

Speciation and Antifungal Susceptibility Testing of *Candida* Isolated from Various Clinical Specimens at a Tertiary Care Centre: A Cross-sectional Study

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ABSTRACT

Introduction: *Candida* species are a normal commensal flora of the human body, colonising the skin, mucous membranes and gastrointestinal tract. However, they may also be associated with superficial and deep-seated fungal infections. In recent years, Non *albicans* *Candida* (NAC) species have emerged as significant pathogens causing severe infections in humans. Commonly used antifungal drugs show significant variation in susceptibility patterns among different *Candida* species. The incidence of drug resistance has been increasing over the last few decades due to the random use of antifungal agents. Therefore, the introduction of newer antifungal agents and changes in drug susceptibility patterns of *Candida* species have made in-vitro susceptibility testing of antifungal agents increasingly relevant for selecting sensitive drugs.

Aim: To identify species of *Candida* isolated from various clinical samples and to perform Antifungal Susceptibility Testing (AFST) using the disc diffusion method.

Materials and Methods: This study was a single-centre cross-sectional investigation involving 100 consecutive, non duplicate *Candida* isolates from various clinical samples obtained from patients at the diagnostic Microbiology laboratory of Karnataka Medical College and Research Institute, Hubballi, Karnataka, India from January 2023 to December 2023. One hundred consecutive clinical specimens (blood, urine, stool, sputum, oropharyngeal swabs, vaginal swabs, wound swabs, pus, Cerebrospinal fluid (CSF), skin and nail samples, and other body fluids) that yielded *Candida* species were included

in the study. *Candida* species were identified by the type and colour of colonies on HiCrome *Candida* differential agar as per the manufacturer's instructions. All isolates were subjected to AFST and interpretation was performed using the disc diffusion method. The data collected were entered into MS Excel and analysis was conducted using statistical software called Statistical Package for the Social Sciences (SPSS) version 28.0. Pearson's Chi-square test was used to compare differences and to find associations between variables.

Results: Out of 100 *Candida* isolates, 38 (38%) were identified as *Candida albicans*, while 62 (62%) were NAC species. The most affected age group was from 1 to 10 years, with 27 (27%) cases. The male to female ratio was 1:1.08. Intensive Care Units (ICUs) reported *Candida* species more frequently, with 51 (51%) cases compared to other areas in the hospital. The predominant source of infection was found to be urine samples, accounting for about 60 (60%). A total of five different *Candida* species were recorded. Among these, *Candida albicans* was the most predominant species, with 38 (38%), followed by *Candida tropicalis* (32, 32%), *Candida krusei* (16, 16%), *Candida parapsilosis* (13, 13%), and *Candida glabrata* (1, 1%). Major resistance was observed to fluconazole (25, 25%) and clotrimazole (6, 6%), while major susceptibility was reported for nystatin (97, 97%).

Conclusion: The emergence of different *Candida* species and the data regarding their resistance patterns may assist clinicians in selecting appropriate antifungal therapy to treat invasive and systemic *Candida* infections.

Keywords: *Candida albicans*, Fungal drug resistance, Non *albicans* *Candida*

INTRODUCTION

Candida is an omnipresent human commensal yeast-like fungus. When the host's resistance is lowered, either locally or systemically, *Candida* can become a pathogen and cause infections [1]. *Candida* species can cause varied clinical manifestations ranging from acute, subacute and chronic to episodic forms. Involvement may be localised or systemic, as seen in endocarditis, septicaemia and meningitis [2,3]. Historically, *Candida albicans* (*C. albicans*) has been the predominant cause of candidiasis. In the 1980s, *C. albicans* accounted for a greater number of nosocomial yeast infections than other species of *Candida*. Over the last few decades, an increase in the prevalence of non *albicans* species has been noted [4]. NAC species such as *Candida tropicalis* (*C. tropicalis*), *Candida krusei* (*C. krusei*), *Candida glabrata* (*C. glabrata*), and *Candida parapsilosis* (*C. parapsilosis*) are less susceptible to azoles, particularly fluconazole [5]. Therefore, correct identification of *Candida* species is essential for early and appropriate antifungal therapy.

Debilitated patients, especially those in ICUs, are at risk for nosocomial infections such as bloodstream infections caused by *Candida* [6]. To help prevent severe illness and death from these infections, and to reduce delays in diagnosis and treatment, awareness of the possibility of fungal co-infection is crucial [7]. To facilitate this, clinical laboratories need to expand their yeast identification capabilities [8].

Conventionally, *Candida* species are identified using the germ tube test, along with sugar assimilation and fermentation tests. Newer methods that have been developed for the speciation of *Candida* isolates include CHROM agar, API systems, Vitek 2 ID systems and molecular methods [9]. Conventional methods are time-consuming and labour-intensive, while automated and molecular methods are often expensive.

