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Original Research Article

Decoding *Candida*-speed and accuracy in species identification: a study from a tertiary care hospital in Western Vidarbha

Rajesh Dawre[1], Nazneen I. Malak[1], Sharmila S. Raut[2], Rupali S. Mantri[1], Saahil Parvez[1]*

[1]Department of Microbiology, Government Medical College, Akola, Maharashtra, India
[2]Department of Microbiology, Government Medical College, Bhandara, Maharashtra, India

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***Correspondence:**
Dr. Saahil Parvez,
E-mail: parvezsaahil@gmail.com

ABSTRACT

Background: *Candida* infections encompass a clinical spectrum ranging from superficial mucocutaneous lesions to life-threatening disseminated candidemia in immunocompromised hosts. Over the years, clinical distribution of *Candida* species has significantly evolved, shifting from the traditional dominance of *Candida albicans* to an increasing isolation rate of diverse non-albicans *Candida* (NAC) species. This study was conducted to isolate *Candida* spp. from various clinical specimens, to speciate and study the distribution of *Candida albicans* and non-*albicans Candida* spp. among various clinical specimens.
Method: A total of 464 samples were included in the study form January 2024 to June 2025. Samples included urine, blood, sputum, high vaginal swab, pus etc. Demographic details of patients were recorded. KOH, gram staining, germ tube techniques were done preliminarily. Culture on Sabourauds dextrose agar, morphology identification by Dalmau plate method and CHROM agar identification was done.
Results: Out of 464 *Candida* spp. isolated, highest infection (40.5%) seen in the 41-60 years age group, with female preponderance. The highest number of *Candida* isolates were obtained from urine samples 37.7% (n=175), followed by 28.2% (n=131) from sputum. non-albicans *Candida* spp. was 244 (53%) as compared to *Candida albicans* 220 (47%). *C. troplicais* predominated from all clinical samples and was commonest NAC spp. from urinary and respiratory tract.
Conclusion: The rise of non-albicans *Candida* species underscores a global shift toward higher drug resistance. Precise speciation is essential to identify these distinct resistance profiles, allowing clinicians to start appropriate empirical and target therapy.

Keywords: *Candida albicans*, tropicalis, CHROM agar, Dalmau plate, Germ tube

INTRODUCTION

Candida species are the constituents of normal flora of human body commonly found in skin, mucous membranes, throughout the gastrointestinal tract and female genital tract. However, specific virulence factors can trigger its transformation into a destructive, opportunistic pathogen. Key mechanisms such as secretion of extracellular hydrolytic enzymes, strong adherence to host tissues and colonization of indwelling medical devices facilitate this transition.[1] Once attached, it forms dense, drug-resistant biofilms that protect it from host immune defenses. Consequently, these specialized survival strategies have established *Candida* as the predominant fungal pathogen responsible for severe septicemia and healthcare-associated infections.[2] The host predisposing factors include extremes of age, pregnancy, diabetes, prolonged administration of antibiotics, steroid therapy, AIDS and in case of Candiduria use of IV catheters, interruption of urine flow, genitourinary tuberculosis.[3] The various infections caused by *Candida* species include oral thrush, skin and nail infections, diarrhoea, urinary tract infection, respiratory tract infection, diabetic and postoperative wound infections, vulvovaginal candidiasis and septicaemia. *Candida albicans* is the commonest species accounting for about

60-80% of infections. However, over the years, indiscriminate use of over-the-counter antifungals contributed significantly to drug resistance and change in trend leading to emergence of non-albicans *Candida* species. These include *Candida glabrata, Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida dubliniensis* and *Candida kefyr*.[4] Species level identification of *Candida* is important owing to the change in drug susceptibility of different *Candida* species, to identify those strains which might be intrinsically resistant to some of the antifungal agents. Speciation of *Candida* isolates is conventionally done by germ tube test which differentiates *C. albicans* and *C. dubliniensis* from other *Candida* spp. CHROM agar which is fast and cost-effective method and Dalmau plate method which is one of the best methods to view the species morphology. Other methods include sugar fermentation, sugar assimilation however both of which are time consuming.[5]

The present study was conducted to isolate *Candida* spp. from various clinical specimens, to speciate and characterise *Candida* spp. and study the distribution of *Candida albicans* and non-albicans spp. among various clinical specimens.

