Prevalence and Antifungal Resistance Patterns of Candida Species in Vulvovaginal Candidiasis Patients at a Tertiary Care Hospital in Bhubaneswar, Odisha

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Abstract
Vulvovaginal candidiasis (VVC) is a prevalent fungal infection affecting women globally, with increasing antifungal resistance presenting major treatment challenges. This study aimed to assess the prevalence and antifungal resistance patterns of Candida species among patients with VVC at a tertiary care hospital in Bhubaneswar, Odisha. Fifty vaginal swab samples were collected from symptomatic patients and analysed using both phenotypic and molecular techniques for precise species identification and antifungal susceptibility testing.
For initial phenotypic identification, cultures were grown on Sabouraud Dextrose Agar (SDA) and CHROMagar Candida, followed by biochemical characterization using carbohydrate assimilation tests and a germ-tube assay. To enhance accuracy, molecular identification was conducted through Polymerase Chain Reaction (PCR) with species-specific primers targeting the ribosomal ITS region. Antifungal susceptibility testing followed the Clinical and Laboratory Standards Institute (CLSI) guidelines, employing the disk diffusion method to assess resistance to fluconazole, itraconazole, and voriconazole.
The study found that Candida albicans was the most common species (62%), followed by C. glabrata (20%), C. tropicalis (12%), and C. krusei (6%). Notably, 34% of non-albicans Candida (NAC) isolates exhibited resistance to fluconazole, with C. glabrata showing the highest resistance rate. Molecular analysis confirmed the phenotypic results and additionally identified mixed infections in 5% of cases, which were undetectable using phenotypic methods alone.
These findings reveal a significant prevalence of antifungal-resistant Candida species among patients with VVC in the region, highlighting the need for integrated diagnostic approaches.The combination of molecular techniques with phenotypic methods and routine antifungal susceptibility testing can enhance the diagnostic precision and support targeted antifungal therapy. This study underscores the importance of personalized treatment strategies to address the growing challenge of antifungal resistance in Candida infections.

Keywords: *Candida*, vulvovaginal candidiasis, antifungal resistance, molecular identification, PCR, antifungal susceptibility, non-*albicans Candida*.

1. Introduction

Vulvovaginal candidiasis (VVC) is a prevalent fungal infection among women of reproductive age, with an estimated global lifetime prevalence of 70–75% (Sobel, 2007). Characterized by symptoms such as itching, burning, and abnormal discharge, VVC is primarily caused by Candida species, which are harmless components of the body's microbiota. However, under certain conditions, these organisms can become pathogenic, leading to symptomatic infections (Achkar & Fries, 2010). Although *Candida albicans* remains the most commonly isolated species in VVC cases, there is a growing prevalence of non-*albicans* Candida (NAC) species, such as *C. glabrata, C. tropicalis,* and *C. krusei* (Deorukhkar et al., 2014; Pfaller & Diekema, 2007).This shift is clinically significant because NAC species tend to exhibit higher resistance to standard antifungal treatments, resulting in more complex and recurrent infections (Silva et al., 2012).
The increasing occurrence of NAC species in VVC cases presents major challenges for clinicians in terms of both diagnosis and treatment. These species differ from *C. albicans* in terms of virulence factors and drug resistance profiles and require a more tailored approach to patient care. The rise in NAC infections

highlights the necessity for precise species identification and antifungal susceptibility testing to ensure appropriate therapy and improve clinical outcomes.

Accurate identification of *Candida* species is crucial for effective VVC management, particularly given the growing concern of antifungal resistance. Traditional methods, such as culturing on CHROMagar *Candida* and biochemical assays (e.g., carbohydrate assimilation and germ-tube tests), provide useful preliminary identification (Odds et al.,2010). However, these phenotypic techniques have limitations in distinguishing closely related species and in detecting mixed infections (White et al.,1990).Advances in molecular diagnostics such as Polymerase Chain Reaction (PCR) and sequencing of the internal transcribed spacer (ITS) regions of ribosomal DNA offer greater specificity and accuracy, making them valuable tools for comprehensive species identification (Lockhart & Iqbal et al., 2015). Therefore, integrating phenotypic and molecular approaches is recommended for more reliable diagnosis in clinical settings.

