

Article

# Isolation and Identification of Different Candida Species From Urinary Tract Catheterized Patients and Its Relation to IL-17

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**Abstract:** Background: The capacity to formation biofilms by yeasts on the surfaces of medical devices, such as urinary catheters, is a significant factor in their potential to induce human disease. Recent studies has demonstrated that biofilms play essential roles in over 65% of microbial infections. Aim of the study: The present study aimed to identify various Candida spp isolated from the urine of catheterized patients and to examine their biofilm formation capability, as well as to evaluate the amount of IL-17 in these patients. Materials and methods: One hundred samples were obtained from patients with Catheter-associated urinary tract infections CAUTIs. The sample collecting technique includes both genders, males and females, of all ages and various disease cases from hospitalized patients at Tikrit Teaching Hospital. The samples were gathered from September 2024 to January 2025. Result: A total of 100 patients with CAUTIs, 67 (67%) were positive for candidia species which were alone or mixed. This study reveals that the isolated yeast species consist of 30% *C. albicans*, 25% *C. parapsilosis*, and 33% *C. tropicalis*. The test results indicated a substantial disparity in the quantity of isolates that generated biofilms compared to those that did not. Among 30 samples, 20 isolates of *C. albicans* exhibited biofilm formation, while *C. tropicalis* demonstrated biofilm formation in only 18 isolates. The isolate of *C. parapsilosis* also produced a biofilm, indicated by the emergence of a black colony. Conclusion: The present study showed increase IL-17 in patients with positive result of candidiasis from CAUTIs were(124±9.44) ng/ml and control(91.85±7.22), at p-value <0.05.

**Keywords:** urinary tract, catheterized, Candida, biofilm, IL-17

## Introduction

There are more than 150 species of Candida. But only 20 of them are known to be dangerous to people, with *Candida albicans* being one of the most common types [1].

*Candida* a dimorphic yeast that is thought to be an opportunistic human pathogen, is present in the flora of the human reproductive, digestive, and respiratory systems and affects 40–60% of healthy individuals. Usually a commensal organism, it can turn harmful in those who have compromised immune systems [2]. Catheter-associated urinary tract infections CAUTIs, is associated with candida infection, which result from extraluminal colonization from the skin either from normal flora that found

in the skin or acquired during hospitalization or from hematogenous seeding of the catheter's vascular lumen during fungemia. While several antifungal drugs can be utilized for prophylaxis, diagnosing candidemia associated with a vascular catheter is challenging to establish prior to its removal [3]. *Candida* usually occurs as a commensal organism in several bodily locations, with its shift to *Candida* species' transition from commensalism to pathogenicity being promoted by diverse virulence factors. This encompasses biofilm formation, the secretion of hydrolytic extracellular enzymes such as proteinases, phospholipases, and hemolysin, adhesion to host tissues and medical devices, and the development of pseudohyphae [4], [5]. *Candida* species have evolved ways to evade the immune system's removal. One method is the protection of PAMPs that have the potential to trigger an immunological reaction [6]. *Candida* species can decrease the development of phagolysosomes and the synthesis of nitric oxide (NO) by macrophages. In addition, *Candida albicans* can increase the probability of survival by causing macrophages to change their inflammatory phenotype from M1 to M2, which is less inflammatory [8].

T-helper 17 (Th17) is distinguished by the secretion of IL-17 and is essential in combating extracellular infections [9]. The connection between both innate and adaptive immunity are facilitated by cytokines. A pro-inflammatory cytokine such as IL-17 significantly stimulates the activation of neutrophils, and migration, generation of interferon by macrophages, as well as chemotaxis of eosinophils [10], [11]. IL-17 is predominantly secreted by Th17 cells, which is synthesized by regulatory T cells, natural killer cells, mast cells, macrophages and neutrophils [12].

When it comes to extracellular bacterial and fungal infections, such as *Klebsiella* and *Candida albicans*, Th17 cells are responsible for providing defense [13]. The presence of these cytokines causes a significant inflammatory response and stimulates the production of chemokines by a variety of target cells [14], [15]. It has been shown that interleukin-17 (IL-17) has an effect on the microenvironment of tumors, as well as the advancement of inflammatory infectious diseases and neurological disorders [16], [17].

## Materials and Methods

### Samples collection

One hundred samples were collected from various patients with urinary tract catheters. The sample collecting technique covered both genders, males and females, across all ages and various disease conditions from hospitalized patients at Tikrit Teaching Hospital. The samples were gathered from September 2024 to January 2025.

### Isolation and culture for different samples

After the patients' urinary tract catheters were removed, the indwelling catheters were sterile-snipped about 6 cm from the tip, next to the bladder and urinary tract, and then brought to the lab in a sterile environment [19].

### Laboratory examination of samples

#### Macroscopic examination and microscopic examination

The first identification relied on colony characteristics observed on Sabouraud Dextrose Agar (SDA) and microscopic analysis as outlined by [20].

#### Diagnosis test using HiCrome™ *Candida* Differential Agar Base

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 [21].

#### Germ tube formation test and Chlamydoconidia formation

This test was used to distinguish the yeast *C. albicans* from other species that are negative for this test. This test done according to [21].

#### Diagnosis using the Vitek device

The VITEK-2 system, along with the Vitek ID card (BioMerieux, France), was utilized in order to carry out additional identification and antifungal susceptibility tests.

## Virulence Factors

### Biofilm formation test

biofilm-forming capabilities were assessed using a Congo Red Agar (CRA) assay. In accordance with [21], the test was planned and executed.

### Collecting blood samples

A sterile, dry medical syringe was used to draw five milliliters of venous blood from 67 patients who had been diagnosed with candidiasis in different hospitals within the Salah Al-Din Governorate. Samples were put in gel tubes and left to congeal for 15 to 20 minutes at room temperature. The serum was then extracted from the tubes by centrifuging them for ten minutes at 3000 revolutions per minute. The serum was then put into Eppendorf tubes for different testing and kept at -20 °C until the immunological tests were carried out. To avoid multiple freeze-thaw cycles that could degrade the quality of the results, all samples were examined concurrently.

### Assessment level of IL-17 by ELISA

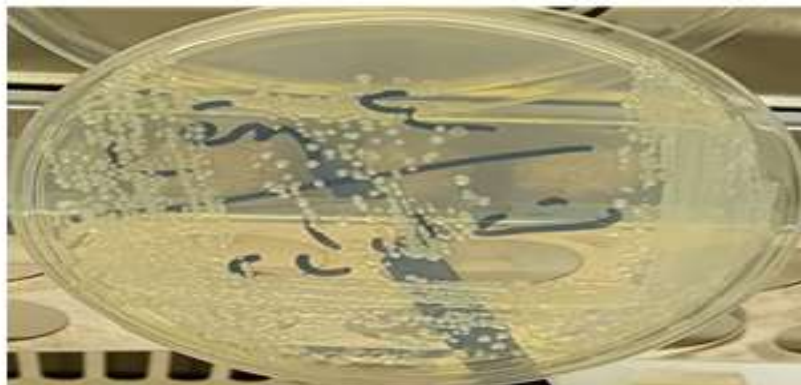
The ELISA method was employed to evaluate serum concentrations. The concentration of IL-17A was quantified utilizing the ELISA method. The plate was pre-coated with antibodies specific to human interleukin-17A. The sample is enhanced with IL-17A content. The concentrations of human IL-17A exhibited a positive correlation with the coloration of the substrate solution. The process concludes with the introduction of an acidic stop solution, followed by the quantification of absorbance at a wavelength of 450 nm.

### Analytical statistics

SPSS 22 was used for statistical analysis. The continuous variable format was means  $\pm$  SE. The link between categorical factors was examined. P-values under or equal 0.05 were significant.

## Result

The results of culture on SDA medium showed colonies that were creamy white, shiny, smooth, oval or spherical in shape and sticky in texture. As shown in Figure (1).



**Figure 1 shows the growth of candida spp on SDA**

The results of *C. albicans* showed a positive reaction after staining with lactophenol blue dye, as the yeast cells showed a spherical to oval or long, single and budding shape. As shown in Figure (2).

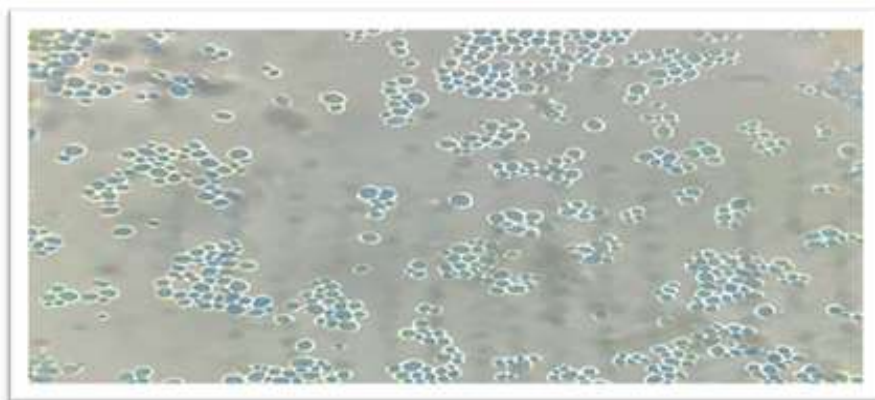


Figure 2 shows the growth of *C. albicans* yeast stained with lactophenol cotton blue (40°C).

According to Table 1, out of a total of one hundred patients who were diagnosed with CAUTIs, 67 (67%) of them tested positive for candidia species, either by themselves or in combination. Thirty percent of the yeast species that were identified in this investigation were *Candida albicans*, twenty-five percent were *Candida parapsilosis*, and thirty-three percent were *Candida tropicalis*. Figure (3) show different species of candida. Colonies of *Candida albicans* looked light green, those of *Candida tropicalis* looked dark blue, and those of *Candida parapsilosis* looked white.

Table 1. Frequency of candida spp in CAUTIs

Type of Candida	Percentage
<i>C.albicans</i>	30 %
<i>C.tropicalis</i>	33 %
<i>C.parapsilosis</i>	25 %
Total	%88

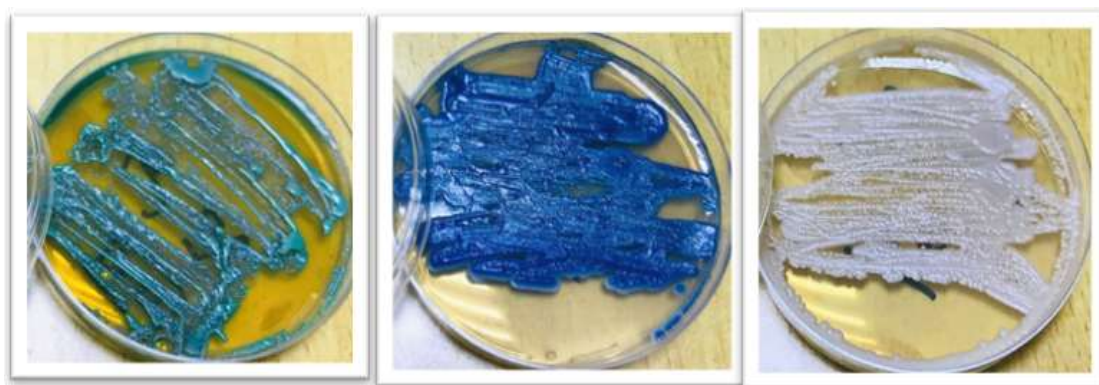
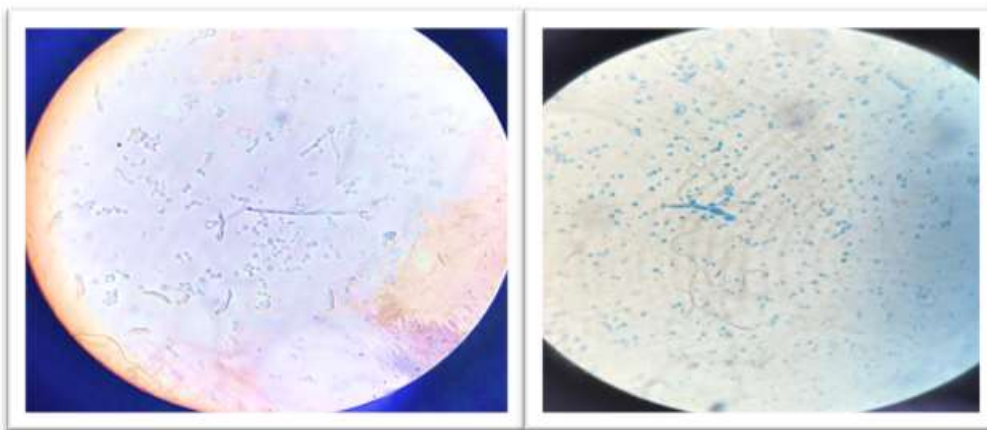