METHODS

The present study is a prospective observational study conducted in the Department of Microbiology at a tertiary care hospital in Akola from January 2024 to June 2025 after approval from the institutional ethics committee (IEC).

Clinical samples suspected of fungal infection received in the microbiology laboratory during the study period were included. Patient's information like age and gender were recorded to observe the individual's susceptibility, specific colonising species and the clinical site of infection. A total of 464 samples received and included were blood, urine, sputum, high vaginal swabs, pus, catheter tips, ear swabs and stool samples. Isolates identify as fungi other than *Candida* species were excluded from the study. The preliminary identification was made by direct microscopy by potassium hydroxide mount (KOH), Gram's stain and negative urea hydrolysis test. The KOH mount was done to observe fungal elements. The concentration of KOH used was 10% and increased to 40% for samples like nail and tissue for homogenisation. It was then observed under low power (10X) and high power (40X) of microscope. Gram staining was performed for the presence of gram-positive budding yeast cells as shown in Figure 1. All the identification tests were performed as per standard mycological procedures.[6] For the isolation of *Candida* species the samples were inoculated on SDA with antibiotics and incubated at 37°C in incubator for 48 hrs. After 2–3 days of incubation on SDA, appearance of smooth, pasty, white-to-cream colonies confirmed the characteristic growth of *Candida* spp. as shown in Figure 2. Speciation was initiated by performing germ tube test also known as Reynold Braude phenomenon. *Candida albicans* gave positive germ tube test (as seen in Figure 3). However, growth at 45 °C and formation of chlamydospore on corn meal agar (CMA) differentiated *Candida albicans* from *Candida dubliniensis*. Germ tube also differentiated between *Candida albicans* and non-albicans *Candida* (NAC) spp. as the latter are germ tube negative.

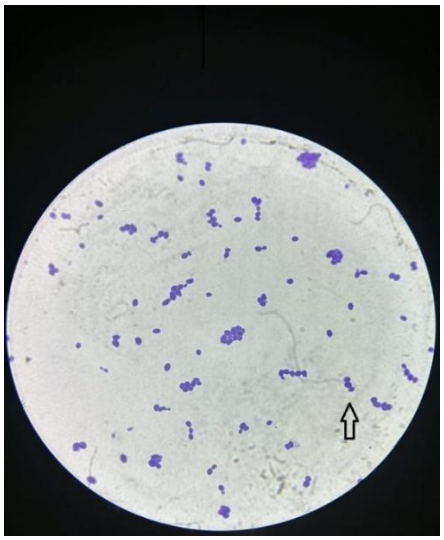

Figure 1: Gram positive budding yeast cells.

Figure 2: White creamy pasty colonies on SDA.

Further speciation of non-*albicans Candida* (NAC) species was done using the Dalmau culture method on cornmeal agar (CMA). As a nutritionally deficient medium, CMA suppresses vegetative growth and stimulates fungal sporulation. This method facilitated the extensive observation of *Candida* spp. morphology like chlamydospore production in *Candida albicans*, pseudohyphae arrangements and blastospore distribution in NAC spp as well, depicted in Figure 4. CHROM agar was used as a differential isolation medium on the basis of

colour given by various *Candida* spp. as per the manufacturer's instructions (Himedia, India). It showed different colours of *Candida* spp. after incubation of 48 to 72 hours at 37°C as shown in Figure 5.

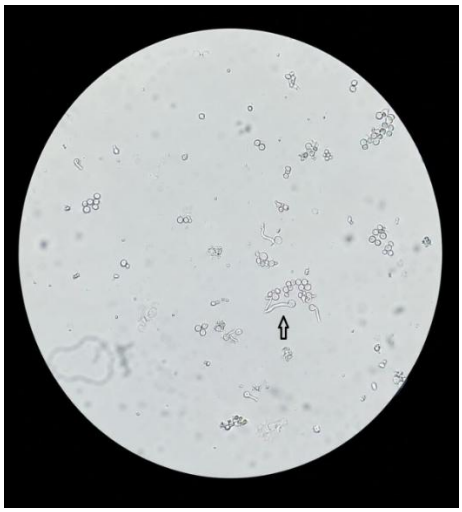

Figure 3: Germ tube positive.

