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Characterization and Susceptibility Pattern of *Candida* Species from Various Clinical Samples in a Rural Tertiary Care Hospital

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Abstract

Fungi infections are becoming more prevalent and burdensome on a global scale leading to an important concern for immunocompromised patients. Hospitals often become infected with serious, invasive *Candida* infections. Higher frequency of *Non-albicans Candida* (NAC) species are found in the hospital setting, and some of these fungi can become opportunistic. Pathogens after a change in the host environment trigger them to move from a commensal to a pathogenic phase. Various clinical symptoms of *Candida* species, which are common human commensals, range from mucocutaneous overgrowth to bloodstream infections. In many hospitals, phenotypic methods are still considered the gold standard method for identification. Among the 112 isolates, *Candida albicans* (n=47; 52.64%) was noted as a significant etiology isolated from clinical samples. Further, *C. albicans* accounted the principal etiology in urine (n=28; 31.36%), and vaginal swab (n=13; 14.56%), followed by *C. tropicalis* (urine: n=15; 16.8% and vaginal swab: n=5; 5.6%). In blood *C. pelliculosa* (n=14; 15.68%) was found to be predominant followed by *C. tropicalis* (n=11; 12.32%). Antifungal susceptibility pattern was performed for (n=51) samples by VITEK AST and 100% susceptibility (voriconazole, and micafungin) was recorded in *C. tropicalis* and *C. albicans*. Whereas, fluconazole resistance was observed in *C. tropicalis* (n=3; 15%), and *C. pelliculosa* (n=1; 11.11%) and amphotericin B resistance in *C. tropicalis* (n=1; 5%) and *C. albicans* (n=1; 9.1%).

Keywords: *Candida,* Identification, Conventional, Antifungal

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INTRODUCTION

For decades, fungal infection incident has increased frequently, especially the infection due to *Candida* spp.[1] Several varieties of *Candida* spp. can be found in nature and various settings, such as hospitals, people, domestic animals, and wild animals.[2] In addition to the nails, scalp, and oral cavity, this *Candida* species can take over the mucosal membranes of the respiratory, vaginal, & digestive systems.[3] When *Candida* species overpower commensal species, non-symptomatic colonization may infect. Depending on the host's surroundings, they are opportunistic and can range from benign to destructive. However, significant systemic *Candida* infections can develop in people with weakened immune systems.[4] Three categories of candidiasis can be distinguished: cutaneous (affecting the skin and its appendages), mucosal (affecting the oropharynx, esophagus, and vulvovaginal area), and systemic (affecting the bloodstream, including candidemia & other types of invasive- candidiasis).[5]

The species that has the potential to induce both superficial and systemic infections is *Candida albicans, C. tropicalis, C. krusei , C. glabrata,* and *C. parapsilosis* are other pathogenic species of *Candida* that cause, respectively, 25%, 8%, 7%, and 4% of candidiasis.[6] NAC species with reduced antifungal resistance have emerged as a major cause of fungemia, despite the fact that *C. albicans* is the species most frequently isolated in hospitals worldwide.[5]

Candida-species' characterisation & susceptibility pattern is essential for managing and guiding appropriate antifungal therapy, optimizing patient outcomes, and halting the development of drug resistance.[7–9] It is crucial to recognise each *Candida* species because they differ from one another exhibit varying levels of virulence and susceptibility to antifungal agents.[10] Identification of isolates that may inherently resist some antifungal medications is dependent upon species-level identification.[8] Due to widespread and prolonged use of azole, *Candida* develops resistance to it.[9] As a tool for drug development studies and a method to monitor the emergence of antifungal resistance in epidemiological research, in-vitro antifungal susceptibility testing is becoming more and more significant in aiding therapeutic decision-making.[11]

While several studies investigated prevalence & the distribution of *Candida*-species in different clinical settings, among rural tertiary care centre, There isn't enough information on their characterization and susceptibility patterns.[9] Rural healthcare facilities often face unique challenges, including limited resources, lack of specialized expertise, and delayed access to diagnostic facilities. These factors can impact the timely identification and management of *Candida* infections, potentially leading to suboptimal treatment outcomes.[9]

Thus, the objective of the present research is to identify the *Candida* species that have been isolated from the various clinical samples taken from patients of a rural tertiary care hospital. Another aim of the investigation is to identify the antifungal susceptibility pattern of these isolates to widely used antifungal drugs.

