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Original article

ASSESSMENT OF SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING IN THE INTENSIVE CARE UNIT: AN OBSERVATIONAL CROSS-SECTIONAL STUDY DESIGN.

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ABSTRACT.

Objectives:
This study aims to investigate the epidemiology of Candidemia in patients admitted to Intensive Care Units (ICUs), focusing on the distribution of species, antifungal susceptibility patterns, and demographic factors.

Methods:
The study was carried out for 1 year at the Department of Microbiology, Rajendra Institute of Medical Sciences in Jharkhand, India, and collected 817 blood samples from ICU-admitted patients with signs of sepsis. Using various tests, including germ tube tests and MALDI-TOF mass spectrometry, the study identified Candida species and conducted antifungal susceptibility tests with the VITEK-2 system. The results, categorized as vulnerable, intermediate, or resistant, provided insights into the incidence rates and sensitivity of Candidemia in the studied population.

Results:
The study revealed a Candidemia incidence of 9.57 % among 292 positive blood cultures from ICU-admitted patients. Non-candida albicans predominated at 71.42 %, with Candida tropicalis species encompassing over 28.57 % of the cases. Antifungal susceptibility testing showed all species were vulnerable to the antifungals employed in this study, with C. krusei displaying innate resistance to fluconazole.

Conclusion:
This study highlights the changing epidemiology of Candidemia, with a notable rise in non-candida albicans species, especially in pediatric patients, particularly infants. Despite these shifts, the identified Candida isolates demonstrated overall susceptibility to tested antifungals, emphasizing the significance of precise species-level confirmation and antifungal vulnerability testing for tailored therapeutic approaches in the ICU setting.

Recommendation:
This study recommends continuous monitoring of local Candida species distribution, presumptive identification, and confirmation for early empirical therapy. Moreover, coupled with regular antifungal susceptibility testing to enhance treatment outcomes in vulnerable patient populations.

Keywords: Candidemia, Non-albicans Candida, ICU, Antifungal susceptibility
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INTRODUCTION.

In critical care, Candidemia, a leading invasive fungal disease, poses a significant challenge in Indian ICUs with a prevalence of around 6.51 cases for every 1,000 hospitalizations, resulting in a national burden of 90,000 cases and an associated mortality rate of 35-75% [1, 2]. With over 14.3 million annual ICU admissions in India, Candidemia affects more than 0.1 million patients, placing a substantial burden on healthcare resources [1]. Candidemia in ICU patients is intricately linked to various medical and surgical risk factors, including exposure to medications with high risk, neutrophil deficiency, central venous lines, severe and long-term illnesses, advanced age, renal dialysis, ventilatory support, and prior surgical history [2]. Interestingly, admission to the ICU itself is a predisposing factor for invasive *Candida* infections,

according to the Centres for Disease Control, emphasizing Candidemia's gravity in the context of nosocomial infections [3]. In recent decades, there has been a discernible surge in nosocomial candidiasis, extending beyond tertiary care centers to impact community hospitals globally [4].

Recently, the epidemiology of Candidemia has been moving away from the prevalence of Candida albicans towards the dominance of non-albicans Candida species [5]. Prominent Non-candida albicans species encompass *C.glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, while newer species such as *Candida lusitaniae* and *C. guilliermondi* have begun to gain recognition [5, 6]. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has become a valuable technique to overcome the limitations associated with traditional methods for identifying various *Candida* species, providing a considerably shorter turnaround time [7]. The Vitek 2 system integrates reagent cards that undergo automatic incubation and interpretation [8].

The increasing population of candidemia patients, particularly the rising incidence rates of individuals affected with non-albicans *Candida* species and the emergence of acquired antifungal resistance, necessitates judicious antifungal prophylaxis and empirical therapy. This study aims to characterize locally prevalent *Candida* strains and assess their antifungal susceptibility, providing essential insights for effective antifungal interventions in at-risk patients.

MATERIALS AND METHODS.

Study design.

The study, conducted at the Microbiology department of Rajendra Institute of Medical Sciences in Jharkhand, India for 1 year, employed a prospective design. It included the collection and analysis of 817 blood samples from ICU-admitted patients with sepsis symptoms, using conventional methods, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF), and the VITEK-2 system for antifungal vulnerability testing.

Inclusion criteria.

The study included patients hospitalized in various ICUs of Rajendra Institute of Medical Sciences exhibiting symptoms of sepsis following the systemic inflammatory response syndrome (SIRS) criteria.

Exclusion criteria.

Patients with a history of receiving antifungal medications within the 30 days preceding the study were excluded from this study.

Study size.

The study cohort included 817 ICU-admitted patients with signs of sepsis whose blood samples were screened for antifungal susceptibility according to the set protocol.

Data collection and analysis.