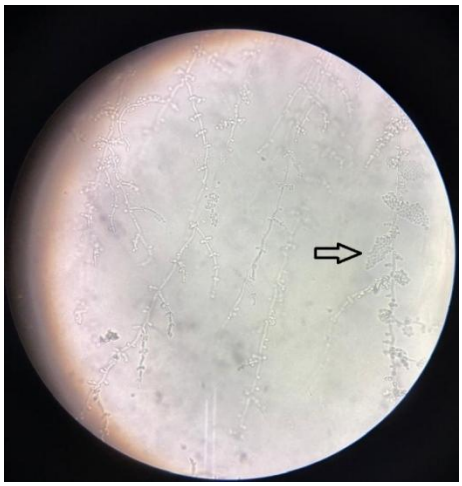

Figure 4: Blastospores in clusters seen in *C. albicans* in Dalmau technique on Corn meal agar.

Figure 5: CHROM agar showing different *Candida* species.

RESULTS

In the present study, a total of 464 *Candida* species were isolated from various clinical specimens. The results are summarized based on demographic profile, specimen distribution and species isolated. *Candida* infection was observed across all age groups ranging from <28 days to >60 years of age, with the highest (40.5%) seen in the 41-60 years age group, with female preponderance across all age groups, as shown in Table 1.

Table 1: Relative risk of abnormal Doppler indices with adverse perinatal outcome.

Age group	No. of patients (%)	Male	Female
<28 days	7 (1.5)	3	4
1-4 years	3 (0.6)	1	2
5-20 years	59 (12.7)	27	32
21-40 years	154 (33.2)	70	84
41-60 years	188 (40.5)	86	102
>60 years	53 (11.4)	23	30
Total	464	210 (45.3%)	254 (54.7%)

Table 2: The distribution of *Candida* species in various clinical specimens.

Name of sample	Total no. of isolates (%)
Urine	175 (37.7)
Sputum	131 (28.2)
High vaginal swab	65 (14.0)
Blood	52 (11.2)
Pus	33 (7.1)
Catheter tip	05 (1.1)
Stool	03 (0.6)

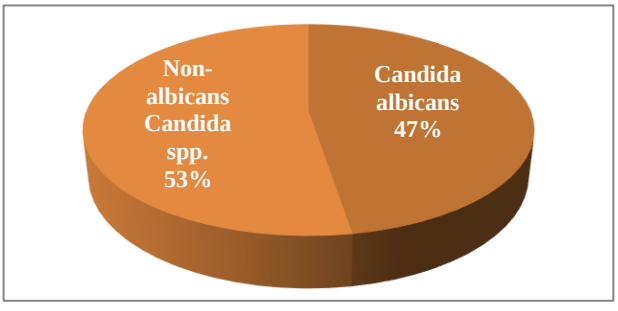

Figure 6: Distribution of *Candida albicans* and non-albicans spp.

The highest number of *Candida* isolates were obtained from urine samples 37.7% (n=175), followed by 28.2% (n=131) from sputum. The distribution of *Candida* species in various clinical specimens is shown in Table 2. Out of 464 samples, *non-albicans Candida* spp. were 244 (53%) as compared to *Candida albicans* 220 (47%) as shown in Figure 6. Amongst the *non-albicans Candida*, *C. tropicalis* was the commonest spp. isolated.

Table 3: CHROMagar and Dalmau culture findings of various *Candida* species.