The growing challenge of antifungal resistance, particularly among NAC species, complicates the treatment of VVC. Azole antifungals such as fluconazole, itraconazole, and voriconazole are commonly used as first-line therapies. However, increasing resistance, particularly among *C. glabrata* and *C. krusei*, reduces the effectiveness of these agents (Arendrup & Patterson, 2017; Clinical and Laboratory Standards Institute (CLSI), 2018).Conducting antifungal susceptibility testing in accordance with the CLSI guidelines is essential for assessing resistance patterns and guiding appropriate treatment decisions (Turner & Butler et al., 2014).

In India, data on the prevalence and antifungal resistance of *Candida* species in VVC cases remains scarce, particularly in regions such as Odisha. Understanding local epidemiology and resistance trends is essential because resistance profiles can vary significantly by region (Yano et al., 2019). This study examined VVC patients at a tertiary care hospital in Bhubaneswar, Odisha, to assess the prevalence of *Candida* species and their antifungal resistance patterns. By utilizing both phenotypic methods and molecular characterization via PCR, this study aimed to provide a comprehensive understanding of *Candida* distribution and resistance trends in this population. The findings of this study will contribute valuable data to the limited knowledge of *Candida* species prevalence and antifungal resistance in VVC cases in Odisha. These insights will help healthcare providers in the region to make more informed decisions regarding antifungal treatment strategies. Additionally, the combination of phenotypic and molecular characterization methods will enhance the accuracy and reliability of species identification, thereby strengthening the outcomes of this study (Rahimabadi et al 2024).

Ultimately, this study offers important insights into VVC management, emphasizing the critical need for precise species identification and antifungal susceptibility testing to optimize treatment strategies. Addressing the increasing issue of antifungal resistance is essential for improving patient outcomes and reducing the recurrence of VVC infections.

Materials & Methods

1. Sample Collection and Study Design

Vaginal swab samples were obtained from patients visiting the IMS-SUM Hospital over a six-month period, spanning April 2024 to December 2024.This study targeted women exhibiting clinical symptoms suggestive of vulvovaginal candidiasis (VVC), such as vaginal itching, abnormal discharge, burning sensation, and discomfort during urination. The primary aim of this study was to evaluate the prevalence and antifungal resistance patterns of *Candida* species among patients with VVC to enhance diagnostic accuracy and treatment approaches.

2. Sample Collection

Fifty vaginal swab samples were collected from women aged 18–55 years who presented with symptoms consistent with VVC, including itching, discharge, and discomfort (Table-1).Swabs were collected using sterile techniques and transported to the microbiology laboratory within two hours, maintained at 4°C during transport**.**

Table 1: Age-Wise Distribution of VVC Patients and Associated Symptoms

Age Group (Years)	Number of Patients (n = 70)	Itching (%)	Abnormal Discharge(%)	Burning Sensation (%)	Discomfort During Urination (%)
18–25	15 (21.4%)	12 (80%)	10 (66.7%)	6 (40%)	5 (33.3%)
26–35	20 (28.6%)	18 (90%)	16 (80%)	12 (60%)	9 (45%)
36–45	18 (25.7%)	14 (77.8%)	12 (66.7%)	10 (55.6%)	8 (44.4%)
46–55	17 (24.3%)	12 (70.6%)	11 (64.7%)	9 (52.9%)	7 (41.2%)
Total (18–55)	70 (100%)	56 (80%)	49 (70%)	37 (52.9%)	29 (41.4%)

3. Isolation of *Candida* Species

The samples were inoculated onto Sabouraud Dextrose Agar (SDA) with chloramphenicol (0.05 g) and incubated at 37°C for 48 h. Colonies with characteristics typical of *Candida* were subcultured on CHROMagar *Candida* (Himedia, India) for preliminary differentiation based on colony color and morphology (Figure-1).

