Journal of Institute of Medicine Nepal
Institute of Medicine, Kathmandu, Nepal

Orginal Article

JIOM Nepal. 2026 April; 48(1):131-137

Distribution of Clinical Isolates of Candida Species among Clinical Specimens in a Tertiary Care Center

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DOI
https://doi.org/10.59779/jiomnepal.1467

Submitted
Feb 25, 2026

Accepted
Apr 8, 2026

ABSTRACT

Introduction
Candida species are the most common cause of mycosis, especially among patients who are immunocompromised, receiving broad spectrum antimicrobial therapy and/or chemotherapy, hospitalized and with diabetes mellitus. The increasing cases of Candida infection by its different species is a subject of concern because each species possess different characteristics features to fail the treatment. Thus, speciation and its prevalence is imperative for the management of candidiasis infection.

Methods
This is a laboratory-based cross-sectional descriptive study. A total of 149 isolates of Candida were identified from 16,234 clinical specimens and those specimens were processed at Department of Microbiology, Tribhuvan University Teaching Hospital. Growth on Sabouraud dextrose agar were evaluated for colony appearance, germ tube test, chlamydospore formation on corn meal agar and Candida speciation from CHROMagar. Data were analyzed using descriptive statistics and all the statistical evaluation were done by using SPSS version 16.0 software.

Results
C. albicans was the most common isolate out of 149 Candida spp. which was 53.70% of total isolates. Similarly, among NAC species C. tropicalis was the most common isolate followed by C. krusei, C. dubliniensis, C. parapsilosis and C. glabrata. The study revealed that the prevalence of isolates is 0.92% in overall specimens.

Conclusion
Although C. albicans is the most common isolate of Candida spp., NAC species infection is increasing especially in case of urine and blood stream infection.

Keywords
Candida; speciation; clinical isolates; CHROMagar; Corn meal agar

INTRODUCTION

Candida species are the most common cause of fungal infections globally, with nearly 200 species, most of which are harmless commensals inhabiting human mucosa, skin, and gastrointestinal tract.[1,2] However, these endogenous opportunists cause both superficial and deep-seated candidiasis, that are notable amongst immunocompromised patients, those with prolonged hospital stay especially in intensive care units, those with recent surgery and history of chronic diseases.[1–5] Virulence factors such as biofilm formation, dimorphism, and enzyme production enable *Candida* spp. to cause disseminated candidiasis leading to morbidity and mortality in even immunocompetent individuals.[6,7] Recent studies show a shift in the etiology of candidiasis, with non-*albicans Candida* (NAC) spp., such as *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida dubliniensis*, now emerging as the major pathogens causing severe infections.[2–4,8] This up rise poses a significant challenge in treatment due to increasing resistance to antifungal agents, that raises question to the empirical antifungal therapy and highlights the need of precise identification of species of *Candida*.[1–3,9] This study aims to address these challenges by focusing on the speciation of *Candida* spp.

METHODS

This laboratory-based cross-sectional study was conducted at the department of Microbiology, of tertiary care hospital in Nepal from August 2023 to January 2024 after obtaining approval from the Head of Department of Microbiology, Institute of Medicine and IRC (75 (6-11) E2 080/081).

Various clinical specimens, including urine, sputum, blood, high vaginal swabs, pus, and body fluids, were cultured on Sabouraud dextrose agar (SDA). Creamy, yeast-like colonies on SDA that showed gram-positive budding yeast cells on Gram's staining were confirmed as isolates of *Candida*. For speciation, germ tube test (GTT) was performed by adding colonies from SDA into 500 microliter blood serum and incubated at two different temperature i.e 37°C and 42°C for 3-4 hours. The germ tube test positive isolates at 37°C could be *C. albicans* or *C. dubliniensis*. Thus, *C. albicans* can further identified if it shows germ tube test at 45°C as well otherwise we considered it as *C. dubliniensis*. Additionally, chlamydospore testing was done where fresh colonies from SDA were stabbed into the cornmeal agar, covering the inoculum with sterile coverslip to create low oxygen tension and incubating at 25°C for 24-72 hours. Similarly, creamy yeast-like colonies were inoculated in CHROMagar and incubated at 37°C for 24 hours and speciation was performed on the basis of color of the colonies as per manufacturer's guideline.[3,8]

All the obtained relevant data were statistically analyzed using SPSS version 16.0 and interpreted according to frequency distribution, percentage. Chi- square test was done in the statistical analysis with P value < 0.05 regarded as significant.