Organism	CHROMagar findings	Dalmau culture findings
Candida albicans	Light green	Pseudohyphae (some true hyphae) with clusters of blastoconidia at the septa; large, thick-walled, single terminal chlamydospores
Candida tropicalis	Metallic blue	Blastoconidia in singles or very small groups along long pseudohyphae (some true hyphae)
Candida parapsilosis	Cream	Blastoconidia singly or in small clusters, along the pseudohyphae which are relatively short and have a crooked or curved appearance.
Candida krusei	Pink	Pseudohyphae with elongated blastoconidia forming a cross matchstick or tree-like appearance,
Candia kefyr	Magenta	Pseudohyphae with elongate blastoconidia, characteristically lined up in parallel, giving the appearance of logs in a stream.

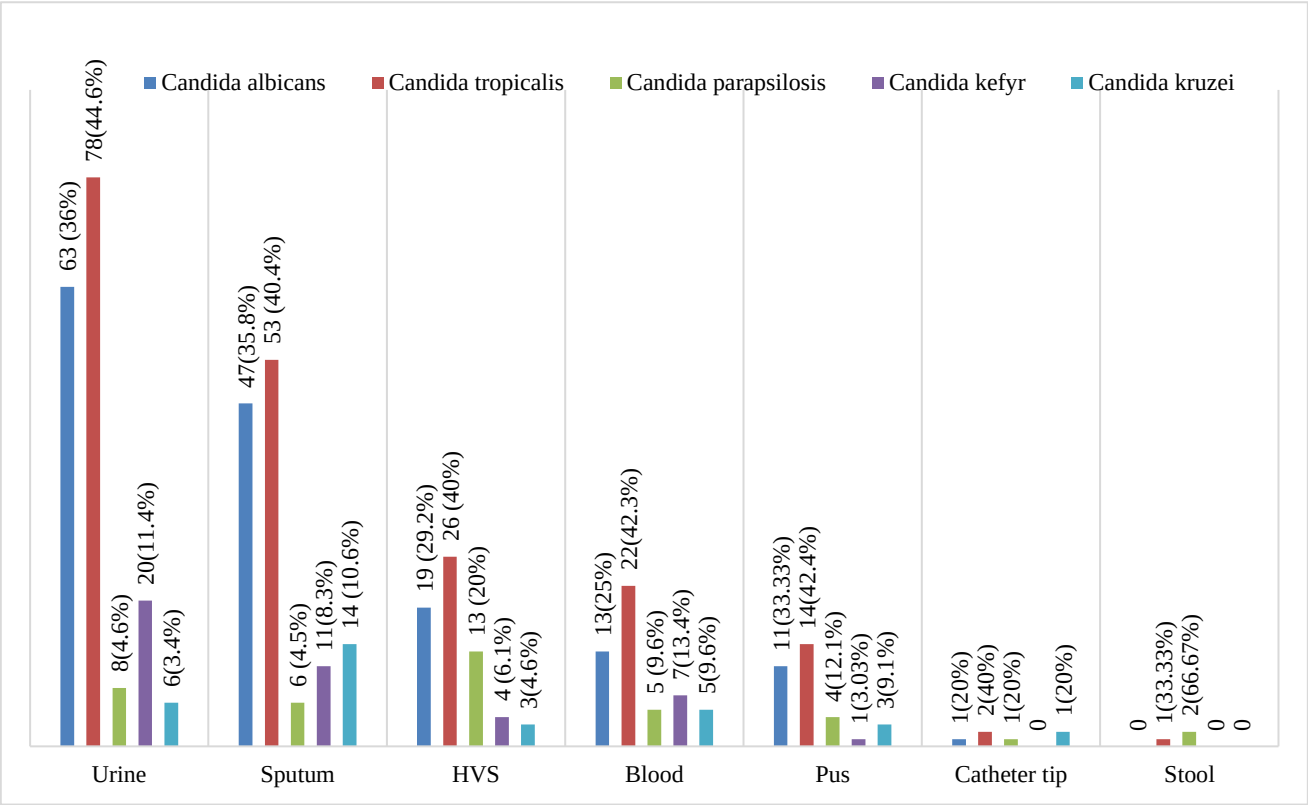

Figure 7: Species distribution of *Candida* spp. in various clinical specimens.