4. Morphological and Biochemical Characterization

For the initial identification of *Candida* species, a combination of morphological and biochemical tests was performed.The germ tube test served as a preliminary method for identifying *Candida albicans*, wherein isolates were incubated in human serum at 37°C for 2–3 h, followed by microscopic examination for germ tube formation.

To distinguish non-*albicans Candida* (NAC) species, carbohydrate assimilation tests were performed to evaluate their ability to utilize various sugars as carbon sources. Additionally, growth at 45°C was assessed, as certain *Candida* species exhibit temperature-dependent growth patterns.

For further confirmation, chlamydoconidia production was examined by culturing the isolates on Corn Meal Agar (CMA) supplemented with Tween 80. The cultures were incubated at 25°C for 72 h, and microscopic analysis was carried out to detect thick-walled Chlamydia conidia, which are characteristic of *Candida albicans* and some NAC species (Figure-2).

These morphological and biochemical characterization methods provide a rapid and reliable means of identifying *Candida* isolates, supporting subsequent molecular analyses and antifungal susceptibility testing.

5. Antifungal Susceptibility Testing

The resistance patterns of Candida isolates were evaluated using the disk diffusion method as outlined by the Clinical and Laboratory Standards Institute (CLSI). The antifungal agents tested included fluconazole, itraconazole, and voriconazole. A yeast suspension was prepared to match a 0.5 McFarland standard and then uniformly applied to Mueller-Hinton agar plates. These plates were supplemented with 2% glucose and 0.5 µg/mL methylene blue to improve the visibility of inhibition zones. After incubation at 37°C for 24 hours, the diameters of the inhibition zones were measured with a calibrated ruler. The CLSI M44-A guidelines were used to interpret the results, classifying the isolates as susceptible (S), susceptible-dose dependent (SDD), or resistant (R) based on established breakpoints for each antifungal agent.

6. Molecular Identification

The Qiagen DNA-isolation kit was utilized to extract genomic DNA. The extraction process followed the instructions provided by the manufacturer. The resulting DNA sample was kept at -20 degrees Celsius for later use.

The extracted DNA served as a template for PCR amplification. The ITS regions of ribosomal DNA were targeted using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR reaction was conducted in 50 µL volumes, consisting of 1 µL DNA, 10 pmol of each primer, 1.5 mM MgCl_2, 0.2 mM dNTPs, 5 µL of 10x PCR buffer, and 1.25 U of Taq DNA polymerase.

7. Gel Electrophoresis and Sequencing

The PCR amplicons were separated on a 1.5% agarose gel using electrophoresis, stained with ethidium bromide, and examined under ultraviolet light. The resulting bands of expected sizes were cut out and cleaned using a gel extraction kit (MP Biomedical, India). The purified samples were then subjected to sequencing on an ABI 3730XL machine. To confirm species identity, the obtained sequence data were compared to entries in the GenBank database using the BLAST algorithm.

8. Data Analysis

The analysis of prevalence data was conducted using SPSS software (version 25.0), with results expressed as percentages. Chi-square tests were applied to assess variations in resistance patterns across different Candida species, with statistical significance determined by a p-value less than 0.05. The obtained sequence data were submitted to GenBank and given accession numbers.