Chrom agar is a differential medium that allows for the selective isolation of yeasts and the identification of colonies of *C. albicans*

and other NAC species. It contains chromogenic substrates that react with enzymes secreted by microorganisms, producing colonies with various colours. These enzymes are species-specific, allowing organisms to be identified to the species level by their colour and colony characteristics [4].

Hence, the present study was undertaken to speciate *Candida* isolated from various clinical samples using HiCrome *Candida* differential agar, which is a novel method, and to perform AFST using the disc diffusion method at a tertiary care hospital. The primary objective was to assess the incidence of different species of *Candida* in various samples received at the Microbiology laboratory, while the secondary objective was to determine the in-vitro susceptibility pattern of *Candida* species to six antifungals: fluconazole, voriconazole, itraconazole, clotrimazole, nystatin and amphotericin B.

MATERIALS AND METHODS

This single-centre cross-sectional study was conducted by the Department of Microbiology for a duration of one year, from January 2023 to December 2023, at Karnataka Medical College and Research Institute, Hubballi, Karnataka, India. A total of 100 consecutive non duplicate isolates from various clinical specimens yielding *Candida* isolates were considered for the study. The study protocols were approved by the ethics committee of the Institute, bearing IEC number: KIMS:ETHICS COMM: 84:2023-24.

Inclusion criteria:

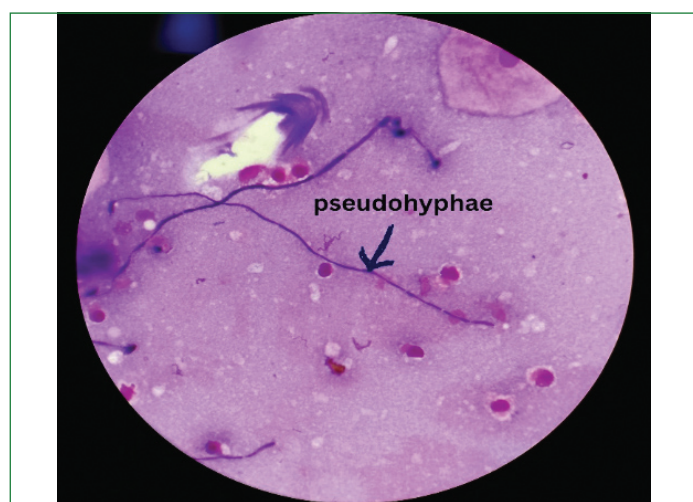
1. *Candida* isolated in pure culture from blood and sterile fluid.
2. Pure or mixed culture from urine, pus, nasal/oral/vaginal swabs, but not more than two isolates showing pseudohyphae along with budding yeast-like cells in a Direct Gram stain.

Exclusion criteria:

1. Stool samples yielding *Candida* species.
2. Mixed culture from urine, pus, nasal/oral/vaginal swabs involving more than two isolates showing pseudohyphae along with budding yeast-like cells in a Direct Gram stain.

Sample size: A non probability sampling technique was used. A total of 100 consecutive non duplicate isolates from various clinical specimens yielding *Candida* isolates were included in the study.

The primary laboratory diagnosis of specimens was performed using wet mounts and Gram stains. All suspected samples were inoculated on Sabouraud's dextrose agar (HIMEDIA- MH063- 500 G, HiMedia Pvt. Ltd., Mumbai) slope, supplemented with chloramphenicol and aerobically incubated at 37°C for 24-48 hours. Any visible growth on the SDA slope was processed for further identification. From the isolated colony, macroscopic examination and Gram staining were performed [Table/Fig-1]. After confirmation of Gram-positive budding



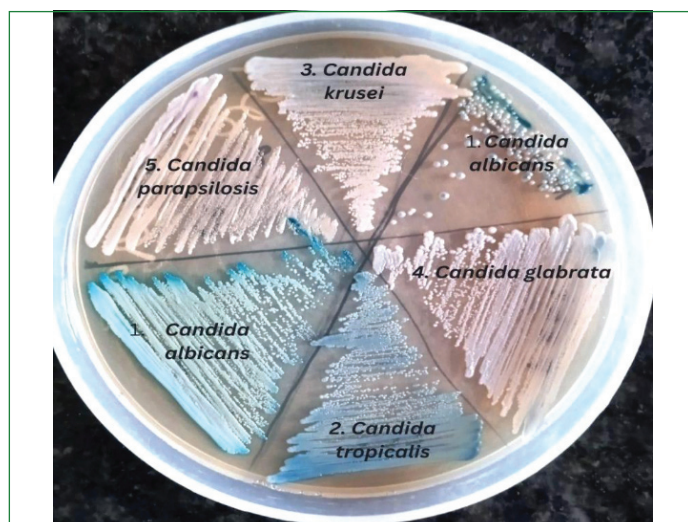
[Table/Fig-1]: Gram staining of *Candida albicans* showing pseudohyphae (Magnification: 100X).

yeast-like cells on microscopy, a germ tube test [Table/Fig-2] was performed to differentiate *C. albicans* and *Candida dubliniensis* from other *Candida* species. *C. albicans* was further differentiated from *Candida dubliniensis* by its ability to grow at 40°C.