Figure 3. Growing colonies of isolated *Candida* species on chroma agar medium, A = *C. albicans*, B = *C. tropicalis*, C = *C. parapsilosis*.

Tests demonstrated that every single *C. albicans* isolate could produce germ tubes, as illustrated in Figure (4) and Table (2). The test demonstrated that all *Candida albicans* and *Candida tropicalis* isolates could produce chlamydial spores under certain conditions, but none of the other species could.

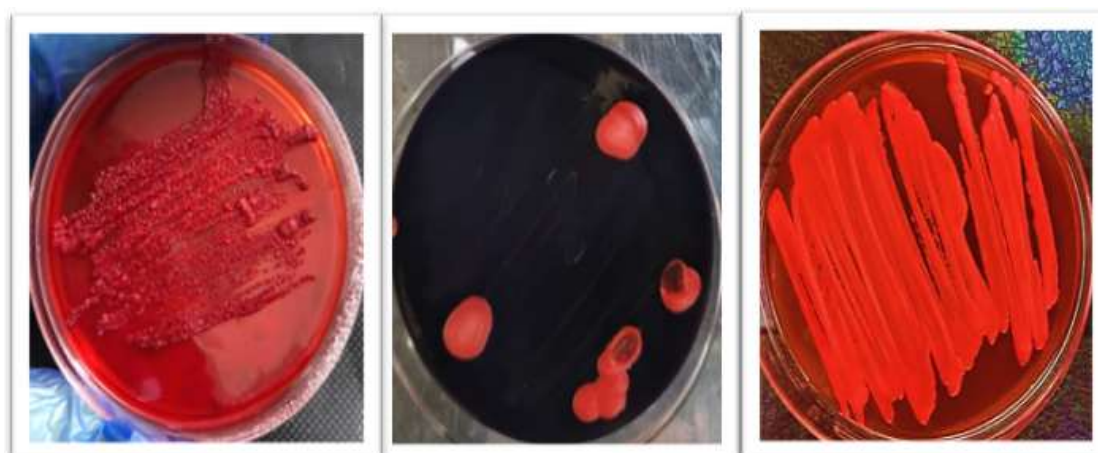
Table 2. Diagnosis of *Candida* species by germination tube test and chlamydial spore formation.

Type of Candida	formation germ tube	formation Chlamydial spore
<i>C.albicans</i>	+	+
<i>C.tropicalis</i>	-	+
<i>C.parapsilosis</i>	-	-



**Figure 4. Diagnosis of Candida species by germination tube test and Formation of chlamydial spores by the yeast *C. albicans* (400 x).**

The test found that there was a statistically significant difference between the numbers of isolates that formed biofilms and those that did not. Twenty of the thirty *C. albicans* isolates formed biofilms; two of these formed black colonies, and three of these crystallized, as illustrated in Figure (5). The results also showed that 33% of *C. tropicalis* isolates were biofilm-forming, with only 18 isolates, out of 15 isolates, 7 of which appeared in the form of black and 8 in the form of crystals. The isolate of *C. parapsilosis* also formed a biofilm, represented by the formation of a black colon.