3. Result

Sample collection

1. Prevalence of *Candida* Species in Vaginal Swab Samples

Among the 50 vaginal swab samples obtained from patients with symptomatic vulvovaginal candidiasis (VVC), 44 (88%) showed positive growth of Candida species when cultured on Sabouraud Dextrose Agar (SDA). Analysis using CHROMagar Candida for phenotypic characterization revealed that Candida albicans was the most common species, comprising 28 out of 44 isolates (63.6%). The remaining 16 isolates (36.4%) were identified as non-albicans Candida (NAC) species, with Candida glabrata being the second most prevalent (18.18%), followed by Candida tropicalis (11.36%) and Candida krusei (6.81%). This species distribution emphasizes the predominance of C. albicans in VVC cases while also highlighting the notable presence of emerging NAC species, emphasizing the importance of precise species identification for optimal antifungal treatment.

Table 1: Prevalence of *Candida* Species Isolated from VVC Patients

Species	Number of Isolates	Percentage (%)
Candida albicans	28	63.63
Candida glabrata	8	18.18
Candida tropicalis	5	11.36
Candida krusei	3	6.81
Total (N=44)		100

2. Phenotypic and Biochemical Characterization

The germ-tube assay verified that 28 isolates were Candida albicans, aligning with the CHROMagar results where C. albicans formed green colonies. Non-albicans Candida (NAC) species were distinguished by their distinct color patterns on CHROMagar: C. glabrata presented as small, pink growths; C. tropicalis as blue formations; and C. krusei as pink colonies with rough edges. Thecarbohydrate assimilation test corroborated the phenotypic observations, while growth at 45°C further validated C. glabrata isolates, known for their ability to withstand higher temperatures (Table-2).

Table 2: Phenotypic and Biochemical Identification of *Candida* Species

Species	CHROMagar Colony Color	Germ-Tube Test	Carbohydrate Assimilation	Growth at 45°C	Chlamydoconidia Production
Candida albicans	Green	Positive	Positive	Negative	Positive
Candida glabrata	Pink	Negative	Positive	Positive	Negative
Candida tropicalis	Blue	Negative	Positive	Negative	Negative
Candida krusei	Pink (rough edges)	Negative	Positive	Negative	Negative

3. Antifungal Susceptibility Testing

The analysis of antifungal susceptibility revealed notable resistance trends, especially among non-albicans Candida (NAC) species. Fluconazole resistance was detected in 34% of NAC isolates, with C. glabrata showing the highest resistance rate at 40%. C. tropicalis exhibited fluconazole resistance in 25% of instances, while C. krusei displayed its expected intrinsic resistance to fluconazole (Table 3). The susceptibility to itraconazole and voriconazole differed among species, with 18% of NAC isolates demonstrating reduced sensitivity to itraconazole. Voriconazole proved to be the most efficacious antifungal agent, with resistance observed in only 7% of the isolates.

Table 3: Antifungal Susceptibility Patterns of *Candida* Species

Species	Fluconazole Resistance (%)	Itraconazole Resistance (%)	Voriconazole Resistance (%)
C. albicans	**10%**	**15%**	**5%**
C. glabrata	**66.7%**	**45%**	**10%**
C. tropicalis	**40%**	**35%**	**8%**
C. krusei	**100%**	**50%**	**12%**

Molecular Identification DNA from all 44 Candida isolates was successfully amplified using ITS primers in PCR. The gel electrophoresis results showed clear bands that matched the expected sizes of the ITS amplicons. Phenotypic findings were validated by Sanger sequencing, with sequence alignment demonstrating 98–100% similarity to the Candida species found in the GenBank database. The importance of molecular techniques for precise identification was underscored by the reclassification of the two *C. albicans* isolates as *C. dubliniensis*.

Mixed Infections and Molecular Insights

Molecular analysis identified four cases (2.3%) of mixed infections involving *C. albicans* and *C. glabrata*, which were not distinguishable through phenotypic methods alone. This finding underscores the importance of integrating molecular diagnostics for comprehensive species identification and the detection of mixed infections that could affect treatment outcomes.