RESULTS

This study encompassed a total of 16,234 clinical specimens out of which 149 i.e. 0.92% were *Candida* spp. Figure 1 shows that most of the *Candida* isolates were obtained from sputum specimen while the only one isolate was obtained from body fluid (p value < 0.05). According to Table 1, majority of the isolates were *C. albicans* (53.69%) while the *C. tropicalis* was the major pathogen among the NAC isolates. It also shows that highest prevalence of *C. albicans* and *C. dubliniensis* were from sputum although *C. dubliniensis* were isolated from sputum and urine specimens only. Most of the *C. tropicalis* were isolated from HVS while *C. krusei* was the only isolate among body fluid. *C. parapsilosis* and *C. glabrata* were isolated from only blood and sputum specimens respectively.

This study determined that the clinical specimens from ICU had the highest prevalence of *Candida* isolates followed by IPD (excluding ICU) and OPD (p value < 0.05). In terms of gender, more clinical isolates of *Candida* were obtained from females (0.95%, 81/8514) compared to males (0.88%, 68/7720) with p value > 0.05. Figure 2 illustrates that the females had higher preponderance among *C. albicans*, *C. krusei*, *C. dubliniensis* and *C. parapsilosis* isolates while *C. tropicalis* and *C. glabrata* showed higher prevalence amongst males.

In terms of age, although only 72 specimens (lowest frequency) were received from age group 91-100 years, the highest prevalence of *Candida* isolate was obtained from this (4.17%) followed by age groups 71-80 years (2.05%), 81-90 years (1.81%), 61-70 years (1.58%) and 51-60 years (1.25%). Age groups 0-10 years and 41-50 years had same prevalence of 0.76% and 11-20 years and 21-30 years also had same prevalence of 0.47%. The least prevalence was noted among age group 31-40 years with 0.37%. These findings were not statistically significant (P-value 0.63). Figure 3 illustrates that in all age groups, *C. albicans* stood out as the major isolate followed by *C. tropicalis*. Additionally, all the six species of *Candida* were isolated from only one age group, i.e. 61-70 years while in three age groups, 41-50 years, 81-90 years and 91-100 years, only two species were identified, i.e. *C. albicans* and *C. tropicalis*.

