

# Antifungal Susceptibility Pattern of Clinical Isolates of *Candida* from a Tertiary Care Hospital in Chhattisgarh, India

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## Abstract

*Candida* continues to be leading cause of morbidity and mortality in large population of immunocompromised and hospitalized patients. Invasive Candidiasis due to non-albicans *Candida* has been on the rise in last few years. Incidence rates vary geographically, often because of different patient populations studied. The present study was conducted to find out the species distribution and antifungal susceptibility of *Candida* species from different sources and patient population of our tertiary care hospital. A total of 103 *Candida* species were isolated from the different clinical specimens of suspected candida infection cases. In this study, it was observed that candidiasis can occur at all ages and in both sexes. 82 (79.6%) isolates were obtained from cases admitted in different inpatient departments of which 51 (49.5%) accounted for isolates from various ICUs mainly NICU. Most of the isolates obtained were from urine samples (44.6%) followed by blood (34.9%). Non albicans *Candida* were isolated at a higher rate (52.8%) than *Candida albicans* (47.5%). Among all species of *Candida* commonest isolate was *C. albicans* (47.5%) followed by *C. tropicalis* (26.21%). Overall high susceptibility to voriconazole and Amphotericin B (99.03%). This study emphasizes the need for monitoring local epidemiologic data and antifungal susceptibility pattern of *Candida* isolates for proper treatment.

**Keywords:** *Candida*, Antifungals susceptibility, non-albicans *Candida*.

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## INTRODUCTION

Developments in diagnostic modalities and therapeutic options has contributed to invasive fungal infections and colonisation in large population of immunocompromised patients and /or those hospitalized with serious underlying conditions. Among these risk groups, *Candida* spp. is a leading cause of morbidity and mortality. There has been increase in the rate of candida infections in different clinical settings throughout the world [1]. Candidiasis which accounts for 66-80% of fungal infections, is a primary or secondary infection involving a member of genus *Candida*. *Candida* species are associated with vast clinical spectrum of human infections ranging from superficial infection of the skin, mucus membranes to life threatening candidemia, and hospital-acquired infections.[2] Invasive candidiasis includes severe diseases such as candidemia, disseminated infections,

CNS infections, endophthalmitis, osteomyelitis. Candidemia which is a bloodstream infection by *Candida* species is the most common and fatal clinical manifestation of invasive candidiasis, and contribute to large number of morbidity and mortality in hospitalized patients [3]. Candidemia is the most common nosocomial bloodstream infection reported from USA and Europe [4] Similarly, *Candida* spp contribute to 10-15% of nosocomial urinary tract infections (UTIs).

Most common precipitating factors of invasive candidiasis are HIV/AIDS, underlying malignancies, invasive interventions, use of broad-spectrum antibiotics, parenteral alimentation, and use of intravascular catheters, long-term hospitalization, and immunosuppressive agents.

Though the *Candida* spp. forms the normal flora of human oral, gastrointestinal and genitourinary

tracts, it causes clinical infection when the host becomes debilitated or immunocompromised. The genus *Candida* includes more than 150 heterogeneous group species out of which approximately 20 different *Candida* species are pathogenic to human.

Although *Candida albicans* remains major cause of candidiasis in spite of its dwindling share. The epidemiology of non-*albicans* *Candida* has been on the rise in last few years.[2] Five species of *Candida* namely, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* are reported to cause more than 90% of invasive infections, although the relative distribution of the species depends on the geographical area, patient population and predisposing conditions, local hospital related factors, and the types of antifungal agents received[5].

The extensive use of antifungals for prophylaxis became the leading cause of colonization of non-*albicans* *Candida* (NAC) species and increasing resistance to antifungal drugs [6].

Changing etiology of candidiasis and emerging antifungal resistance necessitates early identification, speciation and antifungal susceptibility testing to select the appropriate antifungal agent to prevent the treatment failure and also to study the local epidemiology of antifungal resistance.

There are very limited data available on distribution and the antifungal susceptibility of *Candida* spp. in this region. Herein we studied distribution of *Candida* spp. isolated from various clinical specimens at our tertiary hospital, their susceptibility to antifungal agents and associated risk factors. Lack of data in this region so far needs to be addressed.

## MATERIAL & METHODS

We evaluated samples from patients of various age groups being treated for suspected Candidiasis at different departments of our tertiary care hospital in Central India over the period of four months. Detailed clinical and treatment history of patients was recorded. Yeast cultures obtained from blood, urine, sputum, bronchoalveolar lavage, cerebrospinal fluid, pus, peritoneal fluid, high vaginal/ cervical swab were included in the study. The isolation and identification of fungi were performed using standard methods in the microbiology laboratory. Samples were subjected to Gram's stain to look for presence of Gram positive yeast like budding cells with pseudohyphae and KOH mount. The samples were inoculated on Sabouraud's dextrose agar (SDA) with chloramphenicol & Blood agar and incubated at 37 °C and 25 °C [7].