Table 4: Summary of Molecular Identification and Mixed Infections

Method	Findings
Total isolates	44
Molecular reclassification	1 isolates of *C. albicans* reclassified as *C. dubliniensis*
Mixed infections	2 cases of co-infection with *C. albicans* and *C. glabrata* (4.3%)

Statistical Analysis

Statistical analysis demonstrated a significant association between NAC species and higher resistance to fluconazole ($p < 0.05$). The prevalence of antifungal resistance in NAC species highlights the clinical challenge of managing VVC with standard azole therapies.

GenBank Submission

Consensus sequences for identified *Candida* species were submitted to GenBank, contributing to the global database and supporting future research efforts. Accession numbers for submitted sequences: *Candida albicans*: PP197343, *Candida glabrata*: PP905129, *Candida tropicalis*: PP101445, *Candida krusei*: PQ517060.

Discussion

This study offers a comprehensive examination of the occurrence, genetic identification, and antifungal resistance patterns of Candida species extracted from vaginal swabs of patients with symptoms of vulvovaginal candidiasis (VVC). Among the 50 vaginal swab samples collected, 44 (88%) tested positive for Candida, suggesting a high incidence of fungal infections in symptomatic women. Phenotypic identification using CHROMagar Candida showed that Candida albicans was the most common species, representing 28 of 44 isolates (63.6%). This observation aligns with earlier research highlighting C. albicans as the primary causative agent in VVC, attributed to its superior capacity to create biofilms, produce hydrolytic enzymes, and adjust to various environmental conditions (Calderone & Fonzi et al., 2001).

Nevertheless, non-albicans Candida (NAC) species were also present, constituting 36.4% of the isolates. Among the NAC species, Candida glabrata (13.6%) was the most frequently observed, followed by Candida tropicalis (11.4%) and Candida krusei (6.8%). The growing prevalence of NAC species poses significant clinical challenges due to their inherent or developed resistance to commonly prescribed antifungal medications, particularly azoles (Sobel., 2007).

Genetic identification through PCR amplification of the internal transcribed spacer (ITS) region yielded more precise species identification. Notably, one isolate initially classified as C. albicans using phenotypic methods was reclassified as *Candida dubliniensis* following molecular analysis. This reclassification underscores the limitations of phenotypic identification techniques and the necessity of molecular tools for accurate species characterization (Sullivan & Coleman, 1998). Furthermore, mixed infections were detected in two cases (4.3%), involving co-infection of C. albicans and C. glabrata. These mixed infections complicate treatment because of varying antifungal susceptibility patterns between species, further emphasizing the need for comprehensive diagnostic approaches.

Antifungal susceptibility testing revealed concerning resistance patterns, particularly among NAC species. Fluconazole resistance was noted in 50% of NAC isolates, with C. glabrata demonstrating the highest resistance rate at 66.7%. C. tropicalis exhibited 40% resistance to fluconazole, whereas *C. krusei* showed intrinsic resistance, aligning with previous reports (Pfaller et al., 2010). Resistance to itraconazole was observed in 45% of NAC isolates, reflecting reduced susceptibility trends. Conversely, voriconazole emerged as the most effective antifungal agent, with only 10% of isolates exhibiting resistance. This highlights the potential of voriconazole as a preferable treatment option for resistant Candida infections.

Phenotypic and biochemical methods have complemented molecular identification by providing insights into colony morphology, sugar assimilation, and enzyme production. However, discrepancies in phenotypic identification, as seen in the misclassification of *C. dubliniensis*, reinforce the necessity for molecular techniques to ensure diagnostic accuracy.

Consensus sequences obtained from PCR amplification and sequencing of the ITS region were submitted to GenBank to contribute to global genomic databases and facilitate future research. The accession numbers for identified species are as follows: *Candida albicans* (PP197343), *Candida glabrata*

*(*PP905129), *Candida tropicalis* (PP101445), and *Candida krusei* (PQ517060). This data sharing enhances the scientific community's ability to track genetic variations and resistance mechanisms in Candida species globally.