[Table/Fig-2]: Germ tube test of *Candida albicans* (Magnification: 40X).

Simultaneously, all the *Candida* isolates were inoculated on HiCrome *Candida* Differential Agar (HIMEDIA- M1297A- 500G, HiMedia Pvt. Ltd., Mumbai) and incubated aerobically at 37°C for 24-48 hours, allowing for species identification based on the type and colour of the colonies on HiChrom *Candida* Differential Agar, as per the manufacturer's instructions [Table/Fig-3,4] [10].



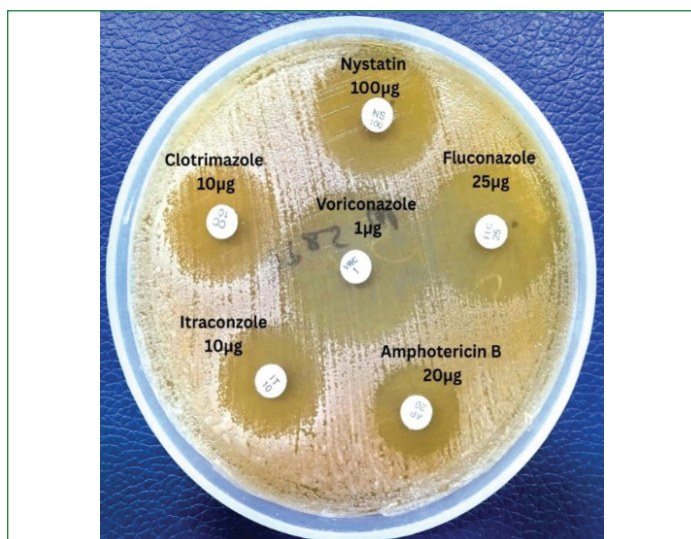
[Table/Fig-3]: HiChrom Differential *Candida* agar showing different species of *Candida*.

<i>Candida albicans</i>	Light green
<i>Candida tropicalis</i>	Metallic blue
<i>Candida krusei</i>	Rose pink
<i>Candida glabrata</i>	White
<i>Candida parapsilosis</i>	Pale cream

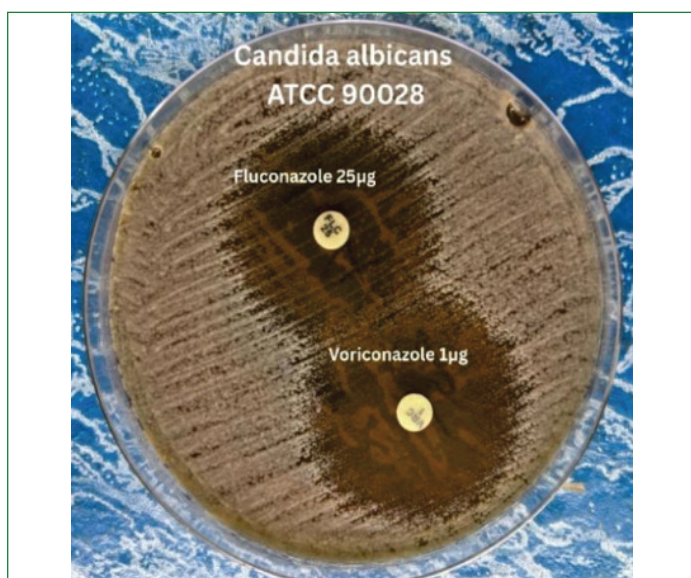
[Table/Fig-4]: Colour of various *Candida* spp. on HiChrom agar for identification [10].

All isolates were subjected to AFST [Table/Fig-5]. The inoculum was prepared by suspending five colonies of growth in 5 mL of sterile saline and comparing the turbidity to the 0.5 McFarland standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller-Hinton agar supplemented with 2% glucose. Antifungal discs (fluconazole, voriconazole, itraconazole, nystatin, clotrimazole, and amphotericin B) were placed on the inoculated media. The zone of inhibition around each disc was measured after incubating the media at 37°C for 24 hours and interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines [11]. Quality control was performed

for AFST with a *C. albicans* strain for fluconazole and voriconazole [Table/Fig-6], and interpretation was done according to CLSI M44-A document guidelines [11]. The MIC was not performed due to a lack of resources and trained personnel.



