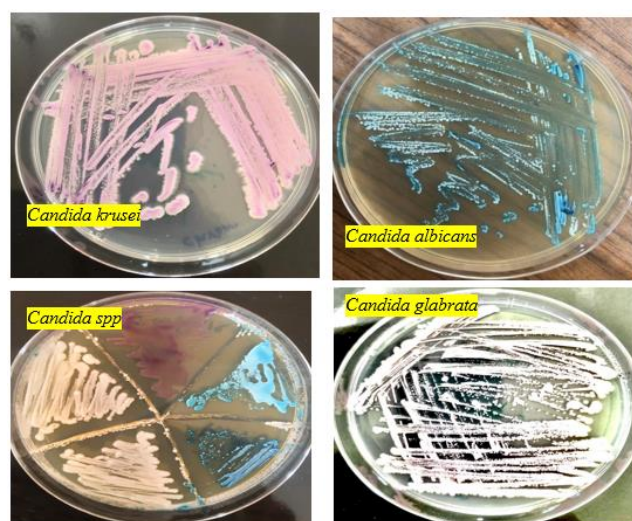


Figure (3) Diagnosis of *Candida spp* on CHROM Agar medium at 37°C for 24-48 hrs.

### Molecular diagnosis

The diagnosis of *Candida* yeasts was carried out using PCR technique to confirm the diagnosis of six isolates of *Candida*, depending on the primers (4), and given that the usual diagnostic methods adopted in the diagnosis of yeasts, including the species belonging to the genus *Candida spp* and based on the determination of phenotypic criteria, were not sufficient, Because of the overlap of these criteria with other species that are classified among the other species of the same genus, in addition to the genetic variation among them, the microorganism farms, even if they belong to a particular group, differ in their phenotypic characteristics and growth characteristics, and this difference does not necessarily mean the difference in The genetic structure, especially between farms and isolates of the same sex and species [18].

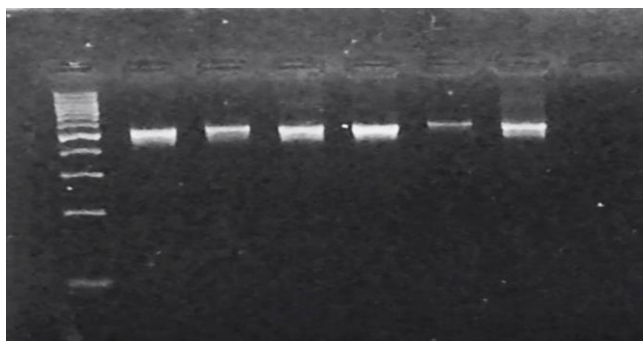


Figure (4): Results of electrophoresis using agarose gel by amplifying the ITS gene of *Candida* spp.

### Results of DNA sequencing analysis

Sequencing was performed for the selected isolates and entered into the NCBI BLAST program to identify the similarity between them and the results in the global gene bank after obtaining the nucleotide sequence of the DNA package of the local isolates and comparing it with the sequences of the same region for some global and local *Candida spp* isolates registered in NCBI BLAST, where *Candida albicans* showed a match between 99.80 to 99.39 percent with the results in the gene bank, and *Candida glabrata* showed a match with the gene bank with a percentage of 99.85% to 98.82%, and for the type *Pichia kudriavzevii* (*Candida krusei*) it was 100%, and this indicates the spread of isolates of this pathogenic fungus widely in the world, where the nucleotide sequence of the DNA was entered into the NCBI BLAST program to find out the similarity between them and the results in the gene bank, as the results of the nitrogen base sequence analysis proved compared to the isolates recorded in NCBI All isolates isolated from patients are isolates of *Candida*, and this result confirms the phenotypic and microscopic diagnosis, as in the table (3), and the nucleotide sequence of multiplexed nucleic acid bundles of *Candida* species, Figure 5 (A,B,C).

