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CHARACTERIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES FROM VARIOUS CLINICAL SAMPLES: A CROSS-SECTIONAL STUDY

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Abstract:
Background: *Candida spp* are the most common opportunistic fungal pathogen causing infections in humans. In recent times, prevalence of candidiasis is on the rise due to indiscriminate use of antibiotics and steroids, AIDS and other immunosuppressive conditions. Some *Candida spp.* are intrinsically resistant to commonly prescribed antifungal drugs leading to treatment failure and episodes of recurrences. Moreover, emerging antifungal resistance in various *Candida* species has made the situation grievous. This mandates early identification and determination of antifungal susceptibility pattern of various *Candida spp.* **Aim:** To characterize *Candida* isolates and to determine their antifungal susceptibility pattern from various clinical samples at a tertiary care hospital. **Material and methods:** All isolates of *Candida spp.* isolated from various clinical specimens were included in this study. Speciation of *Candida spp.* Were performed by germ tube test, HiChrome agar, cornmeal agar and carbohydrate fermentation and assimilation. Antifungal susceptibility testing was done as per standard guidelines. **Results:** Out of total 229 Candida isolates, 28.8% were *C. albicans* and 71.2% were NAC. Among 163 non-albicans Candida spp.; *C. tropicalis* (103) was the most common isolate followed by *C. glabrata* (34), *C. parapsilosis* (19) and *C. krusei* (07). Susceptibility towards Fluconazole was found to be more in *C. albicans* as compared to NAC spp. In this study, NAC species also showed relatively reduced susceptibility to Amphotericin B. **Conclusion:** Prevalence of candidiasis is alarmingly increasing leading to significant morbidity and mortality. Various *Candida spp.* show difference in antifungal susceptibility attributing to treatment failure and episodes of recurrences. Early identification of *Candida spp.* and determination of their antifungal susceptibility pattern are crucial in selecting most appropriate antifungal drug for patient treatment.

Keywords: Antifungal susceptibility testing, Candidiasis, *C. albicans*, *C. tropicalis*

INTRODUCTION:

Fungal diseases remain a significant threat to millions of lives, owing to the challenges in the diagnosis of fungal infections. Factors like geographical areas, socioeconomic conditions and susceptible population have an impact on the epidemiology of fungal infections leading to variation across the globe.[1] Aspergillosis, Candidiasis and Mucormycosis are considered as the most common invasive fungal infections worldwide. These infections are often underestimated, underdiagnosed and underreported as evidenced by a recent study, that about 4.1% of Indian population is affected by an invasive fungal infection.[2] The burden of Candidiasis in India is presumably higher as most of the research studies are limited to ICUs and overall data on Candidiasis is lacking. The infections caused by *Candida spp.* have shown surge over the last decade due to progress in intensive patient care, increasing proportion of elderly and at-risk population like patients of hematological malignancies and transplant recipients.[2] *Candida spp.* can cause diverse clinical manifestations ranging from superficial onychomycosis to invasive blood stream infections (BSIs) and disseminated Candidiasis.[3] Most often Candida infections are endogenous in nature as *Candida spp.* are a part of normal flora found on skin and mucosa as well as in gastrointestinal tract and it establishes itself as a pathogen whenever the host conditions are favorable. Certain risk factors like prolonged use of broad-spectrum antimicrobials and steroids, long term hospital or ICU stay, multiple invasive procedures, HIV/AIDS, diabetes etc. influence the patient susceptibility and act as facilitators for acquiring Candida infection.[4] Candidiasis can be caused by various species which differ widely in their virulence factors and antifungal susceptibility, making the diagnosis and treatment complex. Of the different *Candida spp.*, only six species (*C.*

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albicans, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. auris*, and *C. krusei*) are reported as etiological agents in more than 90% cases of Candidiasis.[3] In recent years, world has faced a surge in non albicans Candida species (NAC), shifting the spectrum from *C. albicans* to NAC spp.[5] Among NAC, *C. tropicalis* is the most commonly reported *Candida spp.* in India that is associated with high morbidity and mortality which could be attributed to emerging antifungal resistance.[3, 5] In virtue of complex detection from the clinical samples, arduous species identification methods and time taking antifungal susceptibility testing, laboratories find it difficult to report promptly. This prolonged turnaround time in turn poses a challenge for clinicians in early initiation of empirical therapy. Diverse epidemiology of Candidiasis across the geographical regions makes perpetual surveillance essential for keeping a track on incidence, spectrum of species causing Candidiasis and their antifungal sensitivity.

So, we conducted this study to characterize Candida isolates from various clinical samples and to determine their antifungal susceptibility pattern at a tertiary care hospital that will enhance the knowledge about prevalent *Candida spp.* and emerging antifungal resistance thereby, helping the clinician in better management of the patients.

MATERIAL & METHODS:

This cross-sectional study was conducted in the department of Microbiology, at a tertiary care hospital, Bareilly, for a duration of one year from July 2022 to June 2023, after approval of institutional ethical committee. Various samples such as pus, blood, body fluids (CSF, BAL, ascitic fluid), urine, vaginal swabs were taken from the patients and were received in Microbiology department for culture and sensitivity. Direct examination was performed by Gram's stain and KOH mount to look for the presence of budding yeast like cells with or without pseudohyphae followed by culture on Sabouraud's dextrose agar (SDA) and Blood agar which were incubated at 37^0 C. Colonies were identified by colony characteristics and Gram's stain. All the Candida isolates grown from the patient samples were included in the study. Further speciation was done by germ tube test (for differentiating *C. albicans* from NAC spp.), corn meal agar inoculation (for conidia, pseudohyphae and chlamydospores production), sugar fermentation, sugar assimilation and CHROMagar (species identification is based on specific colors produced by the chromogenic substrates in the medium for different species). [Figure I, II] Antifungal susceptibility testing (AFST) was performed by disc diffusion method or E- test method (as available) on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/ml of methylene blue as recommended by standard guidelines.[6] The following antifungal agents were used for AFST: Fluconazole (25 µg), Itraconazole (10 µg), Voriconazole (1 µg), Posaconazole (0.002-32 mcg/ml) and Amphotericin B (100 units). The statistical analysis was performed using SPSS version 22 and the data was represented as frequency/percentage.

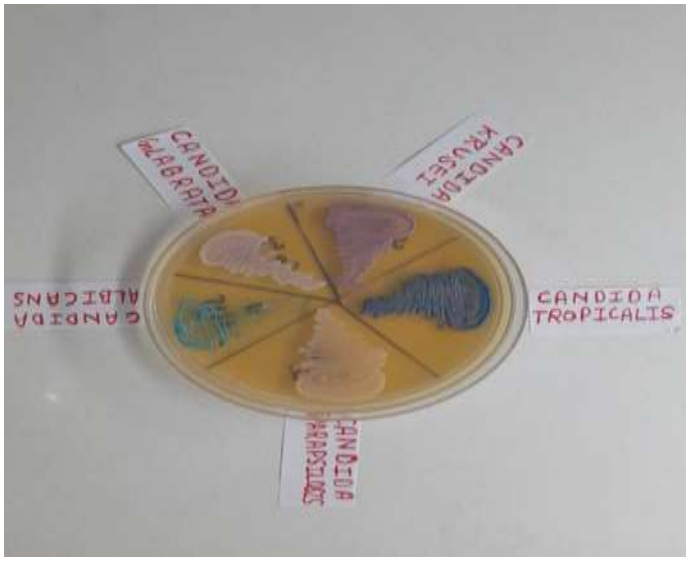

Figure I: Different species of Candida on HiChrome agar

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Figure II: Blastoconidia arranged singly and in small groups along pseudohyphae in *C. albicans* (Cornmeal agar preparation)

RESULTS:

A total of 229 Candida isolates were included in our study which were obtained from various clinical specimens in a duration of one year. Maximum number of Candida isolates were grown from urine samples (49.7%) followed by blood (14.8%) and pus samples (13.9%). [Figure III] Out of the total Candida isolates, 125 were from males and 104 were from females. Highest number of Candida isolates were obtained from adult age group (19-60 years). [Table I] In our study, we have observed NAC (71.2%) preponderance over *C. albicans* (28.8%). Among NAC spp., *C. tropicalis* (44.9%) was the predominant species followed by *C. glabrata* (14.8%), *C. parapsilosis* (8%) and *C. krusei* (3%). [Figure IV] Out of the total Candidemia cases (34), almost half of the cases were caused by *C. tropicalis* while in rest of the cases other *Candida spp.* were isolated as etiological agents. NAC spp. were identified as causative agents in approximately two-third cases of Candiduria; majority of which comprised of *C. tropicalis* (73.2%). [Figure V]

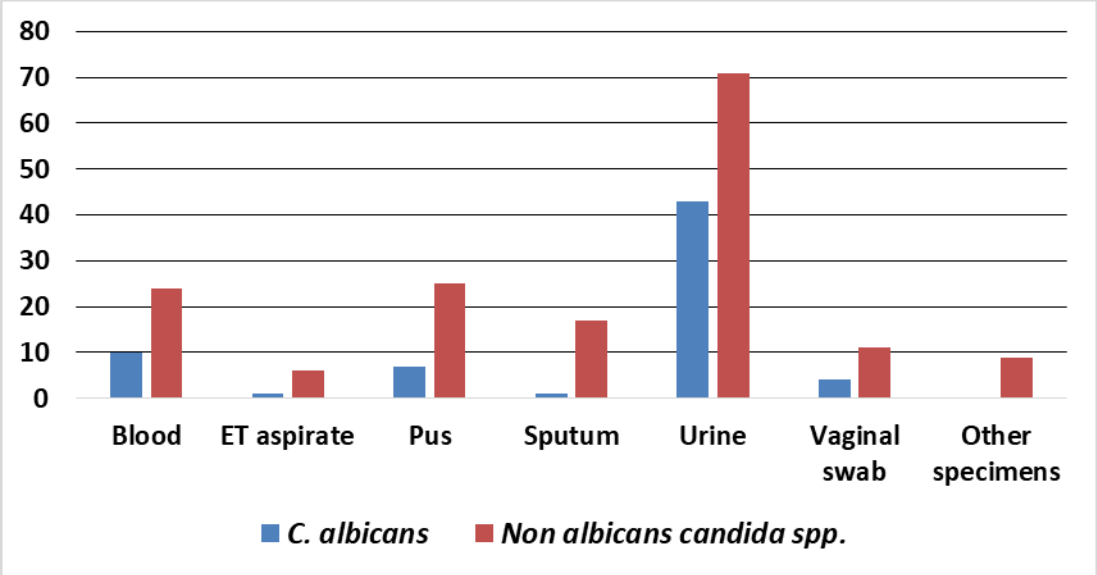

Figure III: Distribution of Candida isolates in various clinical samples

Table I: Distribution of Candida species isolates in different age groups

Age group	No.	*C. albicans*	Non albicans Candida spp.
Pediatric (<18 years)	32	4	28

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Adult (19-60 years)	151	46	105
Geriatric (>60 years)	46	16	30
Total	229	66	163

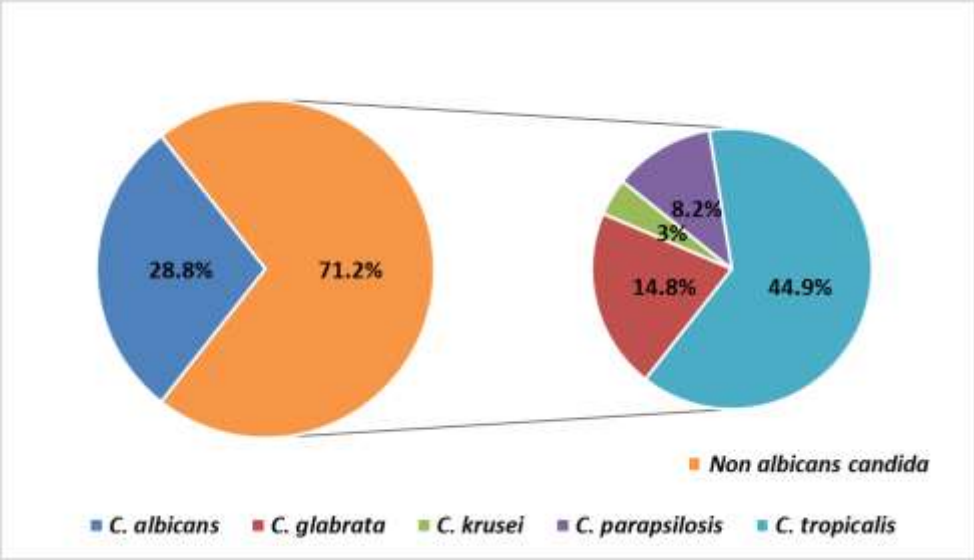

Figure IV: Species wise distribution of Candida isolates

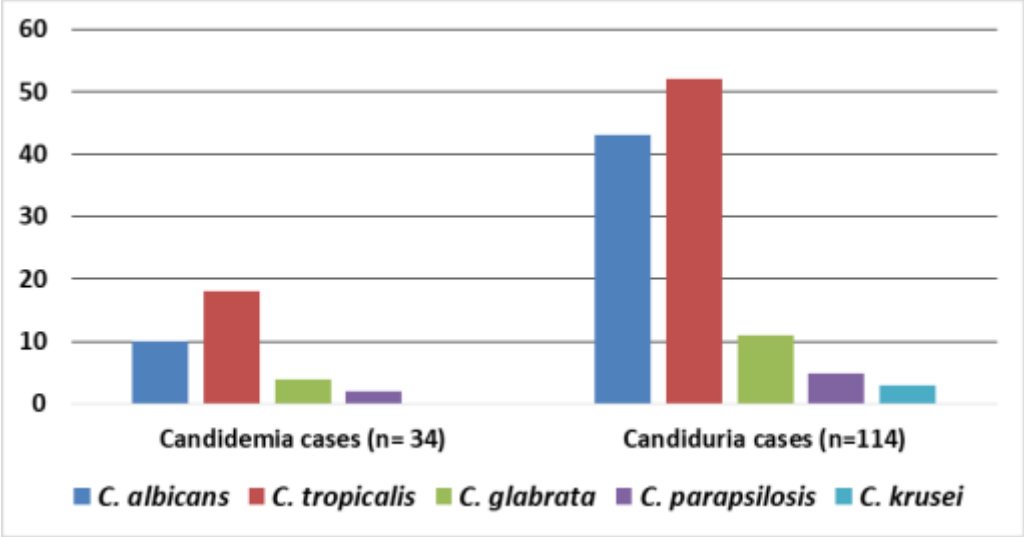

Figure V: Distribution pattern of Candida species in Candidemia and Candiduria cases

On analysing antifungal susceptibility pattern of 229 Candida isolates, we found that all the isolates were susceptible to Posaconazole and minimal resistance (2%) was found against Amphotericin B. Antifungal resistance to Fluconazole, Voriconazole and Itraconazole was detected as 15.2%, 3.4% and 6.1%, respectively. *C. albicans* isolates showed 3%, 4.5% and 4.5% resistance to Fluconazole, Voriconazole and Itraconazole, respectively. While we have observed 14.5% resistance to Fluconazole, 5.8% resistance to Voriconazole, 7.7% resistance to Itraconazole and 3.8% resistance to Amphotericin B in the predominantly isolated *C. tropicalis.* [Table II]

Table II: Antifungal susceptibility pattern of Candida isolates

Species	Total isolates	Fluconazole		Voriconazole		Itraconazole		Amphotericin B		Posaconazole	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
C. albicans	66	97	3	95.5	4.5	95.5	4.5	100	0	100	0

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C. glabrata	34	70.6	29.4	97.1	2.9	94.2	5.8	100	0	100	0
C. krusei	7	57.2	42.8	100	0	85.8	14.2	85.8	14.2	100	0
C. parapsilosis	19	73.7	26.3	100	0	100	0	100	0	100	0
C. tropicalis	103	85.5	14.5	94.2	5.8	92.3	7.7	96.2	3.8	100	0
Total	229	84.7	15.3	95.6	4.4	93.9	6.1	97.9	2.1	100	0

DISCUSSION:

In healthcare settings, Candidiasis is an eminent and growing issue leading to grave complications among the hospitalized patients. This in turn affects recovery and enhances the morbidity and mortality of the patients as well as healthcare cost.[7, 8] In the recent decades, the spectrum of species causing Candidiasis has shifted drastically from *C. albicans* to NAC spp. NAC tends to be more resistant to commonly used antifungal drugs resulting in more chances of treatment failure.[3, 9] This elicits the need of laboratory diagnosis for the correct speciation of Candida isolates along with determination of antifungal susceptibility to make timely and appropriate treatment initiation possible.

In our study, out of 229 Candidiasis cases, we have observed male preponderance (54.6%) over female patients. This observation is in concordance with the studies conducted by Kaur P et al[10] and Jayant S et al[11]. We have found, maximum isolation of *Candida spp.* from 19- 60 years age group population followed by geriatric and paediatric population which is similar to results of study by Pandita I et al[12]. While Kaur P[10] et al and Kaup S[13] et al reported highest isolation of *Candida spp*. from paediatric and geriatric age groups, respectively.

Candida spp. is considered as the seventh most common cause of nosocomial infections. Candiduria and Candidemia are reported as frequent clinical manifestations of invasive Candidiasis. (14) In our study, most of the Candida isolates were isolated from urine samples (49.7%) followed by blood (14.8%) and pus samples (13.9%). These observations correlate well with the studies conducted by pandita I et al[12] and Gade N et al[15] who also reported maximum number of Candida isolates from urine samples followed by blood samples. Furthermore, our observations are backed by previous published literature stating that *Candida spp*. can be elicited as etiological agents in 25% of urinary tract infections in healthcare settings.[16]

Candida spp. are reported as the significant cause of nosocomial blood stream infection in developed countries; likewise, studies from developing countries also depict higher rate of Candidemia.[5, 17] Studies conducted by Kaur P et al[10] and Shukla R et al[18] also found majority of Candida isolates from blood samples.

On speciation of 229 Candida isolates, we have found *C. tropicalis* (44.9%) as the predominant species followed by *C. albicans* (28.8%), *C. glabrata* (14.8%), *C. parapsilosis* (8%) and *C. krusei* (3%). Our results clearly depict higher rate of NAC (71.2%) isolation than *C. albicans* which is concordant with recent studies conducted in various regions of India indicating a shift in trend towards NAC species as the prevalent cause of Candidiasis.[8, 10, 18, 19] Shukla R et al[18] and Ahmed S et al[19] found a predominance of NAC species (75% and 86%, respectively) over *C. albicans* and they have reported *C. tropicalis* (46% and 35.2%, respectively) as the most common species isolated. Bhattacharjee P et al[9] and Jayant S et al[11] also demonstrated preponderance of NAC species; 51.4% and 66%, respectively in their studies, however they have found *C. albicans* (48.6% and 34%, respectively) as the major species associated with Candidiasis. While Gade N et al[15] and Khadka S et al[20] observed contrasting results depicting *C. albicans* (52.4% and 56%, respectively) as the most frequent species isolated in their studies. NAC spp. were reported as 47.6% (Gade N et al[15]) and 44% (Khadka S et al[20]) in their studies.

Susceptibility of *Candida spp.* to antifungal agents differs across various species and geographical areas. In our study, we have observed that *C. albicans* is overall more susceptible to the antifungal agents tested as compared with NAC spp. All the *C. albicans* isolates were 100% susceptible to Amphotericin B and Posaconazole. Furthermore, 95- 97% *C. albicans* isolates were also susceptible to azole group of antifungal agents that are commonly used for the treatment of superficial as well as invasive Candidiasis. Similar susceptibility results of *C. albicans* isolates were reported by Pandita I et al[12] and Edula AR et al[21].

In our study, susceptibility of NAC spp. to a newer antifungal agent, Posaconazole (100%) and a reserved drug Amphotericin B (96.9%) is fairly high. Recent studies also demonstrated higher susceptibility to Amphotericin B among NAC spp.[12, 21] Amphotericin B, despite being nephrotoxic, is a rational alternative agent which can be used in critically ill patients as well as in cases of treatment failure due to antifungal resistance (both acquired and intrinsic).[22] Susceptibility of NAC spp. towards fluconazole varied from 57% to 85%, Itraconazole between 85-

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100% and Voriconazole from 94% to 100%. Comparable susceptibility results were obtained by Edula AR et al[21] and Shukla R et al[18] in their studies. Apart from *C. krusei* which is known to be intrinsically resistant to most antifungal agents, *C. tropicalis* emerged as the other NAC spp. in our study, showing maximum resistance to antifungal drugs.

Conclusion:

In our study we have demonstrated higher rate of isolation of NAC spp., over *C. albicans*, which are notorious of showing antifungal resistance. Moreover, it is a point of concern that isolated NAC spp. showed reduced susceptibility towards commonly used azoles and polyene group of drugs. This necessitates accurate species identification and regular antifungal susceptibility testing before initiation of antifungal treatment for Candidiasis. Furthermore, there is a need of periodic epidemiological and antifungal resistance surveillance as well as formulation of policies for judicious antifungal drug prescription. This will sensitize the clinicians regarding distribution of species in various clinical isolates and their drug susceptibility pattern to help the clinician in selecting timely appropriate empirical therapy.

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