The investigation involved the collection of 817 blood samples sourced from patients admitted to the different ICUs at Rajendra Institute of Medical Sciences. The selected patients showed symptoms indicative of sepsis based on the SIRS criteria. The processing of all blood cultures included a meticulous procedure, with the subsequent differentiation of Candida colonies into both *Candida albicans* and non-*Candida albicans* (NCA). This involved germ tube tests, polenta agar inoculation, and carbohydrate assimilation tests using a twelve-sugar set for both structural and chemical identification, with sugar discs (Cellobiose, Dulcitol, Glucose, Inositol, Galactose, Maltose, Lactose, Raffinose, Melibiose, Trehalose, Xylose, and Sucrose) applied.

Post conventional identification methods, the Candida colonies underwent validation of their species through mass spectrometry. The VITEK-2 automated compact system was employed for investigating the susceptibility of these colonies to various antifungals like Voriconazole, Fluconazole, Amphotericin B, Caspofungin, Micafungin, and Flucytosine. The ensuing results, presented as lowest effective concentrations or minimum inhibitory concentrations (MIC), were classified into vulnerable, intermediate, or resistant categories according to the guidelines.

Statistical analysis.

Statistical analysis involved assessing the variations in antifungal vulnerability between NCA species and *C. albicans* using Fisher's exact test. All statistical computations employed SPSS for calculation, with significance set at $P < 0.05$.

RESULTS/OUTCOMES.

Participants.

Among the 817 blood samples collected from ICU-admitted patients, 292 tested positive, with 28 samples (9.57 %) indicating the presence of Candida species through polenta agar inoculation *via* the Dalmau plate method and carbohydrate assimilation tests. From the tested sample, the majorly isolated species were *Candida albicans*, *C. krusei*, *C. glabrata*, *C. guilliermondii*, *C.*

tropicalis, and *C. parapsilosis* (Table 1). For resolving discrepancies observed in physical characteristics assessments, Vitek-2 compact system and MALDI-TOF mass studies were employed, revealing that 2 out of 5 cases initially confirmed as *C. parapsilosis* by phenotypic methods were, in fact, *C. guilliermondii*.

The predominant organisms identified were *C. tropicalis* and *C. albicans* accounting for 28.57 % of all isolated species. A male dominance of 64 % was observed among cases, with infants (42 %) being the most commonly affected age group. *C. tropicalis* emerged as the most prevalent NCA species, and *C. krusei* was exclusively isolated from younger patients (Table 1).

Table 1: Pervasiveness of Candida species in the patient cohort.

Species	Isolated species		Sex distribution		Age distribution		
	Conventional method	Automated method	Men	Women	Adults	Neonates	Infants
Candida albicans	8 (28.57 %)	8 (28.57 %)	5 (62.5 %)	3 (37.5 %)	3 (37.5 %)	1 (18.75 %)	3 (43.75 %)
Candida tropicalis	8 (28.57 %)	8 (28.57 %)	5 (56.25 %)	3 (43.75 %)	0 (0 %)	4 (56.25 %)	3 (43.75 %)
Candida glabrata	5 (17.85 %)	5 (17.85 %)	3 (70 %)	2 (30 %)	2 (30 %)	1 (20%)	3 (50 %)
Candida parapsilosis	5 (16.07 %)	2 (8.92 %)	2 (60 %)	1 (40 %)	1 (20 %)	2 (40%)	2 (40 %)
Candida krusei	3 (8.92 %)	3 (8.92 %)	2 (60 %)	1 (40 %)	0 (0 %)	2 (60%)	2 (40 %)
Candida guilliermondi	0 (0 %)	2 (7.14 %)	2 (100 %)	0 (0 %)	1 (25 %)	1 (50%)	1 (25 %)

MALDI TOF-MS took 40 minutes to 3 hours for species identification, with a confidence value of 99.9. The timeframe for getting the results of the susceptibility test of antifungals ranged from 11.98 to 18.74 hours, with probabilities between 97 % and 99 % for diverse isolates. MIC values for various antifungals such as Amphotericin B (≤ 0.25), Voriconazole (≤ 0.12), Fluconazole (≤ 0.5), Flucytosine (≤ 1), Micafungin (≤ 0.006), and Caspofungin (≤ 0.12), were also found to align with CLSI reference standards. It revealed that the six isolated species exhibited vulnerability to all 6 tested antifungal agents, except *C. krusei*, which displayed innate resistance to Fluconazole.

DISCUSSION.

Candidemia, a severe bloodstream infection primarily affecting hospitalized individuals, demands precise species-level validation and subsequent antifungal vulnerability testing for prompt and suitable antifungal treatment to mitigate disease and fatality. The present study revealed a 9.57 % prevalence of Candidemia in ICU-admitted patients, with a notable prevalence of non-*Candida albicans* (71.42 %), particularly in patients belonging to the juvenile group (80.35 %). Among the young patients, neonates were frequently affected (42.85 %). Importantly, all isolated species in the current study exhibited susceptibility to the employed antifungals.