Table (3) of the global isolates and their accession numbers in NCBI that were compared with them through the BLAST website, and the percentage of congruence with the isolates under study

Isolated No.	Molecular Diagnostics	Match percentage	Accession Number
6	<i>Candida albicans</i>	%99.80	KP674480.1
39	<i>Candida albicans</i>	%99.39	KP674888.1
50	<i>Candida glabrata</i>	%99.85	OK317687.1
8	<i>Candida glabrata</i>	%98.82	OK317687.1
132	<i>Pichia kudriavzevii</i> ( <i>Candida krusei</i> )	%100	MH545928.1
146	<i>Pichia kudriavzevii</i> ( <i>Candida krusei</i> )	%100	KX015902.1

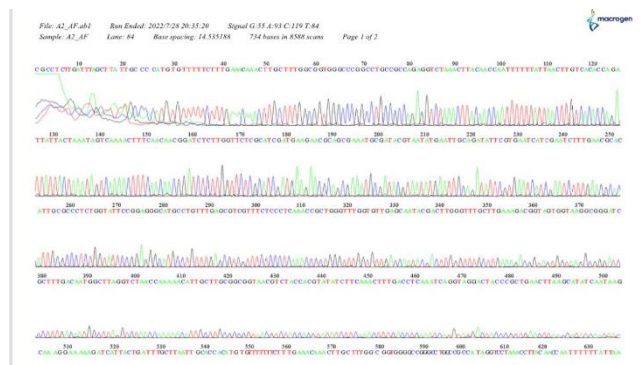


Figure 5-A: The nucleotide sequence of a double-stranded DNA bundle of *Candida albicans*

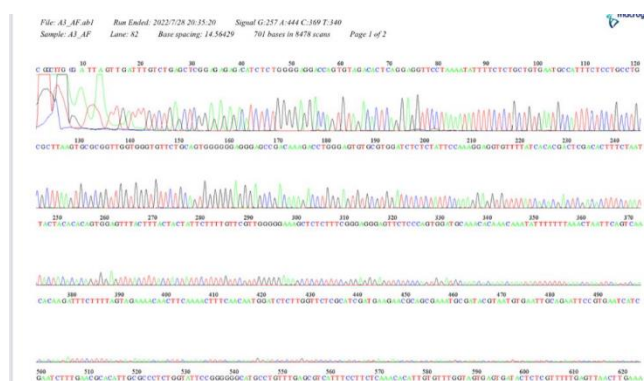


Figure 5-B: The nucleotide sequence of a double-stranded DNA bundle of *Candida glabrata*

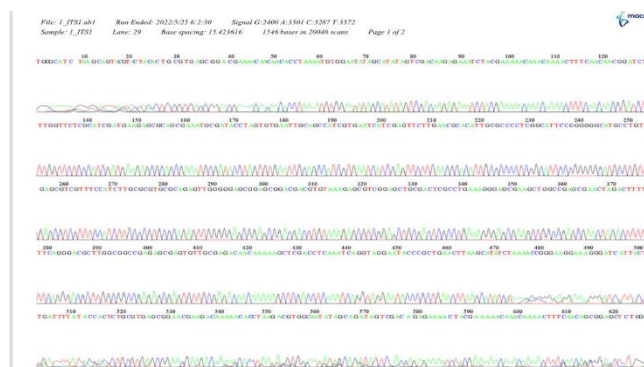


Figure 5-C: The nucleotide sequence of a double-stranded DNA bundle of *Candida krusei*

### REFERENCES

Bouacha, M., Ayed, H., & Grara, N. (2018). Honey bee as alternative medicine to treat eleven multidrug-resistant bacteria causing urinary tract infection during pregnancy. *Scientia pharmaceutica.*, 86(2), 14.

Le, J., Briggs, G.G., McKeown, A., & Bustillo, G. (2004). Urinary tract infections during pregnancy. *Annals of Pharmacotherapy*, 38(10), 1692-1701.

Johnson, J.R., & Stamm, W.E. (1989). Urinary tract infections in women: diagnosis and treatment. *Annals of internal medicine*, 111(11), 906-917.

- Fareid, M.A. (2012). Frequency and susceptibility profile of bacteria causing urinary tract infections among women. *J. New. Scien.*, 5(2): 72-81.
- Obiogbolu, C.H., OKonKo, I.O., Anyamere, C.O.; Adedeji, A.O., Akanbi, A.O., Ogun, A.A., Ejembi, J. and Faleye T.O. (2009). Incidence of urinary tract infections (UTIs) among pregnant women in akwa metropolis, southeastern Nigeria. *J. Scien. Res. Ess.*, 4(8): 820-824.
- Behzadi, P., Behzadi, E., Yazdanbod, H., Aghapour, R., Cheshmeh, M. A., & Omran, D. S. (2010). Urinary tract infections associated with *Candida albicans*. *Maedica*, 5(4), 277.
- Kim, S.J., Ryu, J.H., Kim, Y.B., & Yang, S.O. (2019). Management of *Candida* urinary tract infection in the elderly. *Urogenital Tract Infection*, 14(2), 33-41.
- Alkilani, A.A., El Shalakany, A.H., & Saif, W.Y.M. (2017). Candiduria in catheterized Menoufia patients: emerging microbiological trends. *Menoufia Medical Journal*, 30(3), 892.
- Ghasemi, R.; Farideh G.; Sasan R.; Mohammad R. P.; Ramin N., Mohammad J. J. and Ensieh M. (2020). A comparison of biofiltration performance based on bacteria and fungi for treating toluene vapors from airfow. *AMB Expr.*, 10(8): 1-9.
- White, P.L., Barton, R., Guiver, M., Linton, C.J., Wilson, S., Smith, M., & United Kingdom Fungal Polymerase Chain Reaction Consensus Group. (2006). A consensus on fungal polymerase chain reaction diagnosis?: a United Kingdom-Ireland evaluation of polymerase chain reaction methods for detection of systemic fungal infections. *The Journal of Molecular Diagnostics*, 8(3), 376-384.
- Bhavan, P.S., Rajkumar, R., Radhakrishnan, S., Seenivasan, C., & Kannan, S. (2010). Culture and Identification of *Candida albicans* from vaginal Ulcer and separation of enolase on SDS-PAGE. *International Journal of Biology*, 2(1), 84.
- Ellis CJ, Coppins BJ, Dawson TP, Seaward MRD. 2007. Response of British lichens to climate change scenarios: trends and uncertainties in the projected impact for contrasting biogeographic groups. *Biological Conservation* 140: 217–235.
- Sudbery, P., Gow, N., and Berman, J., (2004). *Trend Microbial*; 38(6): 869-881.
- Anderson, J.M., and, Soll, D.R, (1986). Differences in localization during bud and
- Webb, B.C.; Thomas, C.J. Willcox, M.D. P. Harty, D. W. S. and Knox, K.W. (1998). *Candida*- associated denture stomatitis Aetiology and management: A review. Part 1 factors influencing distribution of *Candida* species in oral cavity. *Austrian Dental J.*, 43(1): 45-50.
- Al\_Bajilan, Asoda Mohammed Noori. (2016). Study of the inhibitory of Snake Venom *Macrovipra lantana* against the Virulence factor of Vaginal *Candida* spp.. 66-67
- Kumar, M. B. and Edward, C.K. (2014). *Robbins and Cotran Review of Pathology*, Elsevier. 6-7.
- Mohieldin, Mohammed Omar, Jigan, Raqiba Ali, 2013, Production of Polgalacturonase enzyme from *Aspergillus niger* heat-tolerant and enzyme-producing mold and diagnosis of the most productive isolate. *Iraqi Agricultural Science Journal*, No. 1, Volume 44, pp. 97-105.