MATERIALS AND METHODS

A prospective investigation examined the identification & susceptibility profile of *Candida* species isolated from the clinical samples. The specimens have been collected and processed in the Jawaharlal Nehru Medical College, Department of Microbiology, Sawangi (Meghe), Wardha (Maharashtra), India, for two years.

Clinical-isolates of *Candida* spp. were obtained from the clinical samples (Blood, vaginal swab and urine), and for the species identification, standard conventional method & VITEK ID were done. Conventional isolation of the *Candida* spp. from urine and the vaginal swab was performed by culturing the sample on a Sabouraud Dextrose Agar (SDA) with chloramphenicol incubating it at 37°C. For the blood sample, BACTEC was used, and after flagging positive, the blood sample from BACTEC bottles was culture on SDA for *Candida* spp. isolation. Different techniques were used for speciation of *Candida* spp. by a conventional method, such as the Germ-tube, Dalmau-Plate technique, biochemical methods, CHROMagar, and further, an automated system like VITEK ID also used for confirmation. Antifungal Susceptibility testing for the *Candida* spp. was performed by the

Table 1. Isolation and frequency distribution of *Candida spp.* from various clinical samples

Specimens	*Candida. albicans*	*C. tropicalis*	*C. pelliculosa*	*C. parapsilosis*	Others*	Total
Urine	28 (31.36%)	15 (16.8%)	-	5 (4.48%)	6 (6.72%)	54 (60.48%)
Vaginal swab	13 (14.56%)	5 (5.6%)	-	-	1 (1.12%)	19 (21.28%)
Blood	2 (2.24%)	11 (12.32%)	14 (15.68%)	-	5 (4.48%)	32 (35.84%)
Others (Sputum, Pus, BAL)	4 (4.48%)	3 (3.36%)	-	-	-	7 (7.84%)
Total	47(52.64%)	34(38.08%)	14 (15.68%)	5 (4.48%)	12(13.44%)	112

**C. glabrata, C. guillermondii, C. krusei, C.lusitaniae*

VITEK AST method based on Minimum Inhibitory concentration (MIC) method.

RESULTS

A total of 112 *Candida spp*. was isolated from various clinical specimens such as urine (n=54; 60.48%), blood (n=32; 35.84%), vaginal swabs (n=19; 21.28%), and others (n=7; 7.84%). The distribution and sources of *Candida spp*. identified by standard conventional method and confirmed by VITEK ID. Among the isolates, *C. albicans* (n=47; 52.64%) was the predominant species identified from clinical specimen. *C. albicans* found to be prevalent species isolated from urine (n=28; 31.36%) and vaginal swab (n=13; 14.56%), followed by *C. tropicalis*. In blood, the most predominant species was *C. pelliculosa* accounted for (n=14; 15.68%) followed by *C. tropicalis* (n=11; 12.32%) (Table 1) (Figure).

Antifungal susceptibility pattern was performed for (n=51) *Candida* spp. by VITEK AST. Based on their Minimum Inhibitory Concentrations (MICs), the isolates were categorised as Sensitive (S), Resistant (R), and Susceptible Dose-Dependent (SDD) to each antifungal drug. Out of 51 *Candida* spp., 11 (21.56%) isolates were *C. albicans* which showed 100% susceptibility to voriconazole, caspofungin, micafungin and flucytosine. Whereas, 9.1% (n=1) isolates showed resistance to amphotericin B and 18.18% (n=2) isolates were SDD to Fluconazole. Similarly, 100% susceptibility to voriconazole, caspofungin, micafungin and flucytosine were observed in case of *C. tropicalis* and 15% (n=3) and 5% (n=1) resistance noted in fluconazole and amphotericin B respectively (Table 2).

DISCUSSION

Over the past decades, there has been a rise in cases of candidiasis, the prevalent source of yeast infections that result in a variety of mucocutaneous disorders that can be serious or non-fatal.[12] Globally, *C. albicans* is the most commonly isolated organism from invasive candidiasis cases.[5] However, growing recovery of the *Non-albicans Candida* (NAC) from patients over the past several decades has given rise to a new hazard. The NAC species with the most reports are *C. tropicalis, C. parapsilosis, C. krusei, and C. glabrata*.[13] The clinical manifestations of infections brought on by numerous NAC spp. typically include some NAC developing resistance over time, becoming established against conventional antifungals, or both.[14,15] Few *Candida* spp. like *C. glabrata* and *C. krusei* are cross-resistant to triazoles like fluconazole. Different pathogenic *Candida* species have comparable clinical symptoms that cannot be identified.[16] *C. auris* causes outbreaks of invasive infections in healthcare facilities, resistance to some other antifungals, and also its intrinsic resistance property can cause multi-drug resistance phenomenon.[17–23]