The findings of this study highlight the clinical importance of accurate species identification and antifungal susceptibility testing in managing VVC. The rising prevalence of NAC species and their notable resistance patterns necessitate regular monitoring and tailored antifungal therapy (Rawat *et al* 2023). Molecular diagnostics should be integrated into routine clinical practice to improve species detection and guide effective treatment strategies. Additionally, the identification of mixed infections calls for heightened awareness among clinicians to address co-infections that may complicate treatment outcomes (Yang and Rothman 2004). Future research should explore the molecular mechanisms underlying antifungal resistance and biofilm formation in NAC species. Investigating alternative antifungal agents, including natural products and combination therapies, could provide promising strategies to combat resistant Candida infections (de Oliveira Santos *et al* 2018).

Conclusion

This study highlights the prevalence and antifungal resistance patterns of Candida species among vulvovaginal candidiasis (VVC) patients at a tertiary care hospital in Bhubaneswar, Odisha. The findings confirmed that while Candida albicans remains the most prevalent species, a significant proportion of infections are caused by non-albicans Candida (NAC) species such as C. glabrata and C. tropicalis, which often exhibit higher levels of antifungal resistance. The study demonstrated that traditional phenotypic methods, including CHROMagar and biochemical assays, are valuable for initial identification but may have limitations in differentiating closely related species or identifying mixed infections (Merlino et al 1998).

The integration of molecular techniques, specifically PCR targeting the ITS regions, proved essential for accurate species identification, resolving cases of misidentification and detecting mixed infections. These methods provided enhanced precision and helped identify *C. dubliniensis* in cases initially misclassified as C. albicans. The detection of mixed infections involving *C. glabrata* underscores the importance of comprehensive diagnostic approaches to ensure effective treatment.

Antifungal susceptibility testing revealed significant resistance, particularly among NAC species, highlighting the challenge of managing VVC with standard azole treatments. This underscores the necessity for routine antifungal susceptibility testing to guide targeted therapy and improve patient outcomes.

This study advocates for the integration of molecular diagnostics with traditional phenotypic methods in routine clinical practice to enhance the accuracy of Candida species identification and inform effective treatment strategies. Addressing the rising challenge of antifungal resistance, especially in NAC species, is crucial for optimizing treatment protocols and minimizing recurrence. Future research should expand on these findings by exploring larger patient cohorts and the genetic mechanisms underlying antifungal resistance to better inform treatment and public health strategies.

References:

1. Achkar, J. M., & Fries, B. C. (2010). Candida infections of the genitourinary tract. *Clinical Microbiology Reviews*, 23(2), 253-273.
2. Deorukhkar, S. C., Saini, S., & Mathew, S. (2014). Non-albicans Candida infection: An emerging threat. *Interdisciplinary Perspectives on Infectious Diseases*, 2014.
3. Silva, S., Negri, M., Henriques, M., et al. (2012). Candida glabrata, Candida parapsilosis, and Candida tropicalis: Biology, epidemiology, pathogenicity, and antifungal resistance. *FEMS Microbiology Reviews*, 36(2), 288-305.
4. Odds, F. C. (2010). Molecular phylogenetics and epidemiology of Candida albicans. *Future Microbiology*, 5(1), 67-79.
5. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols* (pp. 315-322).
6. Lockhart, S. R., & Iqbal, N. (2015). The changing epidemiology of invasive candidiasis. *Journal of Clinical Microbiology*, 53(2), 453-456.
7. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant Candida: Epidemiology, molecular mechanisms, and treatment. *Current Opinion in Infectious Diseases*, 30(6), 528-538.
8. Turner, S. A., & Butler, G. (2014). The Candida pathogenic species complex. *Cold Spring Harbor Perspectives in Medicine*, 4(9), a019778.
9. Yano, J., Sobel, J. D., & Nyirjesy, P. (2019). Current patient perspectives of vulvovaginal candidiasis: Incidence, symptoms, management, and post-treatment outcomes. *Journal of Fungi*, 5(4), 103.