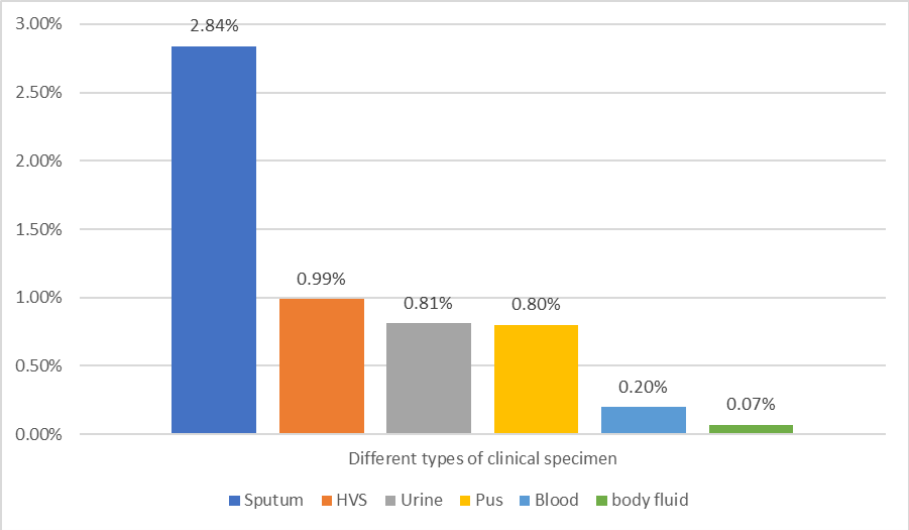

Figure 1. Distribution of Candida spp. among various clinical specimens

Table 1. Distribution of different species of Candida among various specimens

Species of *Candida*	Type of specimen	Number of the specimen with *Candida* isolates	Number of the species of *Candida* isolated	Percentage	*p-value*
C. albicans	Urine	66	29	43.93	0.4494
	Sputum	63	43	68.25	
	Blood	9	3	33.33	
	Pus	6	3	50.00	
	HVS	4	2	50.00	
	Body fluid	1	0	00.00	
	Total	**149**	**80**	**53.69**	
C. tropicalis	Urine	66	29	43.94	0.1028
	Sputum	63	11	17.46	
	Blood	9	2	22.22	
	Pus	6	1	16.67	
	HVS	4	2	50.00	
	Body fluid	1	0	0.00	
	Total	**149**	**45**	**30.20**	
C. krusei	Urine	66	6	9.09	0.1028
	Sputum	63	3	4.76	
	Blood	9	1	11.11	
	Pus	6	2	33.33	
	HVS	4	0	0.00	
	Body fluid	1	1	100.00	
	Total	**149**	**13**	**8.73**	

C. dubliniensis	Urine	66	2	3.03	0.0881
	Sputum	63	4	6.35	
	Blood	9	0	0.00	
	Pus	6	0	0.00	
	HVS	4	0	0.00	
	Body fluid	1	0	0.00	
	Total	**149**	**6**	**4.03**	
C. parapsilosis	Urine	66	0	0.00	0.0823
	Sputum	63	0	0.00	
	Blood	9	3	33.33	
	Pus	6	0	0.00	
	HVS	4	0	0.00	
	Body fluid	1	0	0.00	
	Total	**149**	**3**	**2.01**	
C. glabrata	Urine	66	0	0.00	0.0804
	Sputum	63	2	3.17	
	Blood	9	0	0.00	
	Pus	6	0	0.00	
	HVS	4	0	0.00	
	Body fluid	1	0	0.00	
	Total	**149**	**2**	**1.34**	

Table 2. Distribution of Candida isolates among different departments

Department	**No. of specimen**	**No. of *Candida* isolate**	**Percentage**	***p-value***
ICU	1,196	57	4.76%	0.00001
IPD excluding ICU	7,093	55	0.78%	
Outpatients	7,945	37	0.47%	
Total	**16,234**	**149**	**0.92%**	

