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Speciation and Fluconazole Susceptibility of *Candida* Isolates from Clinical Samples in a Tertiary Hospital in Nepal.

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ABSTRACT

Introduction

Candida species, commonly present as human commensals, are notable opportunistic pathogens responsible for superficial and invasive infections, particularly in hospitalized patients. This study aimed to characterize the distribution and antifungal susceptibility patterns of *Candida* species isolated from various clinical specimens.

Methods

A descriptive cross-sectional, laboratory-based study was conducted in the Clinical Microbiology Laboratory of Nepal Medical College Teaching Hospital between July 2023 and June 2024. Clinical specimens yielding *Candida* species over the one-year period were included. Growth on Sabouraud dextrose agar was analyzed for colony morphology, Gram staining, germ tube production, and urea hydrolysis. Speciation of *Candida* isolates was performed using CHROMagar. Additionally, fluconazole susceptibility testing was conducted following Clinical and Laboratory Standards Institute guidelines.

Results

A total of 72 *Candida* isolates were identified from samples such as sputum, urine, and high vaginal swabs. *Candida* albicans was the most prevalent species 45 (62.5%), followed by non-albicans *Candida* (NAC) species, including C. *glabrata* 14 (19.4%), 9 C. *tropicalis* (12.5%), and 4 C. *parapsilosis* (5.6%). Fluconazole resistance was observed in 22.2% of total isolates of *Candida*, with C. *glabrata* exhibiting the highest resistance 12 (85.7%), whereas 4 (8.9%) of C. *albicans* demonstrated resistance to it.

Conclusion

The findings highlight a shift in epidemiology toward NAC species and raise concerns about rising fluconazole resistance. These results underscore the need for routine species identification and antifungal susceptibility testing to guide effective management strategies. The study advocates for antifungal stewardship programs and ongoing surveillance to address emerging resistance trends in *Candida* infections.

Keywords
Candida; fluconazole susceptibility; non albicans Candida, NAC

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INTRODUCTION

Candida species are common commensals of the human body, residing on the skin, mucous membranes, and gastrointestinal tract.[1] However, they can also cause superficial and invasive fungal infections in both immunocompromised and immunocompetent individuals.[2,3] Notably, *Candida* species are significant nosocomial pathogens responsible for high mortality rates, with most candidiasis cases attributed to *Candida albicans*.[4]

In recent years, the global incidence of nosocomial candidiasis has increased, spreading from tertiary care centers to community hospitals.[4,5] Although C. *albicans* remains the most prevalent species in mucocutaneous and disseminated infections, non-albicans *Candida* (NAC) species—such as C. *tropicalis*, C. *parapsilosis*, C. *glabrata*, and C. *krusei* are increasingly implicated.[6,7]

This shift in *Candida* epidemiology has been observed worldwide, marked by a transition from a dominance of C. albicans to NAC species as primary pathogens.[6-8] Factors contributing to this change include severe immunosuppression, critical illness, prematurity, broad-spectrum antibiotic use, and empirical antifungal therapy.[9]

Antifungal resistance is a growing concern. Susceptibility patterns of antifungal drugs vary significantly among *Candida* species, and the emergence of drug-resistant strains has been reported globally. The indiscriminate use of antifungal agents has exacerbated resistance, highlighting the importance of in vitro susceptibility testing to guide appropriate treatment.[10] Among the available antifungal drugs, fluconazole is widely used and often available over the counter.[11]

Given these challenges, the isolation, identification, and susceptibility testing of *Candida* species from clinical specimens are crucial for effective management of fungal infections.

In this study, we aimed to characterize *Candida* species and evaluate their fluconazole susceptibility patterns in clinical isolates.

METHODS

A descriptive cross-sectional, laboratory-based study was conducted in the Clinical Microbiology Laboratory of Nepal Medical College Teaching Hospital (NMCTH) from July 2023 to June 2024. The ethical approval was obtained from Institutional Review Committee of NMCTH (Ref no: 078-078.079). The study included all samples submitted for culture and sensitivity testing from both outpatient and inpatient departments of NMCTH. Data on isolated *Candida* species were entered into Microsoft Excel 2013, and the frequency of susceptibility to fluconazole was examined for each isolate.

Specimens were processed for the isolation and identification of C.albicans and non-albicans *Candida* species using specific culture methods. Urine samples were cultured on CLED agar, while other specimens such as tracheal aspirates, bronchial washings, sputum, vaginal swabs, body fluids, catheter tips, and cerebrospinal fluid (CSF) were cultured on Blood Agar and MacConkey Agar, followed by overnight incubation at 37°C. Blood was initially inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C aerobically and subcultured was done in blood and MacConkey agar everyday for 5 days. Yeast isolates were identified based on their colony morphology and Gram staining. Growth was considered as significant when colony counts exceeded 105 CFU/ml in urine culture, yeast growth was consistent in repeated cultures for sputum and body fluids, and isolates were obtained from sterile body sides such as catheter tip, CSF or blood. For further differentiation, the Germ Tube Test was performed to distinguish C. albicans from non-albicans *Candida* species.[12-13] Isolates were then subcultured onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24–48 hours. Pure colonies obtained from SDA were subsequently cultured on HiCrome Candida differential agar (Himedia) to differentiate specific non-albicans *Candida* species. After incubating at 37°C for 24–48 hours, colony colors were observed on HiCrome Candida differential agar: C. *albicans* appeared light green, C. glabrata was cream to white, C. *parapsilosis* was white to cream, and C. *tropicalis* exhibited blue to purple colonies.[14]

Fluconazole susceptibility testing was conducted using the disk diffusion method. A yeast inoculum suspension was prepared and adjusted to a 0.5 McFarland standard. Mueller-Hinton agar, supplemented with 2% glucose and 0.5 µg/mL methylene blue, was used as the medium. A sterile cotton swab moistened with the inoculum suspension was streaked evenly onto a 90 mm agar plate, which was allowed to dry for 5–15 minutes before placing a fluconazole disk by Himedia (25 µg/mL) at the center. Plates were incubated at 35–37°C for 18–24 hours, and results were assessed after 48 hours of incubation to ensure adequate growth and clear zone of inhibition for slow-growing *Candida* species. This extended incubation period enhances the accuracy of susceptibility results, particularly for strains that may exhibit delayed growth or reduced sensitivity to fluconazole.[15]

RESULTS

A total of 72 *Candida* species were isolated from sputum 36 (50%), urine 34 (47.2%), high vaginal swabs 1 (1.4%), and endotracheal tubes 1 (1.4%).

Based on growth patterns observed on CHROMagar, four types of *Candida* species were identified.

Table 1. Distribution of frequency of Candida species from various sample

Specimen	*Candida* spp				Total
	C. albicans	*C. glabrata*	*C. tropicalis*	*C. parapsilosis*	
Sputum	21	9	4	2	36
Urine	22	5	5	2	34
High vaginal swab	1	0	0	0	1
Endotracheal tube	1	0	0	0	1
Total	45	14	9	4	72

Table 2. Fluconazole susceptibility pattern of various Candida spp (n=72)

Fluconazole susceptibility	*Candida* spp				Total
	C. albicans	*C. glabrata*	*C. tropicalis*	*C. parapsilosis*	
Sensitive	41 (91.1%)	2 (14.3%)	9 (100%)	4 (100%)	56 (77.8%)
Resistant	4 (8.9%)	12 (85.7%)	0 (0%)	0 (0%)	16 (22.2%)

C.*albicans* was the most frequently encountered species (62.5%), predominantly isolated from urine and sputum. Among the non-albicans *Candida* (NAC) species, C.*glabrata* (19.4%) was the most common, followed by C.*tropicalis*(12.5%) and C.*parapsilosis* (5.6%).

All four types of *Candida* species were found in both sputum and urine samples, with C. *albicans* present across all specimen types. The distribution of *Candida* species across various specimens is detailed in Table 1.

In this study, 22.2% of *Candida* species exhibited resistance to fluconazole. High resistance rate was observed in C. *glabrata* (85.7%) of isolates, followed by C.*albicans*, with resistance seen in 9% of isolates. The fluconazole susceptibility profiles of the different *Candida* species are detailed in Table 2.

DISCUSSION

Candida species are common commensals in humans but can act as opportunistic pathogens, causing significant morbidity and mortality.[2] This study highlights the clinical and microbiological relevance of *Candida* species in a hospital setting. Among the 72 isolates identified, C. *albicans* was the most frequently encountered species (62.5%), predominantly isolated from urine and sputum samples. This aligns with previous studies where C. albicans is reported as the leading cause of candidiasis due to its ability to adhere to epithelial cells and form biofilms.[2,3,16] Similarly other studies conducted in Nepal have also identified C. *albicans* as the predominant *Candida* species.[17,18]

Among NAC species, our study identified C. *glabrata* as the most prevalent. These species were isolated from sputum and urine, with NAC species accounting for a significant proportion of isolates, which differs from other studies in Nepal and India where *C. tropicalis* predominated.[18,19] Conversely, a study by BK et al. in western Nepal reported *C. krusei* as the predominant species.[16] In Europe and the USA, *C. glabrata* has also been consistently identified as the most prevalent NAC species.[20] These variations may be attributed to geographical differences, disparities in diagnostic methodologies, and the increasing use of fluconazole for prophylaxis and treatment, which may influence species distribution and antifungal resistance patterns.[4] As a result, these species present unique challenges in terms of diagnosis, treatment, and resistance management, emphasizing the need for updated clinical guidelines and antifungal stewardship programs.

Antifungal resistance patterns observed in this study raise important clinical concerns. Resistance to fluconazole, a widely used antifungal agent, was seen in 35% of *Candida* isolates. Among NAC species, high resistance rate was seen in C. glabrata (86%) which is consistent with global findings that highlight its intrinsic resistance to azoles.[17-21] In contrast, rate of fluconazole resistance in C. *albicans* was relatively low (9%), which aligns with findings from previous studies by Bhattacharya et al and Pfaller et al that report C. *albicans* as generally sensitive suggesting that it remains susceptible in most clinical cases.[4,23] However studies in immunocompromised populations, have reported slightly higher resistance rate, indicating emerging resistance in specific settings.[4,24]

C. *tropicalis* and C. *parapsilosis* isolates in our study were fully sensitive to fluconazole. This finding is comparable to results from other studies in Southeast Asia and Latin America, where C.

tropicalis and C. *parapsilosis* have demonstrated low resistance rate to fluconazole.[23,24] However, this result may also be influenced by the limited number of isolates of these species in our studies.

The high resistance rates observed in C. *glabrata* call for alternative treatment strategies, especially in cases of invasive infections. Routine antifungal susceptibility testing is essential to guide effective therapy, particularly in settings where NAC species are prevalent.[9]

The presence of *Candida* in sputum and urine raises additional questions regarding the clinical relevance of colonization versus infection. While urine cultures may indicate urinary tract infections, the presence of *Candida* in sputum often reflects colonization rather than active infection, especially in critically ill patients.[22] Thus, clinical correlation is necessary to avoid unnecessary antifungal use and reduce the risk of resistance development.

This study underscores the need for continuous surveillance of *Candida* species distribution and antifungal susceptibility patterns. Antifungal stewardship programs should be implemented to optimize therapy and minimize the emergence of resistance.

These findings suggest the rapid increase in resistance among *Candida* species for ketoconazole and need for speciation and antifungal susceptibility before treatment with antifungal drug.

CONCLUSION

This study highlights the distribution and fluconazole susceptibility patterns of *Candida* species isolated from various clinical specimens in a hospital setting. C. *albicans* was the most prevalent species, followed by C. *glabrata*, C. *tropicalis*, and C. *parapsilosis*. Fluconazole resistance was observed in 35% of isolates, with C. *glabrata* demonstrating alarmingly high resistance. These findings emphasize the importance of routine identification and antifungal susceptibility testing to guide effective therapy, particularly in the context of rising antifungal resistance.

The isolation of NAC species and resistance patterns necessitates the implementation of antifungal stewardship programs and the consideration of alternative therapeutic strategies. Continued surveillance and further research into the mechanisms of resistance are essential to improve the management and outcomes of *Candida* infections in clinical practice.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Study concept, design, data collection, data analysis and manuscript writing: Jyotshna Sapkota; Data collection and manuscript review: Dr. Kiran Aryal, Dr. Ritu Pandey, Dr. Laxmi Kant Khanal; Data analysis and manuscript review: Mr. Ram Prasad Adhikari, Dr. Sushila Khadka; Study design, data analysis and manuscript review: Dr. Deepti Shrestha; Concept review, data analysis and manuscript review: Dr. Manisha Sharma. All authors read and approved the final manuscript.

REFERENCES

1. Gow NA, Yadav B. Microbe Profile: Candida albicans - a shape-changing, opportunistic pathogenic fungus of humans. Microbiology. 2017;163(8):1145-1147. doi: 10.1099/mic.0.000499. PMID: 28809155
2. Brown GD, Denning DW, Gow NA, et al. Hidden killers: Human fungal infections. Sci Transl Med. 2012;4(165):165rv13. doi: 10.1126/scitranslmed.3004404. PMID: 23253612
3. 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;62(1):10-24. doi 10.1099/jmm.0.045054-0. PMID: 23180477
4. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20(1):133-163. doi: 10.1128/CMR.00029-06. PMID: 17223626
5. Guinea J. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect. 2014;20(Suppl 6):5-10. doi: 10.1111/1469-0691.12539. PMID: 24506442
6. Mendoza M, Díaz-González S, Alonso-Sánchez S, et al. Epidemiology of non-albicans Candida species in bloodstream infections in a tertiary hospital in Spain: a 10-year retrospective study. Mycoses. 2019;62(9):711-719. doi: 10.2147/CLEP.S459600. PMID: 39219747
7. Oberoi JK, Wattal C, Goel N, et al Non-albicans Candida species in blood stream infections in a tertiary care hospital at New Delhi, India. Indian J Med Res. 2012 Dec;136(6):997-1003. PMID: 23391796
8. Candidemia Study Group. Epidemiology and microbiology of Candida bloodstream infections: 10-year results from a nationwide surveillance program in Spain. Clin Microbiol Infect. 2012;18(8):785-791. doi: 10.1111/j.1469-0691.2011.03615.x. PMID: 21919971.
9. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1-e50. doi: 10.1093/cid/civ933. PMID: 26679628
10. Rodloff AC, Koch D, Schaumann R. Epidemiology and antifungal resistance in invasive candidiasis. Eur J Med Res. 2011 Apr 28;16(4):187–195. doi: 10.1186/2047-783X-16-4-187
11. Rex JH, Walsh TJ, Sobel JD, et al. Practice guidelines for the treatment of candidiasis. Clin Infect Dis. 2000;30(4):662-78. doi: 10.1086/313749. PMID: 10770728
12. Isenberg HD. Mycology and Antifungal Susceptibility Testing. In:

Gracia LS, Isenberg HD, editors. Clinical microbiology procedure handbook, vol. 2. 2nd ed. Washington, DC: ASM Press; 2004. p. 8.0.1–8.10.7.

13. Kauffman C, Fisher J. Candida urinary tract infections: diagnosis. Clin Infect Dis. 2011;52(suppl 6):S452–6.doi: 10.1093/cid/cir111. PMID: 21498838
14. Murray CK, Beckius ML, Green JA, et al. Use of chromogenic medium for the isolation of yeasts from clinical specimens. J Med Microbiol. 2005;54:981–5. doi: 10.1099/jmm.0.45942-0. PMID: 16157554
15. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI document M100.
16. Arora U, Jha A, Lata H, et al. Distribution and antifungal susceptibility of Candida species in a tertiary care hospital in North India. Indian J Med Microbiol. 2018;36(3):400-403.doi: 10.4103/ijmm.IJMM_17_379. PMID: 30004095
17. Raut S, Koirala A, Paudel S, et al. Prevalence of Candida species in clinical samples and their antifungal susceptibility pattern in a tertiary care hospital of Nepal. J Nepal Health Res Counc. 2021;19(1):1-6.
18. Khadka S, Sherchand JB, Pokhrel BM, et al. Isolation, speciation and antifungal susceptibility testing of Candida isolates from various clinical specimens at a tertiary care hospital, Nepal. BMC Res Notes. 2017;10:218. doi: 10.1186/s13104-017-2522-4. PMID: 28532474
19. Othimani D, Shanthala D, et al. Prevalence and antifungal susceptibility of Candida species in clinical samples from a tertiary care hospital. J Clin Microbiol. 2015;53(3):1242-1247. doi: 10.1128/JCM.03167-14. PMID: 25767244.
20. García-Salazar E, Acosta-Altamirano G, Betancourt-Cisneros P, et al. Detection and molecular identification of eight Candida species in clinical samples by simplex PCR. Microorganisms. 2022;10(2):374. DOI: 10.3390/microorganisms10020374. PMID: 35208828
21. Singh L, Harakuni SU, Basnet B, et al. Speciation and antifungal susceptibility testing of Candida species isolated from clinical samples. Asian Journal of Medical Sciences. 2020;11(4):30-34. https://doi.org/10.3126/ajms.v11i4.28494
22. Koehler P, Stecher M, Cornely OA, et al. Morbidity and mortality of Candida colonization and infections in critically ill surgical patients: A systematic review and meta-analysis. Crit Care. 2019 May 9;23(1):1–12. Doi 10.1186/s13054-019-2435-z. PMID: 31068299
23. Bhattacharya S, Sae-Tia S, Fries BC. Candidiasis and mechanisms of antifungal resistance. Antibiotics (Basel). 2020;9(6):312. Doi: 10.3390/antibiotics9060312. PMID: 32526921
24. Chakrabarti A, Sood P, Rudramurthy SM, et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Int J Med Microbiol. 2015;305(7):552-7. Doi: 10.1007/s00134-014-3603-2. PMID: 25510301