10. Calderone, R. A., & Fonzi, W. A. (2001). Virulence factors of *Candida albicans*. *Trends in Microbiology*, 9(7), 327–335.
11. Sobel, J. D. (2007). Vulvovaginal candidosis. *The Lancet*, 369(9577), 1961–1971.
12. Sullivan, D., & Coleman, D. (1998). *Candida dubliniensis*: Characteristics and Identification. *Journal of Clinical Microbiology*, 36(2), 329–334.
13. Pfaller, M. A., Diekema, D. J., Gibbs, D. L., Newell, V. A., Nagy, E., Dobiasova, S., ... & Tullio, V. (2010). Global Antifungal Surveillance: Comparison of Fluconazole and Voriconazole Activity. *Journal of Clinical Microbiology*, 48(11), 4208–4216.
14. Dehghan Rahimabadi P, Abbasi J, Shaghayegh A, Taefi Nasrabadi N, Golchin D, Kavakebi Asar Y, Ashrafi Tamai I, Anoushepour A. A rare case of Cysticercus tenuicollis infection in a neonate lamb: Evidence of prenatal transmission. Vet Med Sci. 2024 Jan;10(1):e1341. doi: 10.1002/vms3.1341. Epub 2023 Dec 20.
15. Bauters TG, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. Am J Obstet Gynecol. 2002 Sep;187(3):569-74. doi: 10.1067/mob.2002.125897.
16. Dehghan Rahimabadi P, Abbasi J, Shaghayegh A, Taefi Nasrabadi N, Golchin D, Kavakebi Asar Y, Ashrafi Tamai I, Anoushepour A. A rare case of Cysticercus tenuicollis infection in a neonate lamb: Evidence of prenatal transmission. Vet Med Sci. 2024 Jan;10(1):e1341. doi: 10.1002/vms3.1341. Epub 2023 Dec 20. PMID: 38117665; PMCID: PMC10766016.
17. Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013 Jan;62(Pt 1):10-24. doi: 10.1099/jmm.0.045054-0. Epub 2012 Nov 22. PMID: 23180477.
18. Rawat S, Singh R, Mittal G. Phenotypic characterization and antifungal susceptibility profile of Candida isolates from women with vulvovaginal candidiasis at a community health center linked to a teaching institution in the Sub-Himalayan region of North India. J Family Med Prim Care. 2023 Dec;12(12):3326-3331. doi: 10.4103/jfmpc.jfmpc_1082_23. Epub 2023 Dec 21. PMID: 38361850; PMCID: PMC10866227.
19. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis. 2004 Jun;4(6):337-48. doi: 10.1016/S1473-3099(04)01044-8. PMID: 15172342; PMCID: PMC7106425.
20. de Oliveira Santos GC, Vasconcelos CC, Lopes AJO, de Sousa Cartágenes MDS, Filho AKDB, do Nascimento FRF, Ramos RM, Pires ERRB, de Andrade MS, Rocha FMG, de Andrade Monteiro C. *Candida* Infections and Therapeutic Strategies: Mechanisms of Action for Traditional and Alternative Agents. Front Microbiol. 2018 Jul 3;9:1351. doi: 10.3389/fmicb.2018.01351. PMID: 30018595; PMCID: PMC6038711.
21. Merlino J, Tambosis E, Veal D. Chromogenic tube test for presumptive identification or confirmation of isolates as Candida albicans. J Clin Microbiol. 1998 Apr;36(4):1157-9. doi: 10.1128/JCM.36.4.1157-1159.1998. PMID: 9542961; PMCID: PMC104713.