[Table/Fig-5]: Antifungal Susceptibility Testing (AFST) by disc diffusion method *Candida albicans*.



[Table/Fig-6]: *Candida albicans* ATCC 90028 as quality control for AFST.

STATISTICAL ANALYSIS

The data collected was entered into MS Excel and analysis was performed using the statistical software IBM SPSS Statistics version 28.0. Pearson's Chi-square test was used to compare the differences and find the association of susceptibility to antifungal drugs between *C. albicans* and NAC species. A p-value <0.05 was considered significant.

RESULTS

During the study period, a total of 100 consecutive samples yielding *Candida* isolates were considered. The sources of the samples from which *Candida* species were isolated are represented in [Table/Fig-7]. The clinical samples processed included urine, vaginal swabs, blood, endotracheal (ET) secretions, nail clippings, otomycoses debris, ascitic fluid and pus. *Candida albicans* was the predominant species isolated from urine, vaginal secretions and blood samples. *Candida tropicalis* predominated in urine samples, followed by *Candida krusei* in both urine and blood samples [Table/Fig-8].

In the demographic data, all age groups from day 1 to 90 years were included in the study, with the most affected age group being

Samples	n (%)
Urine	60 (60)
Vaginal swab	11 (11)
Blood	21 (21)
ET tube tip	1 (1)
Nail clipping	1 (1)
Otomycotic debris	1 (1)
Ascitic fluid	1 (1)
Pus	4 (4)
Total	100 (100)

[Table/Fig-7]: Isolation of *Candida* species in clinical samples.

from day 1 to 10 years (27, 27%), followed by the age group of 21 to 30 years (23, 23%). Among the paediatric age group, samples from neonates (0-1 month) yielded the maximum number of *Candida* species [Table/Fig-9]. The male-to-female ratio was 1:1.08, with more *Candida* species isolated from samples collected from females (52, 52%) than from males (48, 48%). A total of five different types of *Candida* species were isolated from the 100 samples. *C. albicans* predominated with 38 (38%). Among NAC, *Candida tropicalis* was the most frequently isolated species (32, 32%), followed by *Candida krusei* (16, 16%), *Candida parapsilosis* (13, 13%), and *Candida glabrata* (1, 1%). The area wise distribution of *Candida* species is shown in [Table/Fig-10].

Antifungal susceptibility pattern: The in-vitro susceptibility pattern of *Candida* species to six antifungals-fluconazole, voriconazole, itraconazole, nystatin, clotrimazole and amphotericin B- has been tabulated in [Table/Fig-11a,b]. Drug susceptibility testing was carried out for all 100 *Candida* isolates. Among the 100 isolates, 97 (75%) were susceptible strains, while 25 (25%) were resistant to one or more drugs. Out of 38 *C. albicans* isolates, 36 (94.7%) were sensitive to all antifungals tested, 35 (92.1%) were sensitive to clotrimazole, and 2 (5.2%) were resistant to all antifungal drugs. Among the 62 NAC isolates, *Candida tropicalis* showed 32 (100%) sensitivity to nystatin, while 29-27 (90.6%-84.3%) strains were sensitive to azoles, and 30 (93.7%) were sensitive to amphotericin B. All 16 *Candida krusei* isolates (100%) were sensitive to voriconazole, itraconazole, nystatin, and amphotericin B, but were intrinsically resistant to fluconazole; 13 (81.2%) were sensitive to clotrimazole. All 13 *Candida parapsilosis* isolates (100%) were sensitive to voriconazole, 12 (92.3%) were sensitive to amphotericin B and nystatin, 11 (84.6%) were sensitive to Fluconazole and itraconazole, and 9 (69.2%) were sensitive to clotrimazole. *Candida glabrata* was (100%) sensitive to all antifungal drugs tested. The antifungal resistance pattern was comparatively higher in NAC than in *C. albicans*. The resistance pattern in the study may be due to the increased use of azoles, over-the-counter use of drugs and short-term courses of antifungal therapy prescribed.

On comparison of *C. albicans* and NAC resistance to all six drugs using the Pearson Chi-square test, the p-value was <0.05 (p=0.0012). Thus, there was a statistical significance between the organism and the drugs [Table/Fig-12]. The drugs and organism association revealed a significant difference at the 5% level (p-value <0.001).