DISCUSSION

In recent years, *Candida* species have become prominent opportunistic pathogens responsible for severe healthcare-associated infections. The rise of non-albicans species and emerging drug resistance makes prompt identification upto the species level a mandate. The last few decades have witnessed a sharp increase in the frequency of *Candida* infections, a trend that directly correlates with rising numbers of immunocompromised patients and prolonged hospitalizations."

In the present study, a total of 464 *Candida* species were isolated from various clinical specimens. *Candida* infection was observed across all age groups ranging from <28 days to >60 years of age, with the highest (40.5%) seen in the 41-60 years age group, followed by 21-40 years age group (33.2%). This may be owing to the various independent factors like diabetes mellitus, any surgical procedure, prolonged exposure to broad-spectrum antibiotics during hospitalisations or any other associated risk factors common in the aforementioned age group. This correlates with the study by Sharma et al maximum cases (41.8%) were from adult age group from years 41 to 60 years age group.[7] Female preponderance was seen in the study across all age groups. This correlated with the study by Amar et al which isolated *Candida* species more from

female (60.2%) than male (39.8%) patients in ratio of 0.6:1 (M>F).[8]

The highest number of *Candida* isolates in the study were obtained from urine samples 37.7% (n=175), followed by 28.2% (n=131) from sputum. This correlates with the study by Samyuktha et al and Anita et al which also isolated highest *Candida* spp. from urine (41.6%) followed by sputum (20.4%).[9,10] This indicates *Candida* infection being commonest in urinary and respiratory tract infections. In the study, non-albicans *Candida* spp. was common 244 (53%) as compared to *Candida albicans* 220 (47%). This correlated with the study by Sharma et al who also described *non-albicans Candida* as the predominant spp with majority 75%.[7] Similar to this study, in the study also, *C. tropicalis* predominated among NAC spp. Also, Chander et al and Kaur et al showed higher prevalence by *C. tropicalis*.[11,12]

In the study, highest *Candida* spp. were isolated from urine samples (37.7%, n=175), followed by sputum (28.2%) and HVS (14%). This correlates with the study by Arasi Samyuktha et al and which also showed majority of *Candida* spp. isolated from urine 41.6%, followed by sputum 20.4%, high vaginal swab 14%.[9] Among the urine samples, *C. troplicais* (44.6%) was commonest followed by *C. albicans* (36%), *C. kefyr* (11.4%), *C. parapsilosis* (4.6%) and *C. krusei* (3.4%). These findings correlated with Anita et al who depicted C tropicalis (15/87) as the commonest isolate from urine sample (40/87, 45.9%) followed by *C. albicans* (14/87).[10] In the study, *C. troplicais* (40.4%) was commonest isolated from sputum sample (38.2% n=131) followed by *C. albicans* (35.8%). However, Anita et al showed *C. glabrata* (6/45) and *C. tropicalis* (6/45) were predominant followed by *C. albicans* (4/45) from sputum sample.[10] Similarly, in the study, *C. troplicais* predominated from all clinical samples like HVS (40%), blood (42.3%) pus (42.4%) and catheter tip (40%) also followed by C. albicans. However, in stool sample, *C. parapsilosis* was commonest followed by *C. tropicalis*. Thus, NAC spp predominated across all specimens as compared to *Candida* spp. This NAC spp dominance correlates with study by Naik et al which showed NAC (77.05%) and *Candida albicans* (22.95%).[13]

In the study, speciation methods used were chromogenic agar which is a new and rapid colour based medium. The findings matched with the study by Roopa et al and Pramod et al Dalmau method on corn-meal agar of *Candida* speciation was also used in the study.[14,15] Findings were matched with standard reference textbooks.

CONCLUSION

The increased isolation of *non-albicans Candida* (NAC) species across all specimens in this study mirrors a growing global emergence of these pathogens. Because NAC species exhibit significantly higher resistance to azole antifungals compared to *Candida* albicans, precise species-level identification is critically important. From a clinical perspective, identifying the specific *Candida* species directly guides effective therapeutic choices, prevents treatment failures associated with empiric azole therapy, and optimizes patient outcomes through targeted antifungal stewardship.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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