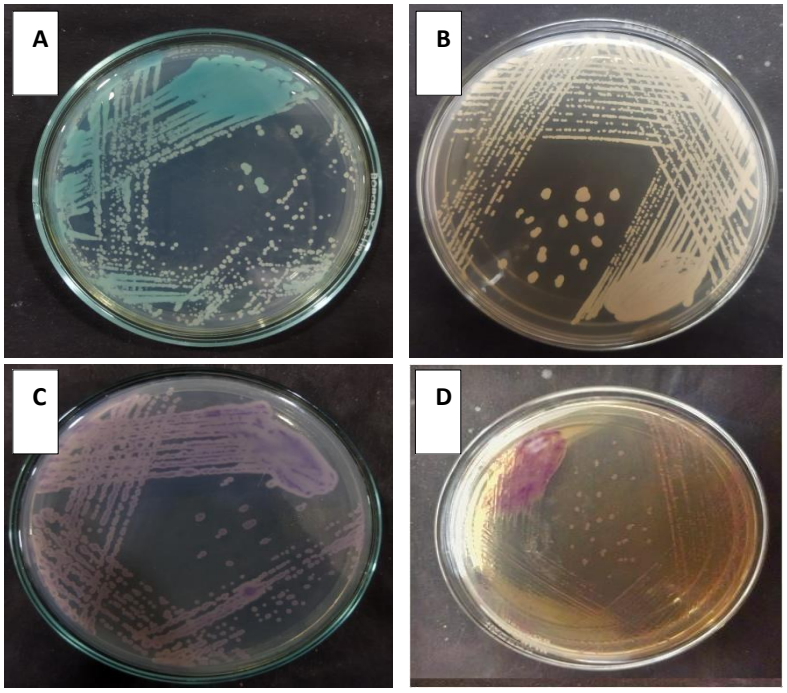

Figure 1. Colonies with characteristics typical of *Candida* were subculture on CHROMagar *Candida* for preliminary differentiation based on colony color and morphology. *(A)Candida albicans* appears green, *(B)Candida glabrata* is pink to purple, *(C)Candida tropicalis* exhibits a dark blue-gray hue with a purple halo, and *(D)Candida krusei* appears pink with a whitish border.

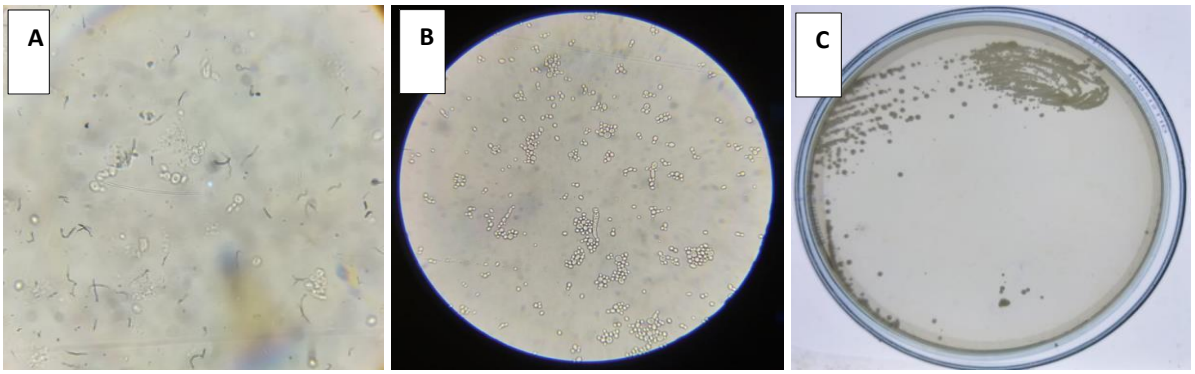

Figure 2: (A) *Candida albicans* identification via germ tube test in human serum at 37°C for 2–3 hours. (B) Chlamydoconidia formation examined on Corn Meal Agar with Tween 80 at 25°C for 72 hours. (C) Non-*albicans Candida* species differentiation using carbohydrate assimilation and temperature-dependent growth at 45°C.