### Identification & Speciation

Suspected colonies of *Candida* on SDA were confirmed by Gram's stain. Further species identification was done by a) germ tube test b) Dalmu

plate culture for Chlamyospore production c) inoculation on chromogenic medium d) carbohydrate utilization patterns by Sugar Assimilation Tests. Germ tube test was carried out by inoculating isolated yeast cells into 0.5 ml of pooled human serum in a small tube and incubation at 37 °C for 2 hours. Germ tubes formation was observed microscopically as tubular elongation extending from the yeast cells without constriction or septa at the point of attachment to the yeast cells. Dalmu plate cultures were done by inoculation on corn meal agar containing 1% Tween 80. Plates were incubated at 30°C for 2-5 days and studied microscopically for the presence of pseudohyphae, chlamyospores & blastospores. Isolated *Candida* species were subcultured onto chromogenic *Candida* medium (HICROME *Candida* agar) and incubated at 37°C for 48 hr. Presumptive species identification was done based on specific colony colors produced by the chromogenic substrates in the medium [Image 1]. All isolates were identified by carbohydrate assimilation tests using the Vitek2 Compact (Biomérieux, France) using Vitek2 cards for identification of yeast and yeast like organisms (ID-YST cards) kits.

Few isolates were sent to Mycology Reference Laboratory, PGIMER, and Chandigarh for confirmation by MALDI-TOFF.

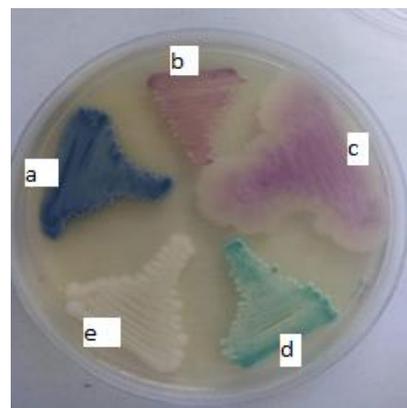


Image-1

*Candida* chrome agar showing growth: a) *C. tropicalis*, b) *C. utilis* c) *C. krusei* d). *albicans* e) *C. lusitanae*

### Antifungal Susceptibility Test

Antifungal susceptibility testing was performed for all the isolates of *Candida* using disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg / ml of methylene blue as per the procedure described in the Clinical and Laboratory Standard Institute (CLSI, 2009) against two antifungal agents. Cell suspensions of individual *Candida* strains were prepared in 5 ml saline solution. The turbidity was adjusted to yield 0.5 McFarland standards. The commercially available antifungal discs of Fluconazole (25 µg), Voriconazole (1µg) were used and zones of inhibition were measured after 20- 24 hours of incubation at 37°C [8]. [Image 2].

Zone diameter interpretative standards for tested antifungal agents against *Candida* species were:

Antifungal agent	Zone diameter (mm)		
	Sensitive	Intermediate	Resistant
Fluconazole (10 µg)	≥19	15-18	≤14
Voriconazole(1 µg)	≥17	14-16	≤13

Anti-fungal susceptibility was also performed with AST YS01 Kits on Vitek 2 compact system. Standard operative procedures as described by the manufacturer were followed. The Antifungal susceptibility values to amphotericin B, fluconazole, voriconazole and caspofungin were interpreted

according to the new species-specific clinical breakpoints defined by the CLSI in 2012 [new CLSI breakpoints] [9]. Species-specific breakpoints for antifungal agents for the most common species of *Candida* isolates.

Antifungal agent	Species	Breakpoints (µg/ml)			
		S	I	R	S-DD
Amphotericin B	<i>C.albicans</i>	<1	-	≥1	-
	<i>C.parapsilosis</i>	<1	-	≥1	-
	<i>C.guilliermondii</i>	<1	-	≥1	-
	<i>C.glabrata</i>	<1	-	≥1	-
	<i>C.krusei</i>	<1	-	≥1	-
	<i>C. tropicalis</i>	<1	-	≥1	-
Fluconazole	<i>C.albicans</i>	≤2	-	≥8	4
	<i>C.parapsilosis</i>	≤2	-	≥8	4
	<i>C.guilliermondii</i>	-	-	-	-
	<i>C.glabrata</i>	-	-	≥64	≤32
	<i>C.krusei</i>	-	-	-	-
	<i>C. tropicalis</i>	≤2	-	≥8	4
Voriconazole	<i>C.albicans</i>	≤0.12	0.25–0.5	≥1	-
	<i>C.parapsilosis</i>	≤0.12	0.25–0.5	≥1	-
	<i>C.guilliermondii</i>	≤2	4	≥8	-
	<i>C.glabrata</i>	-	-	≥1	-
	<i>C.krusei</i>	0.5	1	2	-
	<i>C. tropicalis</i>	≤0.12	0.25–0.5	≥1	-
Caspofungin	<i>C.albicans</i>	≤0.25	0.5	≥1	-
	<i>C.parapsilosis</i>	≤2	4	≥8	-
	<i>C.guilliermondii</i>	≤2	4	≥8	-
	<i>C.glabrata</i>	≤0.12	0.25	≥0.5	-
	<i>C.krusei</i>	≤0.25	0.5	≥1	-
	<i>C. tropicalis</i>	≤0.25	0.5	≥1	-

S-Susceptible R- Resistant I- Intermediate S-DD- Susceptible Dose Dependant

Image-2



Antifungal susceptibility testing of *Candida* isolates by disc diffusion method

**RESULTS**

A total of 103 *Candida* species were isolated from the different clinical specimens during the study period. Most of the isolates obtained were from urine specimens (44.6%) followed by blood (34.9%) and high vaginal swabs (10.6%) [Table 1] [Fig 1] From females, 57 (55.3%) *Candida* were isolated and 46 isolates were from male patients (44.6%). The highest number of isolates was from the age group of 0 - 1 year followed by 21 - 40 yrs. [Table 2].

In our study, among the candida species isolated, most common was *Candida albicans* (47.6%) followed by *Candida tropicalis* (26.2%) and *Candida krusei* (8.7%). [Table 3] In the present study Non albicans *Candida* (NAC) were isolated at a higher rate (52.4%) than *Candida albicans* (47.6%) [Fig 2].

In this study, 82 (79.6%) isolates were obtained from cases admitted in different inpatient departments of which 51 (49.5%) accounted for isolates from various ICUs mainly NICU. Risk factors associated were found to be prolonged hospital stay, use of broad-spectrum antibacterial agents, chemotherapy for underlying malignancy, presence of intravascular catheters, diabetes mellitus, tuberculosis, pregnancy. We identified 32 neonates with candida blood stream infections. Predisposing factors observed in these neonates were extremely low birth weight, very low

birth weight, prematurity, prolonged antibiotic therapy, indwelling catheterization.

In this study, antifungal susceptibility pattern by disk diffusion and microbroth dilution method by Vitek 2 system for fluconazole and voriconazole was found to be in agreement. Overall high susceptibility to voriconazole (100%), Amphotericin B (96.1%), Caspofungin (96.11), and fluconazole (89.3%) was observed. All isolates of the species *C. glabrata* and *C. krusei* and *C. lusitanae* were found to be susceptible to amphotericin B with MIC of 0.5 µg/ml. Only one isolate of *Candida albicans* (2.04%) isolated from blood was found to be resistant to Amphotericin B with MIC of 2 µg/ml. MIC values for all the susceptible isolates ranged from 0.5 to 4 µg/ml for fluconazole, < 0.12 to 0.25 µg/ml for Voriconazole, <0.12 to 0.25 µg/ml for Caspofungin, Amphotericin B <0.25 to 0.5 µg/ml.

**Table-1: Distribution of Candida isolates in various clinical samples**

Source	Number Of Candida Isolates	Male	Female
Blood	36 (34.9%)	24(23.3%)	12(11.6%)
Urine	46 (44.6%)	17(16.5%)	29(28.1%)
Pus	05 (4.8%)	02(1.9%)	03(2.9%)
Sputum	03 (2.9%)	02(1.9%)	01(1.9%)
Peritoneal Fluid	01 (0.9%)	0	01 (1.9%)
HVS	11 (10.6%)	0	11(10.6%)
CSF	01 (0.9%)	01(1.9%)	0
Total	103	46 (44.6%)	57 (55.3%)

**Table-2: Demographic distribution of Candida species isolates**

Age group	Candida albicans (n=49)		Nonalbicans Candida (n=54)		Total
	Male	Female	Male	Female	
<1	03	01	22	08	34 ( 33% )
1- 10YR	02	01	0	0	03(2.9%)
11-20 YR	01	05	0	05	11(10.7%)
21-30	01	14	02	02	19(18.4%)
31-40	02	04	03	04	13(12.6%)
41-50	02	03	01	02	08(7.8%)
51-60	02	01	0	03	06(5.8%)
>60	03	02	02	02	09(8.7%)
Total	16	31	30	26	103(100%)

**Table-3: Species distribution of Candida isolates**

Candida species	Total No of Isolates	Percentage
<i>Candida albicans</i>	49	47.6 %
<i>Candida tropicalis</i>	27	26.2%
<i>Candida krusei</i>	09	8.7%
<i>Candida lusitanae</i>	08	7.8%
<i>Candida utilis</i>	08	7.8%

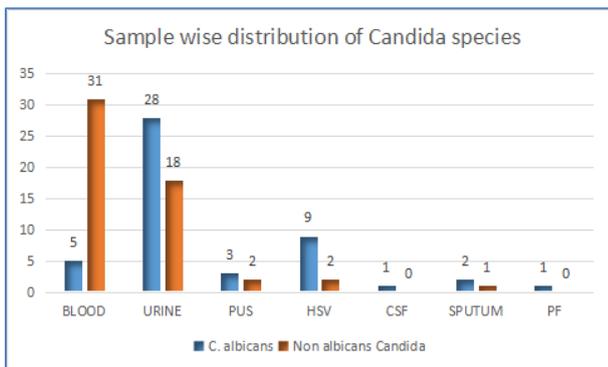
Candida glabrata	02	1.9%
TOTAL	103	100%

**Table-4: Distribution pattern of Candida species in candidemia cases (n=36)**

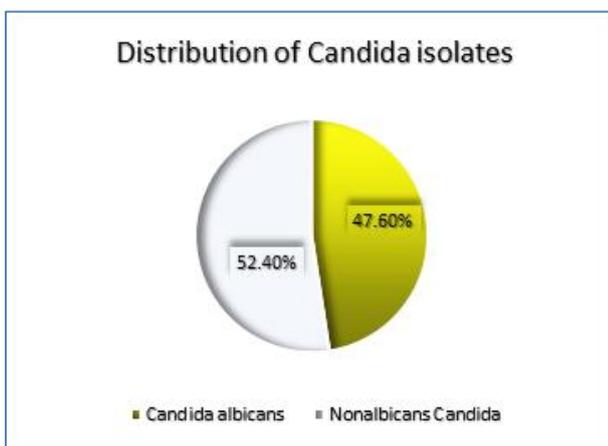
Candida Species	Total No of Isolates	Percentage
Candida albicans	05	13.9%
Candida krusei	06	16.7%
Candida lusitanae	07	19.4%
Candida tropicalis	10	27.8%
Candida utilis	07	19.4%
Candida glabrata	01	2.7%

**Table-5: Distribution pattern of Candida species in candiduria cases (n=46)**

Candida Species	Total No of Isolates	Percentage
Candida albicans	28	60.9%
Candida krusei	03	6.5%
Candida tropicalis	13	28.3%
Candida utilis	01	2.1%
Candida glabrata	01	2.1%



**Fig-1**



**Fig-2**

Fungal infections, particularly those attributed to Candida species, are frequent complications for hospitalized patients contributing to increased morbidity and mortality and healthcare cost. Furthermore there is increasing prevalence of infections caused by nonalbicans Candida worldwide with various degree of susceptibility to routinely use antifungal agents indicating the importance of laboratory diagnoses [10]. In this study, it was observed that candidiasis can occur at all ages and in both sexes. Majority of the patients were less than one year old and those admitted to NICU. The occurrence of Candida spp. infections in infants often involves the colonization of their mucous membranes or skin, which puts them at risk for invasive infections due to changes in their host-parasite equilibrium, prolonged hospital stay and use of broad spectrum antibiotics [11]. Our finding was similar to those reported by Almeida *et al.* [12]

In the present study, the distribution of Candida species in different clinical samples showed the highest number of isolates in urine (44.6%), followed by blood(34.9%), high vaginal swab (10.6%). Our observation is similar with the studies of Furlaneto *et al.* [13] where most of the clinical isolates were from urine specimen (86%) followed by blood (19%) and Shanoo *et al.* [14] where most common clinical sample was urine (38%) followed by sputum (17%) and blood (10%). Pfaller *et al.* [15] found that Candida species was seventh most common cause of nosocomial infection accounting for 25% of urinary tract infections in his studies.

**DISCUSSION**

In current study among 103 *Candida* isolates, the most common isolate was *C. albicans* (47.6 %), followed by *C. tropicalis* (26.2%), *C. krusei* (8.7%), *C. lusitanae* (7.8%), *C. utilis* (7.8%) and *C. glabrata* (1.9%) respectively. Non *albicans* *Candida* were isolated at a higher rate (52.4%) than *Candida albicans* (47.6%) in our study which is in concordance with different studies from various parts of India suggesting non-*C. albicans* are emerging microbial trend in yeast infections [14-19].

The predominant NAC isolated in our tertiary care centre, was *C. tropicalis*. This agreed with the studies conducted by Basu *et al.* [20] and Yang *et al.* [21].

In the present study, *C. tropicalis* was the most frequent (27.7%) species among the cases of candidemia. Several studies from India also reported *C. tropicalis* is the most frequent isolate in cases of candidemia among hospitalized patients [19]. There was significant difference in distribution of candida species in different parts of country [Table 6] In our study, second most commonly isolated species from blood were *C. utilis* (19.4%) and *C. lusitanae* (19.4%). These isolates were confirmed by MALDI-TOF mass spectrometry and were correctly identified by Vitek. *C. utilis* is anamorphic form of *Pichia jandinii*, known for its industrial applications as food additive and rarely associated with disease. There are only few case reports of *C. utilis* fungemia.[22] *C. lusitanae* also an infrequent but emerging cause of nosocomial infections, accounts for only 1% of all candidemias [23]. In our study, these cases of candidemia were observed in neonates admitted to NICU. Underlying risk factors observed were extremely low birth weight, prematurity and use of broad- spectrum antimicrobial therapy. However, we could not find out the environmental

source of these *Candida* species from surveillance cultures during the study period.

A high sensitivity of *C. albicans* to antifungal agents was observed, corroborating other published works. [6,12] In present study, *C. albicans* isolates had a 97.9% susceptibility to Amphotericin B, a polyene antifungal which was comparable to finding by Capoor *et al.* [24]. In contrast to it, Sagarika *et al.* [25] and Mokadas *et al.* [18] reported 100% susceptibility of *C. albicans* to Amphotericin B.

In this study there was no resistance recorded for voriconazole for all the candida species which is in agreement with the study by Samara *et al.* [26] whereas results from the latest ARTEMIS DISK Global Antifungal Surveillance Study of *Candida* species shows 5% resistance for voriconazole [27].

In our study resistance of *Candida* against Fluconazole was more (10.7%) in comparison to other antifungals used in this study. The study by Pfaller *et al.* [27] and Badiee *et al.* [2] had similar findings. Studies from different parts of India [29, 16, 25, 30, 6] reported higher resistance to fluconazole [Table 7]. Indiscriminate use of fluconazole and intrinsic resistance of few nonalbicans candida species contribute to fluconazole resistance. In our study all isolates of *C. krusei* were resistant to fluconazole.

Most *Candida* isolates were susceptible to caspofungin when considering new CLSI breakpoints, except for three isolates of *C. krusei* and one isolate of *C. tropicalis* with an in vitro non-susceptibility rate of 33.3 % and 3.7 % respectively. European studies have also shown rare in vitro resistance to fluconazole, voriconazole and caspofungin among *C. albicans* with increasing trend for fluconazole resistance among nonalbicans *Candida* isolates [26, 28].

**Table-6: Frequency of *Candida* spp. isolation in different studies**

Author	Year	<i>C. albicans</i>	Nonalbicans <i>Candida</i>	<i>C.tropicalis</i>
Basu <i>et al.</i> [20]	2003	45.8%	54.2	24.7%
Yang <i>et al.</i> [21]	2006	69 %	31%	12.9%
Mokadas <i>et al.</i> [18]	2007	39.5%	60.5%	12.5
Adhikary <i>et al.</i> [19]	2011	26.4%	60.2%	39.7%
Shanoo <i>et al.</i> [14]	2017	42%	58%	34%
Sing <i>et al.</i> [6]	2017	25.5%	74.4%	59%
Present study	2019	47.6%	52.4%	27.8%

**Table-7: Comparative study of fluconazole and Amphotericin B resistance among *Candida* species**

Author	Year	Place	Fluconazole Resistance	Amphotericin B Resistance
Roy <i>et al.</i> [29]	2013	Asam	36.2 %	0%
Jayalaxmi <i>et al.</i> [16]	2014	Hyderabad	34.2%	4.2%
Sagarika <i>et al.</i> [25]	2015	Gujarat	15.2%	6.4%
Halder <i>et al.</i> [30]	2016	West Bengal	29%	5.4%
Singh <i>et al.</i> [6]	2017	Uttar Pradesh	4.4%	2.2%
Present Study	2018	Chhattisgarh	10.2%	0.97%

## CONCLUSION

The increasing incidence of candida infections and the emergence of antifungal resistance have emphasized the need for updated laboratory data to guide clinicians in selecting appropriate antifungal therapy.

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