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The Correlation Between *Candida* sp. and Bacterial Co-Infection in Patients with Urinary Tract Infection

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Abstract :

The study included the collection of 120 urine samples, which were later cultured on MacConkey agar, blood agar, and Sabouraud dextrose agar. Among the positive samples, 8 samples showed a co-infection of bacteria with *Candida*. The fungal infection rate constitutes 6.6% of the total samples. Four samples of *C. albicans* were diagnosed, three samples of *C. glabrata*, and one sample of *C. krusei*. Bacterial infections have shown various types of bacteria, including *Escherichia coli, Klebsiella, Staphylococcus aureus, Staphylococcus saprophyticus,* and *Pseudomonas*. We conducted a sensitivity test for types of *Candida spp*. Nystatin, fluconazole, amphotericin, and ketoconazole showed varying sensitivity of the samples to antifungal agents, except for one sample that was resistant to all antifungal medications. This sample was identified and diagnosed as *Candida albicans*. Also conducted a toxicity test for *C. albicans*, and the toxicity rate was 2.21% at a concentration of 200 micrograms/ml and 1.97% at a concentration of 100 µg/ml. Toxicity increases with the increase in the dose of *C. albicans*.

Keywords: Antifungal susceptibility test, Germ tube test, Toxicity test, Vitek2 compact.

العلاقة بين المبيضات والعدوى البكتيرية في المرضى الذين يعانون من التهاب المسالك البولية

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مستخلص:

شملت الدراسة جمع 120 عينة من البول، والتي تم زراعتها لاحقًا على وسط ماكونكي، ووسط الدم، ووسط Sabroied dextrose. من بين العينات الإيجابية، أظهرت 8 عينات وجود عدوى مشتركة بكتيريا مع المبيضات. وتشكل نسبة الاصابة الفطرية 6.6٪ من مجموع العينات. تم تشخيص 4 عينات من *C. albicans*، وثلاث عينات من (*C. glabrata*)، وعينة واحدة من (*C. krusei*). أظهرت الإصابات البكتيرية أنواع عديدة من البكتيريا، بما في ذلك (*Escherichia coli, Klebsiella, Staphylococcus aureus, Staphylococcus saprophyticus, and Pseudomonas*). قمنا بفحص حساسية أنواع (*Candida spp.*). نيستاتين، فلوكونازول، أمفوتيريسين، وكيتوكونازول اضهرت العينات حساسيتها لمضادات الفطريات بنسب مختلفة الا عينة واحدة كانت مقاومة لجميع الأدوية المضادة للفطريات، تم تحديدها وتشخيص العينة على أنها (*Candida albicans*) . كما اجريت فحص سمية الـ (*C. albicans*) وكان معدل السمية يبلغ 2.21٪ عند تركيز 200 ميكروغرام/ مل و1.97٪ عند تركيز 100 ميكروغرام/ مل. تزداد السمية مع زيادة جرعة ال (*C. albicans*) .

الكلمات المفتاحية: اختبار الحساسية المضادة للفطريات، اختبار الأنبوب الجرثومي، اختبار السمية، جهاز فايتك2.

Introduction

A urinary tract infection (UTI) is a prevalent infection resulting from the infiltration of bacteria or yeast into the urinary system. It may be induced by infection in the lower and upper urinary tracts, presenting symptoms such as dysuria and increased urinary frequency with little output *(*Mancuso *et al.,* 2023*)*.

Candida albicans and non-*Candida albicans Candida* (NACA) species are regarded as significant components of the microbial normal flora in the oral cavity, gastrointestinal tract, and vagina of a diverse population of healthy individuals (Sinawe & Casadesus, 2020). Moreover, they inhabit the exterior aspect of the urethral orifice in premenopausal and healthy females. Immune deficits can result in an imbalance between *C. albicans*, NACA yeasts, and the host's natural flora. Under this environment, the commensal yeasts of *Candida* may transform into opportunistic pathogenic bacteria, resulting in candidal urinary tract infections in the host *(*Wagenlehner *et al.,* 2020*)*. The occurrence of *Candida spp.,* particularly *C. albicans*, in urine is referred to as candiduria. Candiduria is classified into asymptomatic (in healthy individuals or patients) and symptomatic versions*(*SHUJA, 2022*)*. Symptomatic candiduria occurs in individuals with cystitis, epididymorchitis, prostatitis, pyelonephritis, and renal candidiasis. Asymptomatic candiduria is predominantly benign and is not classified as a definitive condition. *Candida albicans* is a significant fungal pathogen that can result in candiduria, accounting for 20% of nosocomial infections. Extensive research indicates that *C. albicans* is the predominant cause of candiduria among over 200 *Candida* species (Afzelius *et al.,* 2024*;* Gharaghani *et al.,* 2021).

Urinary tract candidiasis is recognized as the most prevalent nosocomial fungal infection globally(Hassan Shahryari *et al.,* 2024). *Candida albicans* is the predominant etiological agent of nosocomial fungal urinary tract infections; nevertheless, a fast alteration in the distribution of *Candida species* is occurring. The rise in urinary tract candidiasis has resulted in the emergence of antifungal-resistant *Candida species*. (Rhodes & Fisher, 2019).

The aim of this study was to isolate and identify *Candida* spp and the co-infection microbe from patients have

urinary tract infections (UTIs) and study the toxicity of *candida albicans*.

Methods

Collection of samples : Urine specimens were collected in a sterile container from patients with urinary tract infections in Balad city, Salah al-Din Governorate, from August 1, 2023, to March 1, 2024, at Balad General Hospital, Iraq. All specimens were promptly cultured on media for bacteria and yeast, including blood agar, MacConkey agar, SDA agar, and chromogenic agar plates. The plates were incubated under aerobic conditions at 37°C following the manufacturer's guidelines. A Gram stain was conducted for microscopic analysis. A series of tests were used to classify bacteria and candida genera, as well as their associated species.

Vitek2 compact : The VITEK 2 compact device was used for the accurate identification of bacterial isolates. This system depends on a series of biochemical tests done together, using VITEK2 gram-negative (GN), VITEK2 gram-positive (GP), and VITEK2 BCL cards. Primarily dependent on a set of biological components, it provides precise outcomes within a few hours (Tshabuse *et al.*, 2022).

Diagnosis of *candida* : The isolated strains were cultivated on Sabroied dextrose agar media (SDA), and the culture were left at a temperature of 37°C for a period of 2-4 days. Specific features of the dorsal colonies were examined in order to detection shape and color (Hemaid *et al.*, 2021).

Growth on Chrom Agar media : The Chrom Agar plates were inoculated with yeast colonies obtained using a sterile loop by streaking and thereafter incubated at 37 °C for 48 hours, following which the growth and coloration of the colonies were examined (Bayona *et al.,* 2020).

Gram stain for *Candida* : The Gram stain process is obtaining a sample from a broth or plate by either pouring 1-2 loopfuls of broth onto a clean slide and let it to air dry, or by combining tap water with a colony and leaving it to air dry. Subsequent to drying, apply heat to fix the slide, ensuring its adherence to the sample. Crystal violet staining requires saturating the slide with the stain and allowing it to incubate for 1 minute prior to washing with water. Iodine staining entails the application of an iodine solution to the slide, followed by washing with water. Decolorization entails applying a

Gram stain decolorizer to the slide for 3 seconds, followed by washing with water. Contra staining entails the application of safranin stain for a duration of 30 seconds, followed by washing with water. The slide is subsequently dried and analyzed under a microscope with an oil immersion objective (Moss & Musher, 2021).

Germ tube test : The test was conducted by introducing human serum into a plane tube containing isolates of *candida albicans* and allowing it to incubate at 37°C for one hour, and observing the growth under a microscope. If the fungi are positive, germ tubes, long strands formed by fungal cells, will appear (Bhumbla & Gupta, 2021).

Antifungal susceptibility test : The sensitivity of 8 clinical isolates of *C. albicans.* to nine antifungal was tested by disk diffusion method. As follows: (Rex *et al.*, 1993) according to what was stated in the Disc diffusion method that used Nystatin, Fluconazole, Amphotericin, and Ketoconazole.

The isolates were activated in Mueller Hinton media following the placement of fungus utilizing a loop onto the medium. The antifungal disks were subsequently placed with forceps to the agar surface and incubated at 37°C for 24 to 48 hours. The outcomes were then documented by measuring the width of the inhibitory zone in millimeters surrounding each disc. It was compared to the standard rates for the width of the inhibition zone (Jeon *et al.*, 2021)

Toxicity test : The toxicity was tested with a hemolysis experiment as describe in (De Jong *et al.,* 2020). Ten ml of blood was drawn from a healthy 30-year-old guy. Combine 30 microliters of a 0.2 mg/ml solution of *Candida albicans* with 0.2 ml of blood, and mix gently for 5 seconds. Introduce 20 ml of physiological saline solution to avert hemolysis, then centrifuge using an apparatus. Centrifuge at 3000 rotations per minute for 10 minutes. The measurement obtained using a spectrophotometer at a wavelength of 540 nm is denoted by the sign AA.

Administer 30 microliters of DMSO to the saline solution and blood in the same proportion utilized for the Positive control component. The measurement was conducted using a spectrometer at a wavelength of 540 nm, denoted by the symbol AB. The hemolysis rate was established at 100% by diluting the blood with a volume 100

times bigger than itself. Distilled water was analyzed using a spectrometer at a wavelength of 540 nm, denoted as (A100H), and the following equation was used (Bai *et al*., 2024).

Hemolysis% = (AA - AB) / (A100%H-AB) *100%

Statistical Analysis

The results analysis statistically using SPSS version 22, significant differences between means were assessed using one-way analysis of variance (ANOVA), followed by the Duncan test. The level of significance was set at $p < 0.05$.

Results and Discussion

A total of 120 urine samples were randomly collected for the evaluation of urinary tract infections in patients. The specimens were cultivated on MacConkey Agar and Blood Agar, adhering to the manufacturer's guidelines for the preparation of the suitable growth media. The cultivated specimens were thereafter placed in an incubator maintained at 37º C for 24 hours. Urinary tract infections were diagnosed using a general urinary examination (GUE). A diverse array of bacteria was identified in the 34 positive samples. Among these, 8 samples demonstrated co-infection with *Candida spp*. Direct experiments were conducted to assess the hemolytic and lactose-fermenting properties of bacteria on MacConkey agar and blood agar. Following the discovery of the bacteria, diagnostic tests were performed to further examine their biochemical characteristics.

The samples underwent biochemical analysis, encompassing assays for Indole, Catalase, Coagulase, and Oxidase. The test findings revealed the presence of bacteria including *E. coli, Klebsiella spp., Staphylococcus aureus, Staphylococcus saprophyticus, and Pseudomonas*.

Bacterial diagnosis by VITEK 2 system : The VITEK 2 identification system detects medically relevant bacterial species within 15 hours utilizing fluorescent-based technology, which is sensitive and provides findings without the need for formal evaluation (Wu & Hulme, 2021).

Through this technique, we examined 34 positive samples for bacteria, 11 of which were *E. coli*, 11 *s. aureus*, 3 *pseudomonas*, 3 *s. saprophyticus* and 6 *klebsiella spp.* As for the fungal examination, 8 samples were positive, including 4 samples C. albicans, 3 *C. glabrata*, and one specimen of *C. krusei* (Table 1).

Table 1: Co- infection isolates of Bacterial and fungal species.

No.	Bacterial species	Candida species
1.	*S. Saprophyticus*	*C. albicans*
2.	*S. Aureus*	*C. albicans*
3.	*S. Aureus*	*C. glabrata*
4.	*E. coli*	*C. albicans*
5.	*S. Saprophyticus*	*C. glabrata*
6.	*E. coli*	*C. krusei*
7.	*S. Aureus*	*C. albicans*
8.	*E. coli*	*C. albicans*

Diagnosis of candida species by CHROM agar medium: Growth identification on Chrom Agar culture medium is one of the effective and rapid biochemical tests in diagnosing *Candida spp* at the color level after inoculation and incubation compared to other tests, as the test results showed colonies of different colors on Chrom Agar medium. The *C. albicans* colony showed a light green color, while the *C. lusitaniae* yeast appeared in pale pink to creamy. As for the *C. krusei* type, its colonies appeared in white to creamy, as show in (figure 3) and this agrees with both(Shaik *et al*., 2016; Edward Kumar *et al*., 2007; Nadeem, 2007 and David *et al*.,2014).

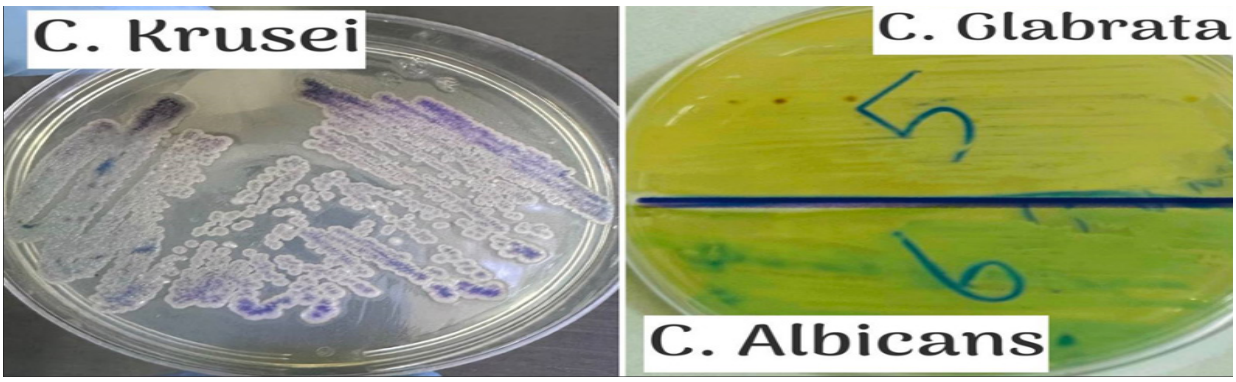

Figure (1) candida species differentiation by Hicrom agar

Antifungals Susceptibility : Evaluating the susceptibility of *Candida spp*. to various antifungal agents. (Table 2) presents the antifungal sensitivity of the examined *Candida* isolates, with a total of 8 isolates analyzed across the three species. Among the isolates, it was determined that *Candida* ex-

hibited resistance to Nystatin, with 25.0% being susceptible, 37.5% moderately sensitive, and 37.5% resistant. Among the *Candida spp.* samples, 50% were susceptible and 50% were resistant to Fluconazole, whereas 50% were susceptible to Amphotericin, with 37.5% exhibiting intermediate sensitivity and 12.5% being resistant. Of *Candida spp*., 37.5% are vulnerable, 12.5% are moderately sensitive, and 50% are resistant to Ketoconazole. One of these samples, which was diagnosed as *candida albicans*, was resistant to all antifungal on which other experiments were conducted.

Table 2: Anti-fungal Susceptibility for *candida* spp.

sample	KT	AP	FLC	NS
1	++ S	+++S	+++S	+S
2	++S	+S	+++S	+++S
3	R	++S	+++S	++S
4	+S	+S	R	+S
5	R	R	R	R
6	R	++S	R	+S
7	R	++S	R	R
8	++S	+S	++S	R

S+: Positive Sensitivity, S++: High Sensitivity,
S+++: Superior Sensitivity, R: Resist.

Gram staining of *candida* spp. : They show Gram-positive characteristics, staining purple, and consist of oval or round yeast cells. Extended formations that resemble hyphae but are broader and have constrictions at the septa (Sharma *et al.*, 2022).

Germ tube test for *C. albicans* isolated : In the cases group, all 8 positive Candida cultures exhibited a germ tube production test result of 100%, as assessed macroscopically. The Candida albicans isolates exhibited filamentous structures during their development in human blood serum. These results are consistent with (Abdullah *et al.*, 2022).

Toxicity test of *Candida albicans* : In this experiments, the toxicity obtained indicate the toxicity of exposure to *C. albicans* (Bouma & Schakel, 2002). A larger concentration of the chemical correlates with an increased risk of blood deterioration. At a concentration of 20 µg/ml, the degradation rate was 2.21%, whereas at a concentration of 10 µg/ml, it was 1.97%. Tox-

icity escalates with heightened exposure to *C. albicans*. The hemolysis rate was ascertained to be 100% by diluting the blood with a volume 100 times larger; this finding is consistent with (Mogavero *et al*., 2022).

Conclusions

Bacteria, including *Escherichia coli*, *Staphylococcus aureus,* and *Staphylococcus saprophyticus*, and *Candida* species including *C. krusei, C. lusitaniae,* and *C. albicans,* are the causative agents of UTI that can be isolated from randomly selected urine samples. *C. albicans* displays elevated toxicity (hemolysis) in human blood, with significant antifungal resistance to ketoconazole, amphotericin, fluconazole, and nystatin, with resistance levels escalating in correlation with increased doses of *C. albicans*.

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