DISCUSSION

Candida species significantly vary in their antifungal susceptibility. Therefore, it is very important to identify the specific *Candida* species isolated in the laboratory from different clinical samples to select the most effective antifungal therapy. Fungal infections caused by *Candida* species have increased in frequency over the last few decades. In this study, a total of 100 consecutive samples yielding *Candida* isolates were considered from various clinical specimens. The majority of *Candida* species were isolated from urine and sputum, covering 90%, which indicates a higher incidence and distribution of *Candida* species causing urinary tract and respiratory tract infections.

Species	Urine	Blood	Vaginal secretions	Pus	Sputum	Nail clipping	Ascitic fluid	Total (%)
<i>C. albicans</i>	22 (22%)	9 (9%)	6 (6%)	1 (1%)	0	0	0	38 (38%)
<i>C. tropicalis</i>	27 (27%)	1 (1%)	3 (3%)	0	0	0	1 (01%)	32 (32%)
<i>C. krusei</i>	7 (7%)	5 (5%)	1 (1%)	2 (2%)	0	1 (1%)	0	16 (16%)
<i>C. parapsilosis</i>	3 (3%)	6 (6%)	1 (1%)	2 (2%)	1 (1%)	0	0	13 (13%)
<i>C. glabrata</i>	1 (1%)	0	0	0	0	0	0	1 (1%)
Total	60 (60%)	21 (21%)	11 (11%)	5 (5%)	1 (1%)	1 (1%)	1 (1%)	100 (100%)

[Table/Fig-8]: Sample- wise distribution of *Candida* species.
C= *Candida*

Age group		Males		Females		Number of cases (%)
1-10 years	0-1 month	11 (11%)	17 (17%)	5 (5%)	10 (10%)	27 (27%)
	1 month-1 year	4 (4%)		2 (2%)		
	2-10 years	2 (2%)		3 (3%)		
11-20 years		1 (1%)		5 (5%)		6 (6%)
21-30 years		4 (4%)		19 (19%)		23 (23%)
31-40 years		5 (5%)		4 (4%)		9 (9%)
41-50 years		4 (4%)		3 (3%)		7 (7%)
51-60 years		5 (5%)		5 (5%)		10 (10%)
61-70 years		8 (8%)		2 (2%)		10 (10%)
71-80 years		3 (3%)		3 (3%)		6 (6%)
81-90 years		1 (1%)		1 (1%)		2 (2%)
Total		48 (48%)		52 (52%)		100 (100%)

[Table/Fig-9]: Age-wise distribution of samples.

Area of hospital	Number of cases (%)
OPD	9 (9)
Ward	34 (34)
ICU	51 (51)
Labour room	6 (6)

[Table/Fig-10]: Area-wise distribution of *Candida* species.

Species	Fluconazole			Voriconazole			Itraconazole			Clotrimazole		
	S	I	R	S	I	R	S	I	R	S	I	R
<i>C. albicans</i> (38)	36 (94.7%)	0	2 (5.3%)	36 (94.7%)	0	2 (5.3%)	36 (94.7%)	1 (2.6%)	1 (2.6%)	35 (92.1%)	1 (2.6%)	2 (5.3%)
<i>C. tropicalis</i> (32)	27 (84.4%)	0	5 (15.6%)	29 (90.6%)	0	3 (9.4%)	28 (87.5%)	3 (9.4%)	1 (3.1%)	21 (65.6%)	7 (21.9%)	4 (12.5%)
<i>C. krusei</i> (16)	0	0	16 (100%)	16 (100%)	0	0	16 (100%)	0	0	13 (81.3%)	3 (18.7%)	0
<i>C. parapsilosis</i> (13)	11 (84.6%)	0	2 (15.4%)	13 (100%)	0	0	11 (84.6%)	1 (7.6%)	1 (7.6%)	9 (69.2%)	3 (23.1%)	1 (7.7%)
<i>C. glabrata</i> (1)	1 (100%)	0	0	1 (100%)	0	0	1 (100%)	0	0	0	1 (100%)	0
Total (%)	75 (75%)	0	25 (25%)	95 (95%)	0	5 (5%)	92 (92%)	5 (5%)	3 (3%)	78 (78%)	15 (15%)	7 (7%)

[Table/Fig-11a]: Antifungal susceptibility profile of *Candida* species for azoles.
S= Sensitive; I= Intermediate; R= Resistant

Species	Nystatin			Amphotericin B		
	S	I	R	S	I	R
<i>C. albicans</i> (38)	36 (94.7%)	0	2 (5.3%)	36 (94.7%)	0	2 (5.3%)
<i>C. tropicalis</i> (32)	32 (100%)	0	0	30 (93.8%)	2 (6.2%)	0
<i>C. krusei</i> (16)	16 (100%)	0	0	16 (100%)	0	0
<i>C. parapsilosis</i> (13)	12 (92.3%)	0	1 (7.7%)	12 (92.3%)	0	1 (7.7%)
<i>C. glabrata</i> (1)	1 (100%)	0	0	1 (100%)	0	0
Total (100)	97 (97%)	0	3 (3%)	95 (95%)	2 (2%)	3 (3%)

[Table/Fig-11b]: Antifungal susceptibility profile of *Candida* species for polyenes.
S= Sensitive; I= Intermediate; R= Resistant

Drugs	<i>Candida albicans</i>		Non-albicans <i>Candida</i> (NAC)	
	Sensitive	Resistant	Sensitive	Resistant
Fluconazole	36 (94.7%)	2 (5.2%)	39 (62.9%)	23 (37%)
Voriconazole	36 (94.7%)	2 (5.2%)	59 (95.1%)	3 (4.8%)
Itraconazole	36 (94.7%)	1 (2.6%)	56 (90.3%)	2 (3.2%)
Nystatin	36 (94.7%)	2 (5.2%)	61 (98.3%)	1 (1.6%)
Clotrimazole	35 (92.1%)	2 (5.2%)	43 (69.3%)	5 (8%)
Amphotericin B	36 (94.7%)	2 (5.2%)	59 (95.1%)	1 (1.6%)
Chi-square	10.448			
p-value	0.0012 (<0.05)			

[Table/Fig-12]: Comparison of resistance pattern of *C. albicans* and NAC to six anti-fungal drugs by the Pearson Chi-square test.

In studies conducted by Madhumati B et al., and Raj Kumari S et al., a comparatively higher proportion of *C. albicans* was isolated than in the current study [15,16]. As depicted in the table, studies conducted by Roopa C et al., Kanna BV et al., and Devi S et al., showed that *C. albicans* predominated compared to NAC [17-19].

Although *Candida* was isolated more frequently from female patients (52, 52%) than from males (48, 48%), there was not much difference in the male-to-female ratio. Similar findings were reported in the studies by Ekpo IA et al., and Goel R et al., [20,21]. However, a study conducted in Sweden by Lindberg E et al., showed a male preponderance [22]. Most of the *Candida* isolates among female and male patients were cases of Urinary Tract Infections (UTI) and sepsis, respectively.

Studies	Number of <i>Candida albicans</i> isolated (%)	Number of NAC isolated (%)
Present study	38 (38)	62 (62)
Yashavanth R et al., [12]	20 (30.3)	46 (69.7)
Awari A [13]	40 (36.6)	69 (63.3)
Golia S et al., [14]	40 (36)	72 (64)
Madhumati B and Rajendran R, [15]	46 (46)	54 (54)
Rajkumari S and Adhikaree N, [16]	71 (45.5)	85 (54.5)
Roopa C and Biradar SK, [17]	69 (50.7)	67 (49.3)
Vignesh Kanna B et al., [18]	26 (51)	24 (49)
Devi LS and Maheshwari M, [19]	31 (52)	29 (48)

[Table/Fig-13]: Comparative analysis between the present study and the studies conducted in the past [12-19].

In the present study, the prevalence of *C. albicans* was 38 (38%), and NAC was 62 (62%), which was comparable to studies conducted by Yashavanth R et al., Awari A, and Golia S et al., [Table/Fig-13] [12-19].

Studies	Fluconazole	Voriconazole	Itraconazole	Clotrimazole	Nystatin	Amp B
Present study	75 (75%)	95 (95%)	92 (92%)	78 (78%)	97 (97%)	95 (95%)
Roopa C and Biradar SK, [17]	76 (55.8%)	--	--	--	136 (100%)	136 (100%)
Manikandan C and Asmath A, [25]	4 (18.2%)	--	21 (95.5%)	--	--	21 (95.5%)
Jayalakshmi L, [26]	57 (54.2%)	--	79 (75.2%)	75 (71%)	104 (99%)	103 (97.1%)
Khadka S et al., [23]	64 (64%)	--	--	82 (82%)	--	--
Yashwanth R et al., [12]	22 (66.6%)	24 (72.7%)	--	--	--	30 (91%)
Adhikary R and Joshi S, [27]	51 (75%)	68 (100%)	--	--	--	63 (92%)
Khan M et al., [28]	36 (33%)	92 (85.2%)	46 (42.6%)	23 (21.3%)	27 (25%)	--

[Table/Fig-14]: Comparison of susceptibility of *Candida* isolates for antifungal drugs of present study with other studies [12,17,23,25-28].

The majority of *Candida* isolates were obtained from urine samples (60, 60%), which was consistent with the study by Khadka et al., [23]. However, in studies conducted by Madhumati B et al., Roopa C and Biradar SK and Devi S and Maheshwari M, the highest incidence of *Candida* was observed in high vaginal swabs, followed by urine and sputum samples [15,17,19].

In this study, the paediatric age group (27, 27%) was the most affected, followed by the 21-30 years age group (23, 23%), whereas in other studies, the most affected population was above 60 years [12,21]. Among the paediatric age group, the maximum number of *Candida* isolates was obtained from neonates through blood cultures, implying that *Candida* is one of the major aetiologies of neonatal sepsis. The second-highest affected age group was 21-30 years, in which most patients were young females suffering from UTIs. Most cases were from patients admitted to the ICU, similar to the findings of a study by Alfouzan WAM, [24]. This is likely attributed to the fact that most patients in the ICU are catheterised, critically-ill, and on broad-spectrum antibiotics.

The AFST pattern showed that *Candida* isolates were more susceptible to Nystatin (97, 97%) and Amphotericin B (95, 95%) than to azoles (Fluconazole: 75, 75%), which was similar to the studies conducted by Yashavanth R et al., Roopa C et al., Khadka S et al., Manikandan C et al., Jayalakshmi L et al., and Adhikary R et al., [Table/Fig-14] [12,17,23,25-27]. Resistance to azoles was higher in the NAC group compared to *C. albicans*, which aligns with the findings of Yashavanth R et al., and Khadka S et al., [12,23]. In the study conducted by Khan M et al., on vulvovaginal candidiasis, the susceptibility to fluconazole was only 36 (33%), which was not in line with the present study [28].

Among the azoles, the highest susceptibility was shown to voriconazole and itraconazole (92, 92%), followed by clotrimazole (78, 78%) and fluconazole (75, 75%). Among the frequently isolated species of *Candida*, the highest susceptibility was observed in *C. albicans* compared to *C. tropicalis*. However, in a study conducted by Vijaya D et al., azoles demonstrated comparatively better in-vitro activity, especially against *C. albicans* [10].

The findings of this study can serve as an effective guide for clinical management and antifungal stewardship, as it includes data on the distribution of *Candida* species and their emerging resistance to antifungal agents. Analysing the laboratory data on the diversity of *Candida* species, their distribution in different clinical samples, and their susceptibility pattern in a tertiary care centre would help clinicians initiate appropriate antifungal treatment.

Limitation(s)

Minimum Inhibitory Concentration (MIC) testing was not performed for antifungal susceptibility due to a lack of resources and trained personnel. Speciation of *Candida* from chromogenic differential agar was not confirmed with additional tests. Predisposing factors influencing the distribution of *Candida* species in different areas of the hospital (e.g., hospital wards, ICU settings) were not studied. The sample size of this study was small, and a larger sample size would be needed to strengthen the findings, especially for more specific species like *Candida glabrata*. This individual institution data does

not represent the total population, so it has limitations regarding extrapolation to multiple centres. Quality control for AFST was not performed with all the drugs and species involved in the study due to a lack of quality control strains.

CONCLUSION(S)

The present study statistically represents the body sites affected by different species of *Candida* and their antifungal resistance patterns. The susceptibility pattern of antifungal agents varies with each species of *Candida*. Therefore, continuous surveillance, epidemiological studies and clinical investigations performed locally at a tertiary care centre can provide a database to monitor different *Candida* infections. The fungal antibiogram profile could guide clinicians in formulating effective strategies to control invasive and systemic *Candida* infections.

REFERENCES

- Anaissie EJ, McGinnis MR, Pfaller MA. Clinical mycology. Edinburgh? Churchill Livingstone/Elsevier; 2009.
- Chander J. Textbook of medical mycology. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd; 2018: 212-230.
- Collier L, Brian WJ Mahy, Balows A, Sussman M, Duerden BI, Hausler WJ, et al. Topley and Wilson's Microbiology and microbial infections. 5. London: Arnold; 1998:423-460.
- Horvath LL, Hospenhal DR, Murray CK, Dooley DP. Direct isolation of *Candida* spp. from blood cultures on the chromogenic medium CHROMagar *Candida*. J Clin Microbiol. 2003;41(6):2629-32.
- Binesh LY, Kalyani M. Phenotypic characterization of *Candida* species and their antifungal susceptibility from a tertiary care centre. J Pharm Biomed Sci. 2011;11:01-04.
- Nucci M, Barreiros G, Guimarães LF, Deriquehem VA, Castiñeiras AC, Nouér SA. Increased incidence of candidemia in a tertiary care hospital with the COVID 19 pandemic. Mycoses. 2021;64(2):152-56.
- Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: A systematic review and meta-analysis. J Infect. 2020;81(2):266-75.
- Pfaller MA. Epidemiology of candidiasis. J Hosp Infect. 1995;30:329-38.
- Jain N, Mathur P, Misra MC, Behera B, Xess I, Sharma SP. Rapid identification of yeast isolates from clinical specimens in critically ill trauma ICU patients. J Lab Phys. 2012;4(01):030-34.
- Vijaya D, Harsha TR, Nagarathnamma T. Candida speciation using chrom agar. J Clin Diagn Res. 2011;5(4):755-57.
- Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts: Approved Guideline. Document M44-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
- Yashavanth R, Shiju MP, Bhaskar UA, Ronald R, Anita KB. Prevalence of *Candida* species among uropathogens and their antifungal susceptibility pattern in a tertiary care hospital. J Clin Diagn Res. 2013;7:2459-61.
- Awari A. Species distribution and antifungal susceptibility profile of *Candida* isolated from urine samples. Int J App Basic Med Res. 2011;18:228-34.
- Golia S, Reddy KM, Karjigi KS, Hittinahalli V. Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity. Al Ameen J Med Sci. 2013;6(2):163-66.
- Madhumati B, Rajendran R. Evaluation of chrom agar in speciation of *Candida* species from various clinical samples in a tertiary care hospital. Int J Curr Microbiol App Sci. 2015;4(9):463-72.
- Rajkumari S, Adhikaree N. Speciation of *Candida* using CHROMagar from various clinical specimens and their antifungal susceptibility pattern at a tertiary care hospital. J Coll Med Sci-Nepal. 2020;16(2):107-11.
- Roopa C, Biradar SK. Isolation of *Candida* and its speciation in various samples in a tertiary care hospital in North Karnataka, India. Int J Curr Microbiol App Sci. 2015;4:996-1000.
- Vignesh Kanna B, Amar Kumar G, Swapna M, Joshy ME. Isolation and identification of candida species from various clinical samples in a tertiary care hospital. Int J Res Med Sci. 2017;5(8):3520-22.

- [19] Devi LS, Maheshwari M. Speciation of *Candida* species isolated from clinical specimens by using Chrom agar and conventional methods. *Int J Sci Res Pub*. 2014;4(3):01-05.
- [20] Ekpo IA, Kechia FA, Iwewe YS, Nguemoum AD, Nangwat C, Dzoyem JP. Species distribution and antifungal susceptibility profile of *Candida* spp isolated from urine of hospitalized patients in Dschang District Hospital, Cameroon. *Int J Biol Chem Sci*. 2017;11(3):1212-21.
- [21] Goel R, Singh S, Gill AK, Kaur A, Kour I. Speciation, characterization and antifungal susceptibility pattern of *Candida* species. *Int J Contemp Med Res*. 2018;5(5):E1-E4.
- [22] Lindberg E, Hammarström H, Ataollahy N, Kondori N. Species distribution and antifungal drug susceptibilities of yeasts isolated from the blood samples of patients with candidemia. *Scientific Reports*. 2019;9(1):3838.
- [23] Khadka S, Sherchand JB, Pokhrel BM, Parajuli K, Mishra SK, Sharma S, et al. Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal. *BMC Research Notes*. 2017;10:01-05.
- [24] Alfouzan WAM. Epidemiological study on species identification and susceptibility profile of *Candida* in urine. *Fungal Genom Biol*. 2015;5:124. Doi: 10.4172/2165-8056.1000124.
- [25] Manikandan C, Amsath A. Characterization and susceptibility pattern of *Candida* species isolate d from urine samples in Pattukkottal, Tamilnadu, India. *International Journal of Pure and Applied Zoology*. 2015;3:17-23.
- [26] Jayalakshmi L. Isolation, speciation and antifungal susceptibility testing of *Candida* from clinical specimens at a tertiary care hospital. *Sch J App Med Sci*. 2014;2(6E):3193-98.
- [27] Adhikary R, Joshi S. Species distribution and antifungal susceptibility of candidaemia at a multi super speciality center in southern India. *IJ Med Microbiol*. 2011;29(3):309-11.
- [28] Khan M, Ahmed J, Gul A, Ikram A, Lalani FK. Antifungal susceptibility testing of vulvovaginal *Candida* species among women attending antenatal clinic in tertiary care hospitals of Peshawar. *Infection and Drug Resistance*. 2018:447-56.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Feb 04, 2025
- Manual Googling: Jun 05, 2025
- iThenticate Software: Jun 20, 2025 (21%)

ETYMOLOGY: Author Origin**EMENDATIONS:** 7**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 31, 2025**Date of Peer Review: **Mar 19, 2025**Date of Acceptance: **Jun 16, 2025**Date of Publishing: **Jul 01, 2025**