A study in Karnataka (2014) by Tellapragada *et al.*[24] Vulvovaginal Candidiasis (VVC) was most frequently caused by *C. albicans* (61%) and *C. glabrata* (20%), whereas *C. tropicalis* (46%) was predominant in bloodstream infections (BSI). Another study by Mohandas *et al*. in Manipal reported 50% *C. krusei* and 25% *C. albicans* from the symptomatic UTI cases. Further, they also demonstrated a frequency of 74.19% *C. albicans* and 25.8% NAC from the salivary sample.[25] In all clinical samples, *C. krusei* isolate was predominant, while the *C. albicans* isolates (41.37%) were

Table 2. Antifungal susceptibility testing of *Candida* species

Antifungal agent Susceptibility pattern n=51	Fluconazole			Voriconazole			Caspofungin			Micafungin			Amphotericin-B			Flucytosine		
	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R
C. albicans (n=11)	9 (81.81%)	2 (18.18%)	0	11 (100%)	-	-	11 (100%)	-	-	11 (100%)	-	-	10 (90.90%)	-	1 (9.1%)	11 (100%)	-	-
C. tropicalis (n=20)	17 (85%)	-	3 (15%)	20 (100%)	-	-	19 (95%)	1 (5%)	-	20 (100%)	-	-	19 (95%)	-	1 (5%)	18 (90%)	1 (5%)	1 (5%)
C. pelliculosa (n=9)	7 (77.77%)	1 (11.11%)	1 (11.11%)	9 (100%)	-	-	8 (88.88%)	1 (11.11%)	-	9 (100%)	-	-	9 (100%)	-	-	9 (100%)	-	-
C. parapsilosis (n=4)	4 (100%)	-	-	4 (100%)	-	-	4 (100%)	-	-	4 (100%)	-	-	4 (100%)	-	-	4 (100%)	-	-
Others (n=7)*	3 (42.85%)	-	4 (57.14%)	7 (100%)	-	-	5 (71.42%)	-	2 (28.57%)	7 (100%)	-	-	6 (85.71%)	-	1 (14.28%)	6 (85.71%)	-	1 (14.28%)

Others (n=7)*- *C. glabrata, C. guillermondii,C. krusei,C. Lusitaniae, C. rugosa;* S-Sensitive; SDD-Susceptible dose-dependent; R-Resistant

commonly isolated from respiratory tract infection (RTI).[25] Also, a study from Nepal (2017) stated 90% of the *Candida* spp. were reported from sputum and urine, showing a higher prevalence of *Candida* spp. from UTI and RTI.[10] Nowadays, a standard genotypic method for *Candida* spp. identification is generally used due to being less time-consuming, but conventional techniques are considered the gold standard.[26] Numerous studies have recommended using CHROM agar *Candida* for the primary isolation of *Candida* spp. and their rapid identification.[27] The additional advantage of using CHROMagar for primary isolation is the ability to identify different species of *Candida* from the clinical sample containing mixed species of *Candida*.[28] In our study, all conventional techniques were used to identify *Candida* isolates like Germ tube, CHROMagar, biochemical methods and Dalmau plate technique in which, 32.14% were identified as *C. albicans, followed by C. tropicalis* (12.5%), *C. pelliculosa* (4.46%), *C. parapsilosis* (0.89%), *C. glabrata* (1.78%), *C. krusei* (1.78%) *C. rugosa* (0.89%). Rajeevan et al.,[29] in Tamil Nadu stated that in blood sample 75% of NAC species were reported. In our study, out of 112 isolates, 12.5% of *C. pelliculosa* was the principal etiology identified from blood, and afterward, *C. tropicalis* (9.82%). In contrast to our findings, other studies from India reported *C. tropicalis* (35-45%) as the major causative agent isolated from blood.[30-32] To date, multiple risk factors such as low birth weight, low gestational age, long-term hospitalization, sustained blood alkalosis, surgeries, and inappropriate use of antibiotics are noted for increased cases of *C. pelliculosa*. Hospital-acquired infections due to *C. pelliculosa* were documented in various clinical units such as pediatric intensive care units, nurseries, surgical intensive care units, and hematology wards.[33,34]