The observed incidence rate exceeds that reported in studies conducted in Kolkata and northern India [9, 10]. Moreover, different studies across India have shown variations in the incidence of Candidemia. For instance, Xess *et al* reported a 6 % incidence from a health center in North India, while Verma *et al* noted an incidence of 1.61 % [11, 12]. Kumar *et al* reported a 5.7 % incidence in children with malignancies of the blood in southern India [13]. Sahni *et al* also carried out a study on candidemia in New Delhi and reported an incidence of 6.9 % [14].

The prevalence rate of C. albicans in this study is 28.57 % among all species, a figure notably lower than reported in other studies [15-17]. Among NCAs, C. tropicalis stands out as the major causative species of Candidemia, consistent with findings in various studies [1, 9, 11, 15, 18-20]. Another significant causative organism identified in this study was *C. parapsilosis*, an opportunistic pathogen associated with candidemia, consistent with findings from various other studies [11, 21-23].

The findings of this study also unveiled *C. glabrata* as a noteworthy pathogen, aligning with reports from other studies conducted in India [24-26]. The isolation of *C. krusei*, constituting 8.92 % of all species, mirrors findings in work done by Kaur *et al* as well as Gandham *et al* [10, 27]. In yet another investigation, the major causative species predominantly found in males was identified as *C. guilliermondii*, with an incidence of 7.14 % [25]. Age and sex were considered variables in this study, revealing a higher frequency of Candidemia in the pediatric age group, consistent with findings in studies conducted in north India [9-11, 13]. However, studies in other regions of India concluded that the majority affected were adults [5, 12].

The isolates were identified through conventional phenotypic and chemical tests. But, for infrequently observed species that are challenging to identify accurately, MALDI-TOF mass studies were used as an alternative technique for the characterization of *Candida* species [28, 29]. In this study, 2 isolates initially validated as *C. parapsilosis* were identified as *C. guilliermondii* using MALDI-TOF MS. Given the increasing variety of antifungal drugs, precise choice of antifungal treatment is essential. The conventional broth microdilution method is costly, demanding, and challenging for microbiology laboratories. Therefore, the VITEK-2 system proves to be a dependable approach for conducting antifungal vulnerability tests on yeast species.

CONCLUSION.

The current study focussing on the assessment of speciation and antifungal susceptibility testing in the ICU of a health center in eastern India for candidemia reveals that candidemia emerges as a noteworthy contributor to bloodstream infections in the facility, with non-candida albicans prevailing as the primary pathogen. Predominantly observed among pediatric patients, all identified isolates exhibited susceptibility to antifungal treatments. The commonly occurring isolates in this study were *C. albicans*, and *C. tropicalis*, in equal proportions, besides *C. glabrata* which was identified in a smaller cohort of the patients. Initial identifications were conducted through traditional methods, with subsequent confirmation using automated systems to address any ambiguities.

LIMITATIONS.

This study has several limitations, including its single-center design, potentially restricting the generalizability of findings. The reliance on conventional and automated methods, along with the study's duration and sample size, may impact the comprehensive understanding of *Candida* infections and their sensitivity to antifungal drugs.

RECOMMENDATIONS.

The study recommends the implementation of routine surveillance for Candidemia in ICU settings, emphasizing the demand for species-level validation and antifungal vulnerability testing to guide appropriate and timely therapeutic interventions. Additionally, future multicentre studies with larger sample sizes can provide a more nuanced understanding of Candida infections and contribute to refining treatment strategies.

LIST OF ABBREVIATIONS.

ICU- Intensive Care Units
MALDI-TOF MS- Matrix-assisted laser desorption ionization-time of flight mass spectrometry
SIRS- Systemic Inflammatory Response Syndrome
NCA- Non-Candida Albicans

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CONFLICT OF INTEREST.

No conflict of interest.

REFERENCES.

1. Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M, Chhina D, Rao R, Eshwara VK, Xess I, Kindo AJ. Incidence, characteristics, and outcome of ICU-acquired candidemia in India. Intensive care medicine. 2015 Feb; 41:285-95.
2. Guery BP, Arendrup MC, Auzinger G, Azoulay É, Borges Sá M, Johnson EM, Müller E, Petersen C, Rotstein C, Sganga G, Venditti M. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part II. Treatment. Intensive care medicine. 2009 Feb; 35:206-14.
3. Sardi JD, Pitangui ND, Gullo FP, Almeida AM, Mendes-Giannini MJ. A mini-review of Candida species in hospital infection: epidemiology, virulence factor, and drugs resistance and prophylaxis. Tropical Medicine & Surgery. 2013:1-7.
4. Singhi S, Deep A. Invasive candidiasis in pediatric intensive care units. The Indian Journal of Pediatrics. 2009 Oct;76:1033-44.
5. