Original Article

A Phenotypic Approach to Genitourinary Candidiasis: Correlating CHROMagar Color Profiles and Germ Tube Status with Specific Risk Factors for Targeted Intervention

Zahraa Majid Jillab [*], Ali Fadel Razouki

Department of Medical Biotechnology, College of Biotechnology, Al-Qasim Green University, 51013, Babylon, Iraq.
[*] Corresponding author: zahraajillab@gmail.com

Submission: December 11, 2025 Accepted: March 13, 2026 Published: March 31, 2026

Abstract

Background: Genitourinary candidiasis, including vulvovaginal candidiasis (VVC) in women and various forms of the fungus *Candida* found in urine (candiduria) in men and children presents a serious, increasing global economic burden. **Objectives:** To identify and characterize the different Candida species isolated from vaginal swabs and urine samples using phenotypic methods. **Materials and Methods:** 27 samples were found to be positive for *Candida* species from the total 120 culured. Of the positive samples, 26 were vaginal swabs and one was a urine sample. Each of the samples was then cultured on CHROMagar™ *Candida* and incubated for 48 hours. Species were identified by characteristic colony coloration and morphology, supported by germ tube test. In addition, exploratory risk factor analysis was conducted to assess the association between identified species and specific clinical and demographic factors. **Results:** We found 5 species of *Candida* including *C. albicans* (59.3%, n=16), *C. tropicalis* (18.5%, n=5), *C. krusei* (11.1%, n=3), *C. glabrata* (7,4%, n=2) and *C. dubliniensis* (3,7%, n=1). *C. tropicalis* isolates varied in color presentation including metallic-blue, whitish-copper, and white-metallic blue phenotypes. *C. dubliniensis* showed characteristic dark green color that was easily separated from the light green *C. albicans* species. Conclusion: *C. albicans* was the most commonly identified species (59.3%), followed by *C. tropicalis* (18.5%), *C. krusei* (11.1%), *C. glabrata* (7.4%) and *C. dubliniensis* (3,7%). Species identification was 100% concordant on germ tube and CHROMagar™ Candida. The *C. albicans* were most common in regards to UTIs, pregnancy and COVID-19 history, while the *C. tropicalis* had a strong predilection for UTIs (80%). A combination of these phenotypic approaches accurately identified the species in a rapid, inexpensive manner that is necessary to guide appropriate antifungal therapy.

Keyword: *Candida* spp, Germ tube, CHROMagar, Risk factors, Vulvovaginitis.

Introduction

Genitourinary candidiasis, ranging from vulvovaginal candidiasis (VVC) in women to possibly asymptomatic infections (candiduria) in men and children, is an important and increasingly common global health problem [1, 2]. These infections are caused by opportunistic yeasts that belong to the *Candida* genus, which are commensal organisms in the mucocutaneous membranes of most normal individuals. However, in the presence of certain predisposing factors these yeasts may proliferate and become pathogenic, resulting in symptomatic disease [3]. Although *Candida* albicans was previously the

most commonly isolated species, there is an important epidemiological shift to a more prevalent frequency of NAC (non-albicans Candida) species worldwide, including *C. glabrata*, *C. tropicalis* and *C. krusei* [4]. This trend is clinically relevant because NAC species often demonstrate distinct antifungal susceptibility profiles, including intrinsic or acquired resistance to azole drugs commonly used for primary treatment, which complicate therapeutic approaches [5]. This transition from commensalism to pathogenesis is strongly linked to several host-specific risk elements. In women, pregnancy, the use of hormonal contraception and antibiotic therapy are the most important triggers for VVC [6]. The common risk factors are urinary catheters, underlying urogenital abnormalities, diabetes mellitus and broad-spectrum antibiotic use [7] in adult male population and pediatric population. A detailed understanding of these relationships is essential for forecasting infection dynamics, but they may vary markedly over geography [8]. Thus, intervention in an acute setting requires identification of the causative *Candida* species both accurately and expediently for successful clinical management. Simple methods available today, such as the germ tube test, permit presumptive identification of *C. albicans* [9] but do not differentiate among species within NAC. This limitation emphasizes the necessity for more suitable tools such as CHROMagar™ Candida, a differential medium that enables the presumptive identification of multiple species depending on their characteristic color on the plate [10]. Denominated that despite some prior studies conducted in Iraq which have described the epidemiology of *Candida* spp. in certain clinical settings [11], a significant knowledge gap remains. In deepening therapy of *candida* infections, the previous studies on identification of *Candida* species locally have not been correlated systematically according to particular bit status in host. However, most studies reviewed either identification or prevalence alone and did not correlate specific species (*C. glabrata* vs. *C. albicans*) with a specific predisposing condition (diabetes vs. antibiotic usage) in the Iraqi community [12]. Addressing this gap is critical for developing targeted, evidence-based clinical practice guidelines that can be applicable to our home health setting. This study thus attempts to fill in the above gap. Specifically, we aim to use a phenotypic approach as follows: To identify the types of *Candida* species isolated from vaginal swabs and urine samples, assess the utility of CHROMagar and germ tube test in their identification and finally correlate between these two observations with risk factors in patients. Linking the noticeable characteristics of *Candida* isolates to specific predisposing conditions would help develop targets to a more proactive therapeutic approach in Iraq.

Materials and Methods

Sample Collection

One hundred twenty clinical specimens were obtained from children, women and men attending Al-Shomali Hospital and licensed private laboratories in Al-Shomali District between 16 September 2025 to 11 October 2025.. Urine samples of male and pediatric patients were collected in sterile urine containers and vaginal swabs were taken aseptically using sterile cotton swabs from female patients. All samples were transported to the Microbiology Laboratory and processed among 2 hours post collection.

Sabouraud dextrose agar culture and microscopy

We collected and cultured 120 samples on Sabouraud Dextrose Agar (SDA) at ADivision of

tulipm diagnostics (p) Ltd,spain together with 0.05g/L chloramphenicol to inhibit bacterial contamination Plates were incubated at 35° C for 24 to 48 hours. If colonies were observed for a yeast-like term, they would be examined microscopically by wet mount preparation after incubation to confirm yeast cells. The pure isolates were then resuspended into Brain Heart Infusion (BHI) broth containing 20% (v/v) sterile glycerol and stored at -20°C until needed.

Germ tube test

This test was conducted to distinguish *C. albicans* from the other species of Candida. Inoculum from a 24-hour-old pure yeast colony was suspended in 0.5 mL of pooled human serum in a test tube for the assay. Following mixing the suspension was incubated for 2 to 3 hours in a 37C water bath. After incubation, a drop of the suspension was placed on a clean glass slide, and then covered with coverslip and examined microscopically by oil immersion (100x) objective [12].

CHROMagar™ *Candida*

To identify *Candida* isolates received via the laboratory, particularly non-albicans species from purified yeast isolates were subcultured onto CHROMagar™ *Candida* medium (CHROMagar, Paris, France) for further identification. To obtain individual colonies, a single well-isolated colony from the Sabouraud Dextrose Agar plate was streaked onto the surface of CHROMagar plate. Plates were incubated aerobically at 35–37°C and inspected at 24 h and 48 h. The presumptive species identification was determined based on colony color and morphology according to the manufacturer-supplied interpretation guide.

Ethical Approval

This study was ethically approved by Ministry of health, Babylon Health Directorate, Training and Human Development Center with official permission letter No 1339 (2025/9/15). All procedures were performed in compliance with approved ethical standards and patient data were treated anonymously.

Results

Confirmation of *Candida* isolates

Total 120 clinical samples were processed for isolation of *Candida* species. Of these, 27 samples (22.5%) demonstrated fungal growth and presence of *Candida* (figure 1). The other 93 samples (77.5%) were found to be negative for the fungal growth.

Figure 1: Growth of *Candida* isolates on SDA, showing creamy, smooth colonies.

Patient demographics and general risk factors

Candida isolates were recovered from samples from all 27 female patients, but none was cultured from men and children. Therefore, demographic and risk factor analysis were conducted on this cohort of 27 women. The age distribution of these patients is shown in Table 1. The ages of the patients were in the range of 17–60 years old. For the 26 - 35 and 36 - 45 years age groups, close to equal but high prevalence of

infection (each accounting for 29.6% of all cases) was also seen in any one of the eight isolates. The group aged ≤17 years was next (22.2%; 6 isolates; Table), followed by the youngest age group (age 17–25 years; 6 isolates; 22.2%). The oldest age group (46–60) and the last one with 5 isolates represented only 18.5% of all cases, being also the smallest proportion. Figure 2 demonstrates the distribution of identified risk factors in our patient population. Urinary Tract Infection (UTI) was the most prevalent risk factor, with a reported incidence of 12 cases (44.4%). Antibiotics were the second most used factor, in 5 patients (18.5%), followed closely by the use of contraception (n=4, 14.8%), a history of COVID-19 infection (n = 4, 14.8%) and diabetes (n = 4; 14.8%). Other predisposing conditions were pregnancy (n=3, 11.1%), catheterization (n=2, 7.4%), and presence of renal sand (n=1, 3.7%).

Table 1: Patients demographics by age.

Age group(years)	Vaginal swab	%
17 – 25	6	22.2%
26 – 35	8	29.6%
36 – 45	8	29.6%
46 – 60	5	18.5%
Total	27	100%

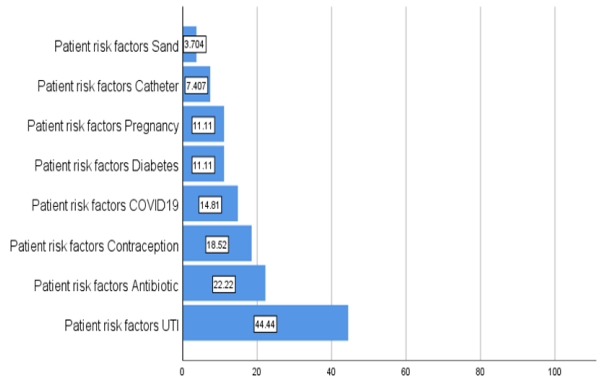

Figure 2: Percentage distribution of risk factors.

Germ tube test result

Among the 27 total *Candida* isolates that were subjected to germ tube test, 16 isolates (59.3%) turned out to be positive. Under the microscope, these positive isolates were seen to form short non-septate filamentous hyphae (germ tubes) directly from the yeast cell wall (Figure 2). This successful result gives a presumptive identification of these isolates as *C. albicans*. The other 11 isolates (40.7%) were negative and showed no germ tube formation even after the incubation period.

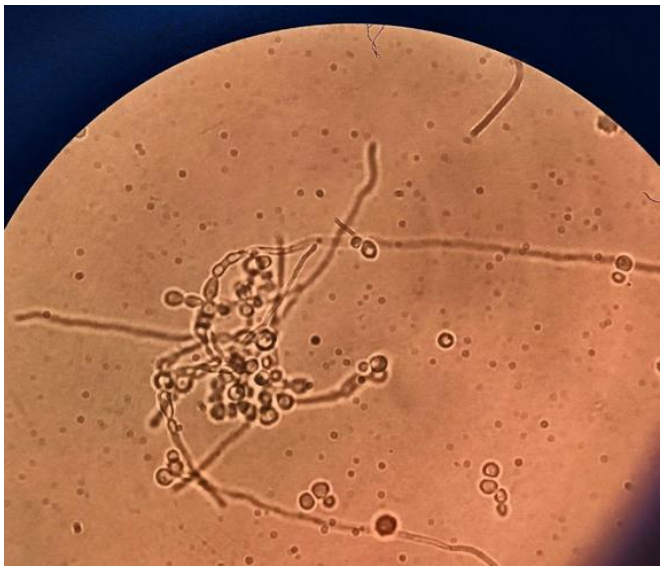

Figure 2: Microscopic view of positive germ tube test for *C. albicans*

Identification of *Candida* Species by Colony Morphology on CHROMagar™

Presumptive identification 27 unique yeast isolates were identified from their distinct colony types after being incubated for 48 hours on CHROMagar™ *Candida* medium (figure 4). These species-level identifications were then compared with the germ tube test, which had been performed prior to use of the chromogenic medium, in order to improve accuracy of identification among *Candida* species. The species most frequently recovered was *C. albicans*, represented by 16 isolates. These

isolates produced green colonies that were consistent in color from light-green to deep emerald-green and, importantly, all sixteen of them tested positive on the germ tube test. This perfect correlation of the green phenotype and positive germ tube result gives us a unique identification of these isolates as *C. albicans*. Interestingly, a 17th isolate was further identified which generated a green colony noted as dark-green. However, the germ tube test was negative for this isolate. This particular combination of a green colony on CHROMagar and a negative germ tube result identified the isolate presumptively as *C. dubliniensis* (1 isolate), thereby differentiating it from *C. albicans*. The remaining non-albicans species (all of which were germ tube-negative) were identified as shown in the table below: Isolates of *C. tropicalis* were found in 5 isolates with large, smooth colonies and color variations from cream to a characteristic white-metallic blue. 3 isolates of *C. krusei* formed large, flat colonies, with a rougher appearance that were pink in color. 2 isolates were identified as *C. glabrata*, which produced smooth- and creamy-white opaque colonies.

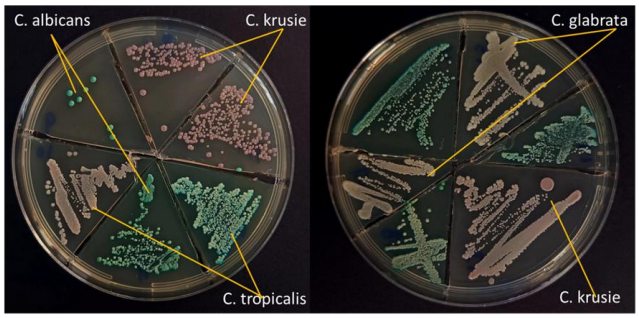

Figure 3: Colony morphology of *Candida*. spp on CHROMagar *Candida* after 48 hours of incubation.

Correlation of *Candida* species with risk factors

Analysis was performed to assess the relation between the identified *Candida* species and specific risk factors of those 27 patients. Table 2 gives a detailed breakdown of these correlations. The most common species, *C. albicans* with 16 isolates showed a wide distribution associated with the diverse risk factor. Urinary Tract Infection (UTI) was the most common association seen, present in 6 out of 16 *C. albicans* cases. Other important factors were pregnancy (3 isolates) and previous COVID-19 infection (3 isolates). It was noted with diabetes in association with antibiotic use (2 isolates) and catheterization (2 isolates). *C. tropicalis* (n=5) predominantly affected UTI patients. This factor was found in 4 of the 5 cases (80%) and one patient presented with renal sand. The other isolate was linked to contraception use. The three isolates identified as *C. krusei* were each associated with a different primary risk factor, including diabetes mellitus, the use of antibiotics, and the combination of contraception as well as UTI occurring in the same patient; therefore, no single risk factor predominated for this species in our cohort. *C. glabrata* was isolated from 2 patients, one with a history of contraception and one with a history of COVID-19 infection. Finally, a single isolate of *C. dubliniensis* was recovered from urinary tract infection (UTI).

Table 2: *Candida* species distribution based on risk factors.

Candida spp.	UTI	Pregnancy	COVID-19	diabetes	Antibiotic use	Catheterization	contraception
C. albicans	6	3	3	2	5	2	2
C. tropicalis	4	0	0	0	0	0	1
C. krusei	1	0	0	1	1	0	1
C. glabrata	0	0	1	0	0	0	1
C. dubliniensis	1	0	0	0	0	0	0

Discussion

C. albicans was the most frequent species (59.3%) observed, which is in agreement with more recent studies demonstrating that *C. albicans* continues to be the leading isolate in vulvovaginal candidiasis accounting for 75-95% of cases according to Rafat et al. [13]. On the other hand, our study demonstrated a more prevalent of *C. tropicalis* (18.5%), which might be in accordance with the rising incidence of non-albicans *Candida* (NAC) species [14]. The rising prevalence of NAC species within our study is consistent with global increases and bears important consequences for antifungal therapy, as these species frequently demonstrate distinct susceptibility profiles compared to *C. albicans* [15]. The *C. tropicalis*-dominant profile of this Iraqi population implies region-specific patterns of epidemiology, especially regarding the commonly noted increased prevalence of *C. tropicalis* in tropical and subtropical areas. CHROMagar™ *Candida* uniquely identified all five species by distinctive colors: *C. albicans* (light-green), *C. tropicalis* (metallic-blue), *C. krusei* (pink), *C. glabrata* (creamy-white) and *C. dubliniensis* (dark-green) [16,17]. The dark green color of *C. dubliniensis* set it apart from the lighter green of *C. albicans* [17]. The *C. tropicalis* isolates studied here showed variation in color presentations, normal metallic-blue along with atypical colorations of whitish-copper and white-metallic blue. Such color variation has longtime been documented Bloch et al. [18] demonstrated that depending on the strains used and growth conditions, *C. tropicalis* can show colors between blue-gray and blue-greenish or metallic blue type. This feature is likely associated with *C. tropicalis* physiology and its ability to switch from yeast to a filamentous form [19]. Corrêa-Moreira et al. [20] also mentioned that C.tropicalis can present blue-gray, bluish-gray to blue-green or metallic blue coloration potentially due to phenotypic plasticity and differences of enzymatic substrates utilization in different isolates. However, when taking into account average isolate coloration in CHROMagar appears to be blue-tinged it does not interfere with identification at the species level. The germ tube was a complementary confirmatory method that offered additional evidence for the species determined by CHROMagar™ Candida. Both of the isolates among the 16 presumptive *C. albicans* organisms isolates that had been tested positive using germ tube test confirmed its identity as *C. albicans* (Based on its green phenotype, they were presumed to be *C. albicans*). Agreement between species identification on chromogenic mediums and germ tube test, further adds confidence to the reliability of species identity. (C) The germ tube test is used to differentiate *C. dubliniensis* from *C. albicans*. One isolate which was germ tube negative had confirmed *C. dubliniensis*. While *C. dubliniensis* are biochemically capable of germ tube production, the serum type and culture conditions utilized highly influence its capability to do so. The earlier studies indicated that *C. dubliniensis* produced germ tubes reliably in rabbit serum or fetal bovine serum compared to fewer productions by challenging of the organism with human serum under standard conditions [21]. The concentration-dependent influence of serum on germ tube formation, coupled with the unique dark-green pigmentation pattern generated using CHROMagar are useful phenotypic features for distinguishing *C. dubliniensis* from *C. albicans*. The 3 germ tube-negative isolates identifiable as *C. glabrata*, *C. krusei* and *C. tropicalis* species were accustomed (not performing) since these are intrinsically germ tube negative species. The individual species association with the patient risk factors provides an important biologic and clinical perspective. The most frequent corelated

factors with *C. albicans* were urinary tract infection (UTI) (37.5%), pregnancy (18.8%) and history of COVID-19 (18.8%). It was attributed that *C. albicans* has the highest capacity to produce virulence determinants such as proteinases and phospholipases important for tissue and biofilm formation, which probably led *C. albicans* outnumbered the rest of UTI cases. The association with pregnancy is consistent with well-known data showing that hormonal changes associated to pregnancy, in particular elevation of estrogen levels create an environment favorable for growth *Candida* due to better adhesion to cells from vaginal epithelium and increased temperature and formation hyphae [22]. The associations of *C. albicans* and *C. glabrata* with a history of COVID-19 may also reflect the immunosuppressive effects mediated by SARS-CoV-2 infection or ongoing use of immunosuppressive medications in convalescence that impair T-cell immunity and predispose patients to certain opportunistic fungal infections [23]. *C. tropicalis* demonstrated an exceptionally high (80%) preference for UTI, suggesting a true tropism for urinary epithelium that may be associated with its distinctive adhesion mechanisms and enzymatic profiles. *C. krusei* has heterogeneous associations consistent with its opportunistic phenotype and intrinsic resistance to azole antifungals, which enables it to thrive in a number of predisposing risk factors ranging from diabetes [24] to antibiotics. Notably, the detection of *C. dubliniensis* from the urogenital tract of a patient with UTI is striking since this species has traditionally been linked to disease in immunocompromised hosts presenting primarily with oral candidiasis [17]. This finding suggests that this species has a broader distribution in the urogenital tract than historically recognized. This study demonstrates the successful use of CHROMagar™ *Candida* for rapid species identification in resource limited settings. The association of species with risk factors also provides epidemiologic insight. However, (i) this work has a small sample size of n=27 and there is no generalization; and (ii) it is restricted only to vaginal samples from females, limiting its applicability. Additional characterization of these yeasts, including antifungal susceptibility testing and molecular confirmation, would confirm our results.

Conclusion

We have shown that testing with CHROMagar™ *Candida* followed by germ tube testing represents a practical, low-cost phenotypic strategy for rapid species identification in the clinical laboratory. *C. albicans* was the most prevalent species (59.3%), followed by the emerging non-albicans species *C. tropicalis* (18.5%), which parallels a worldwide epidemiological trend. All 16 *C. albicans* isolates were confirmed, and germ tube testing' showed that colours at the others were indeed due to *C. dubliniensis* (verified by its negative germ tube result). The high specificity of *C. tropicalis* to urinary tract infections (80%) and *C. albicans* to pregnancy and history of COVID-19 suggests the species choice for pathogenic mechanism and clinical relevance. The increasing incidence of severe bloodstream infections with *C. krusei*, in the context of widening azole resistance, emphasizes the need for accurate species identification to ensure appropriate antifungal therapy is given to patients infected with organisms showing intrinsic (for example *C. krusei*) and emerging species whose prevalence is on the increase. More studies involving more subjects, molecular confirmation and antifungal susceptibility testing are needed to support our findings and settle the grounds for local therapeutic guidelines.

References

[1] Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Microbiol Rev. 2010;23:253-73.

[2] Willems HM, Ahmed SS, Liu J, Xu Z, Peters BM. Vulvovaginal candidiasis: a current understanding and burning questions. J Fungi. 2020;6:27.

[3] Mahalingam SS, Jayaraman S, Pandiyan P. Fungal colonization and infections—interactions with other human diseases. Pathogens. 2022;11:212.

[4] Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida infection: an emerging threat. Interdiscip Perspect Infect Dis. 2014;2014:615958.

[5] Li X, Sholeh M, Moghadam MA, Ostadrahimi M, Hosseini SS. Global prevalence and trends of fluconazole resistance in non-albicans Candida species: a systematic review and meta-analysis. BMC Infect Dis. 2026.

[6] Disha T, Haque F. Prevalence and risk factors of vulvovaginal candidosis during pregnancy: a review. Infect Dis Obstet Gynecol. 2022;2022:6195712.

[7] Sobel JD, Fisher JF, Kauffman CA, Newman CA. Candida urinary tract infections epidemiology. Clin Infect Dis. 2011;52: S433-6.

[8] Arendrup MC, Patterson TF. Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. J Infect Dis. 2017;216:S445-51.

[9] Arafa SH, Elbanna K, Osman GE, Abulreesh HH. Candida diagnostic techniques: A review. J Umm Al-Qura Univ Appl Sci. 2023;9:360-77.

[10] Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med. 2010;5:2144.

[11] Khalaf HY, Noomi BS, Aiub MM, Mohammed AY, Aifa S. Incidence and characterization of Candida species belonging to Iraqi hospitalized patients. Access Microbiol. 2025:001050-v1.

[12] Leber AL. Clinical microbiology procedures handbook. John Wiley & Sons; 2020.

[13] Rafat Z, Alipour N, Esmaeili E, Mirhasani F, Yaseri M, Haghighat F, et al. Vulvovaginal candidiasis: a global perspective through systematic review and metaanalysis. Microb Pathog. 2026: 108284.

[14] Nadeem SF, Hussain MS, Rasheed F, Ahmad I, Ahmad SS. Unveiling the spectrum of nosocomial candiduria: a cross-sectional analysis in a tertiary care hospital of Punjab, Pakistan. Pak J Med Dent. 2025;14:190-7.

[15] Nguyen MD, Ren P. Trends in antifungal resistance among Candida species: an eight-year retrospective study in the Galveston–Houston Gulf Coast region. J Fungi. 2025; 11:232.

[16] Bentz ML, Le N, Min B, Nunnally NS, Sullivan V, Tran M, et al. Evaluation of CHROMagar Candida Plus for the detection of C. auris with a panel of 206 fungal isolates and 83 colonization screening skin-swabs. Microbiol Spectr. 2024;12:e03564-23.

[17] Gómez-Gaviria M, Baruch-Martínez DA, Mora-Montes HM. Exploring the biology, virulence, and general aspects of Candida dubliniensis. Infect Drug Resist. 2024:5755-73.

[18] Bloch A, Bogiel T, Prażyńska M, Gospodarek-Komkowska E. Usefulness of chromogenic media in the identification of Candida spp. yeasts compared to mass spectrometry. Methods Protoc. 2025;8:98.

[19] Kadosh D, Mundodi V. A re-evaluation of the relationship between morphology and pathogenicity in Candida species. J Fungi. 2020;6:13.

[20] Corrêa-Moreira D, da Costa GL, de Lima Neto RG, Pinto T, Salomão B, Fumian TM, et al. Screening of Candida spp. in wastewater in Brazil during COVID-19 pandemic: workflow for monitoring fungal pathogens. BMC Biotechnol. 2024;24:43.

[21] Kim TH, Park BR, Kim HR, Lee MK. Candida dubliniensis screening using the germ tube test in clinical yeast isolates and prevalence of C. dubliniensis in Korea. J Clin Lab Anal. 2010;24:145-8.

[22] Bataineh MT, Cacciatore S, Semreen MH, Dash NR, Soares NC, Zhu X, et al. Exploring the effect of estrogen on Candida albicans hyphal cell wall glycans and ergosterol synthesis. Front Cell Infect Microbiol. 2022;12:977157.

[23] Akhtar N, Wani AK, Tripathi SK, Prakash A, Mannan MA. The role of SARS-CoV-2 immunosuppression and the therapy used to manage COVID-19 disease in the emergence of opportunistic fungal infections: A review. Curr Res Biotechnol. 2022;4:337-49.

[24] Cao X, Liu J, Sun T, Duan F, Song N, Liu Z, et al. Species distribution, drug resistance, and risk determinants of Candida UTIs: a five-year retrospective study in Beijing. Infect Drug Resist. 2025:5917-26.