**Figure 5. The ability of Candida species to produce biofilm on Congo red agar medium, A: Biofilm producer, B: Biofilm producer in crystal form, C: Non-biofilm producer.**

The present study showed increase IL-17 in patients with positive result of candidiasis from CAUTIs were (124±9.44) ng/ml and control (91.85±7.22) ng/ml, at (p-value <0.05). As shown in table (3)

**Table 3. Level of IL-17 in patients with positive result of candidiasis and control**

parameters	Patients	Control	P-value
IL-17 ng/ml	124±9.44	91.85±7.22	0.01

**Discussions**

The ability of Candida species to stick to surfaces and form surface-attached microbial communities, often known as biofilms, is a significant component that contributes to their virulence. The antimicrobial drugs that are effective against Candida biofilms are more effective than those that are effective against planktonic Candida. They play a significant part in the development of infections that are caused by medical devices, including stents, shunts, implants, and a variety of catheters, as

well as infections that occur in hospitals [22]. There is a clear correlation between the presence of a urinary catheter and the development of *Candida* infections in the urinary system [23]. The National Nosocomial Infections Surveillance (NNIS) data revealed that *C. albicans* was responsible for 21% of CAUTIs, compared to 13% of non-catheter-related infections. Infections associated with biomaterials represent an increasingly concerning issue due to their significant resistance to antimicrobial treatment [22]. Recent studies indicate that numerous *Candida* species can adhere to polymeric surfaces, such as urinary catheters, and generate biofilms.

The current investigation revealed that the yeast isolates comprise *albicans*, *C. parapsilosis*, and *C. tropicalis* with percentage (30,25,33)%. Another study [23] isolates *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis* with percentage (25, 25, 6.25, 43.75)% respectively. These results may stem from the frequent use of urinary tract catheters, even in needless situations, which creates a conducive environment for bacteria to develop biofilms, hence increasing their aggressiveness and resistance. This may result in a higher occurrence of CAUTIs.

Multiple studies have indicated that a significant proportion, if not the majority, of candidemia cases are associated with catheters. The most extensive prospective investigation on candidemia identified a catheter as a contributing factor in (72% )of patients [25]. Predisposing factors for *Candida* infections involve immunocompromising medicines, antibiotic use, implanted devices as intravascular catheters, and age. *Candida* infections can also be caused by a combination of several other factors. It is [26]. The pathogenesis of *Candida* infections that are associated with catheters is a complicated process. In the process of pathogenesis, several factors play a significant role. These factors include the length of time that the catheter is implanted, the type of catheter material, and the characteristics of the organism. It is possible for organisms to originate from the layer of skin, infusate, or local sites; nevertheless, all of these sources are typically caused by a disruption in the gastrointestinal system [27]. A mortality rate of 41% was shown to be connected with catheter-related candidemia infections in patients who retained the vascular catheter, according to a research study that was conducted across many centers and involved 427 patients who were diagnosed with candidemia [28]. It is impossible to manage biofilms in medical settings due to their inherent characteristics, which make them extremely difficult to deal with. The retention of vascular catheters that have been colonized by *Candida* species has been shown to correspond with prolonged fungemia, increased rates of antifungal treatment failure, an increased risk of metastatic effects, and mortality, according to research on CAUTIs [29], [30], [31].

The present study showed increase mean $\pm$ SD in level of IL-17 in infectious patients with urinary catheterization. The present study agrees with [32] which showed increase IL-17 in catheterized patients. The generation of IL-17 by TH 17 cells is crucial for host defense against *Candida* species. These cytokines encourage the recruitment and activation of neutrophils, which in turn activate epithelial cells and secrete antifungal  $\beta$ -defensins.

It is thought that IL-17 encourages *Candida* in the body by directly binding to a shortage of food [33]. Another study [34] indicated that IL-17A therapy could enhance the survival of mice following invasive candidiasis.

## Conclusion

It is important to note that urinary tract catheters serve as an additional source of biofilm formation and infection transmission. Opportunistic *Candida* spp as *C. albicans*, *C. parapsilosis*, and 33% *C. tropicalis* were isolated from CAUTIs. The isolates showed positive results for biofilm formation displayed resistance to most antibiotics. Finally, this study concluded increase IL-17 in patients with positive result of candidiasis and control.

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