Antifungal susceptibility testing (AST) of *Candida* isolates by MIC method helps the clinician in the treatment of critically ill patients and is further helpful for generating clinical guidelines for the better management of treatment. A study from Karnataka (2014) found that all *Candida* isolates were susceptible to flucytosine and amphotericin B, but reduced susceptibility was noted in voriconazole for *C. parapsilosis* (64%) and *C. tropicalis* (79%). Fluconazole intermediate-susceptible isolate was

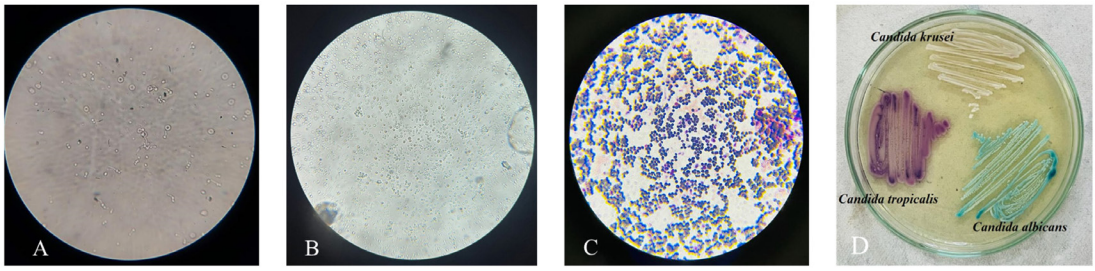

Figure. Pictorial representation of *Candida* spp.
A- Positive germ tube**; B-** Negative germ tube**; C-** Gram staining showing gram positive budding yeast cell **; D-** Growth of various *Candida* species on CHROM Agar

seen in 6% *C. albicans* isolated from Vulvovaginal Candidiasis.[24] The present study reports *C. albicans* and *C. tropicalis* showed 100% susceptibility to voriconazole, and micafungin. In agreement to previous findings,[24] we also found intermediate susceptibility to *C. albicans* (18.18%) whereas, resistance to fluconazole noted for *C. tropicalis* (15%) and *C. pelliculosa* (11.11%).

In our study a high prevalence of NAC was noted in blood sample among which the most predominant etiology was *C. pelliculosa.* As in literature, Most *C. pelliculosa* infections occur in preterm children with exceptionally low birth weights as the rare fungus dwells mostly in soil, lakes, fermented foods, and industrial pollution. Correct identification for *Candida* spp. plays significant role in diagnosing the disease and its prevalence. A study by Kaur *et al*., (2016) (Saudi Arabia) studied the comparison of a yeast identification by conventional techniques and VITEK-2. Out of 172 *Candida* spp., Vitek 2 appropriately recognized 90.12% of *Candida* spp., 2.32% were identified correctly with low discrimination, and 7.56% isolated were misidentified. In our study, VITEK identified the isolates to their species level with proper discrimination. A study by Ahmad et al. (2012) (Kuwait)[35] showed that molecular methods are less time-consuming and accurate than the conventional and VITEK systems. Our study focused on the identification of isolates through VITEK and conventional methods. The AST of various isolates in our study resistance was shown by *C. albicans* (9.1%) to amphotericin-B, whereas *C. tropicalis* isolates (15%) were resistant to fluconazole and (5%) to amphotericin B followed by *C. pelliculosa* (11.11%) showed resistance to fluconazole. The rest of the isolates were susceptible dose-dependent and susceptible to the tested antifungals as stated in the study, whereas a study from Nepal showed 86% resistance to the isolated *Candida* species where ketoconazole demonstrated the highest level of resistance. Further sensitive isolates were reported in clotrimazole, miconazole and fluconazole correspondingly.[10]

The lack of molecular methods for identifying and isolating *Candida* spp. limits our investigation and may result in the lack of some species that warrant attention. In our study, we performed VITEK ID and VITEK-AST for small number of sample. Thus, for studying the prevalence, there is a need for screening a larger number of samples.

CONCLUSION

Our study observed *C. albicans* & *C. tropicalis* as the predominant organism isolated from urine and vaginal swabs, whereas *C. pelliculosa* and *C. tropicalis* were major etiology in blood. Antifungal susceptibility pattern showed 100% susceptibility to voriconazole, and micafungin in *C. tropicalis* and *C. albicans*. Resistance to fluconazole noted in *C. tropicalis* and *C. pelliculosa* and amphotericin B resistance recorded in *C. tropicalis* and *C. albicans*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics committee at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha, India, wide reference number DMIMS(DU)/IEC/May-2020/8843.

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