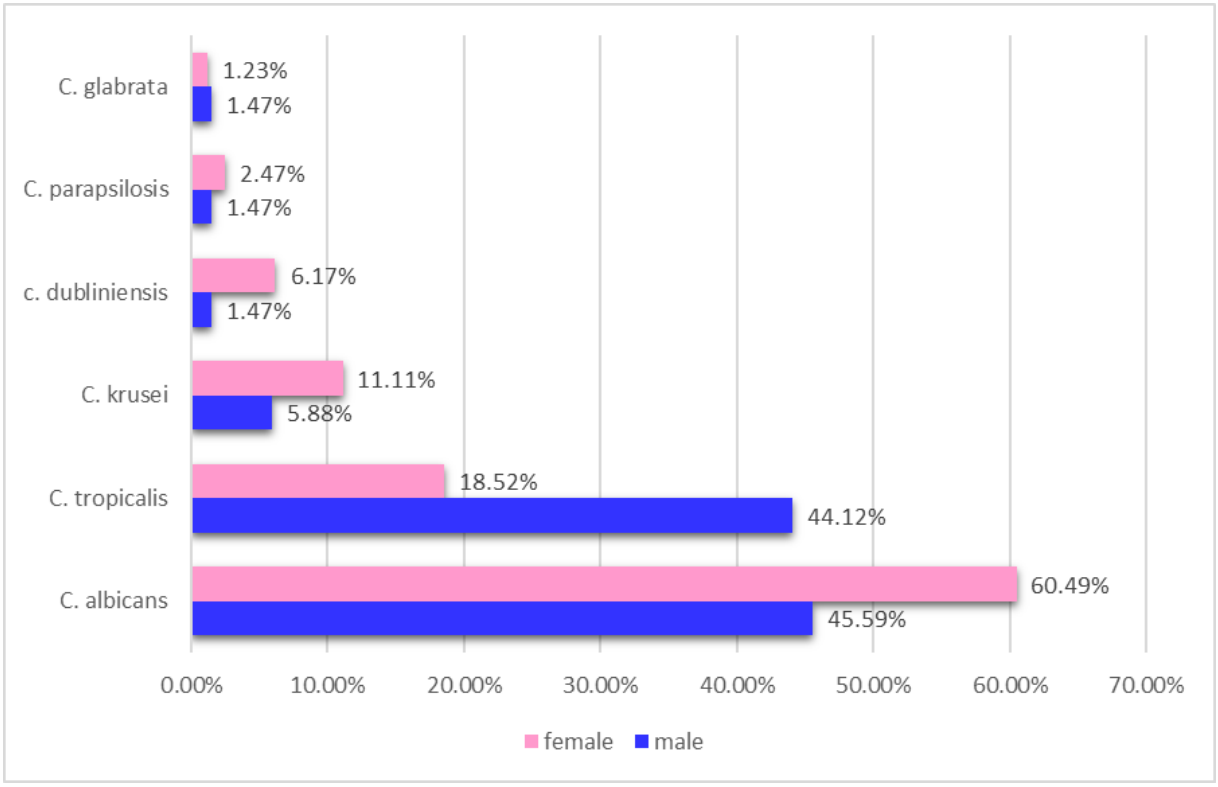

Figure 2. Gender-wise distribution of different species of Candida isolates

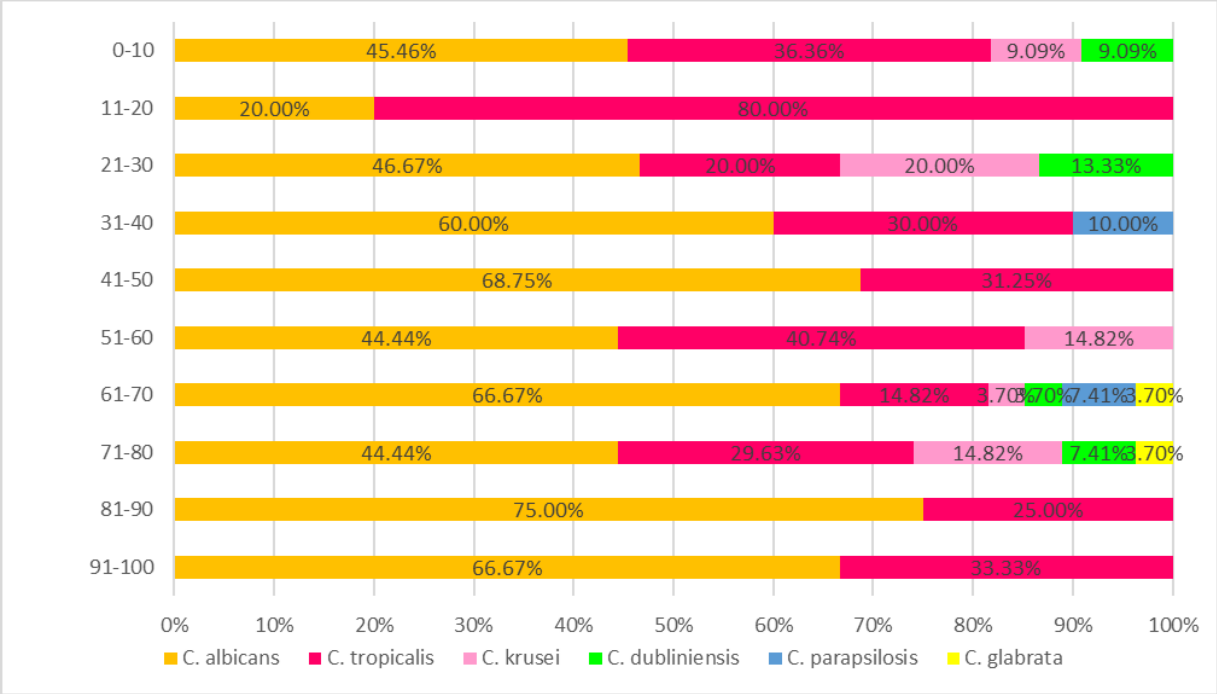

Figure 3. Age-wise distribution of different species of Candida isolates

DISCUSSION

This study, conducted at Tertiary care Hospital of Nepal, aimed to determine the prevalence and distribution of *Candida* spp. across various clinical specimens. Out of 16,234 total samples processed, 149 *Candida* isolates were identified, accounting for a prevalence of 0.92%. This figure is relatively lower than those reported by previous studies such as Goyal et al., Khadka et al., Basu et al., and Samal et al., which documented higher *Candida* isolation rates in different clinical settings.[2,9,14,15] The variation in prevalence may be attributed to differences in geographical location, patient demographics, or methodological factors such as sampling size and selection criteria. On the basis of specimen type, *Candida* spp. were most frequently isolated from sputum (2.84%), followed by high vaginal swabs (0.99%), urine (0.81%), pus (0.80%), blood (0.20%), and body fluids (0.07%). However, in terms of total isolates, the majority were derived from urine samples (44.29%) and sputum (42.28%), while the rest were recovered from blood (6.04%), pus (4.02%), high vaginal swabs (2.68%), and body fluids (0.67%). These findings were consistent with Khadka et al. and Ghimire et al., but diverged from Devi et al., likely due to differences in sample sizes and study durations.[2-4]

Species-wise distribution showed that *C. albicans* was the most frequently isolated species overall, particularly dominating sputum and pus specimens, while both *C. albicans* and *C. tropicalis* were prevalent in urine and high vaginal swabs. *C. albicans* and *C. parapsilosis* were most common in blood, whereas *C. krusei* was the sole species recovered from body fluids. This distribution aligns with findings by Khadka et al. but contrasts with Tri Waibawa et al. and Bilal et al., who observed differing dominant species in their respective studies.[2,19,18] The finding of *C. albicans* (53.70%) in this study supports the pattern seen in studies by Khadka et al. in Nepal (56.00%), Devi et al. in India (52.00%), Papon et al. in the USA (63.80%), Ghimire et al. in Nepal (69.00%), and Samal et al. (70.00%) .[2-4,15,16] Additionally, Khadka et al. previously reported prevalence of NAC as 44.0% in 2017 from the same study site compared to 46.30% noted in this study, suggesting a slight rise in NAC infections over time, which may indicate a shifting trend.[2] Nevertheless, more longitudinal studies are necessary to confirm this observation. Notably, studies by Basu et al. (2003), Goyal et al. (2016), Bhattacharjee, and Bilal et al. (2022) found higher proportions of NAC species—66.67%, 54.20%, 51.43%, and 50.64%, respectively—indicating possible regional or environmental influences.[9,14,17,18]

The gender-wise distribution of Candida isolates showed a slightly higher prevalence in females than in males, with females contributing 54.36% of the total isolates. *C. albicans* remained the predominant species in both genders. These findings were in line with Goyal et al., Ghimire et al., and Muzaheed et al., though other studies by Khadka et al. and Bhattacharjee reported a higher prevalence among males.[2,4,9,17,21]

Age wise distribution of *Candida* isolates reported similar finding from the study conducted in a tertiary care hospital in India in 2016 by Bhattacharjee with 51-60 years of age groups showing highest *Candida* isolates.[17] Similarly, Goyal et al. in 2016 reported *C. albicans* as a commonest pathogen for Candiduria of age group above 60 years.[9] In 2022, Muzaheed et al. also reported age group between 70-80 were prone to have *Candida* infection which is similar to the finding of this study.[21] However the study conducted in tertiary care hospital in India by B S G Sailaja and P D Prasad in 2019 reported more isolates were isolated from 30-40 age groups.[24] The maximum number of isolates were isolated from more than 50 years of age groups and new born this could be due to immune-compromised population or due to predisposing factors.

In terms of department, the highest number of *Candida* isolates came from ICU patients (38.25%), followed by inpatient departments excluding ICU (36.91%) and outpatient departments (24.84%). This pattern closely matches results from Gajdács et al. and Wisplinghoff et al., likely due to factors such as increased disease severity, prolonged hospitalization, immunosuppression, and extensive use of broad-spectrum antibiotics among ICU and hospitalized patients. These findings underscore the importance of continuous surveillance, species-level identification, and antifungal stewardship to manage Candida infections effectively.[22,23]

Limitations

This study does not differentiate between colonizers and true pathogens amongst the isolates that might lead to overdiagnosis of candidiasis.

CONCLUSION

Among the 0.92% of clinical isolates of *Candida* spp., *C. albicans* was the most common species and among NAC, *C. tropicalis* was the most common isolate. In agreement to other reports of uprise in NAC infections, urine and blood stream infections had NAC as the most prevalent isolate. *Candida* infection is more common in female than male. The characteristics feature of each isolates were found to be complementary to each other in different methods which were analyzed by following standard microbiological procedure.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to all the patients from whom these clinical isolates were obtained and the Head of the Department and entire staffs of Department of Microbiology of this tertiary care hospital, and IOM-IRC for granting ethical approval.

Financial Support

None. The necessary supplies were provided by Department of Microbiology of the institute.

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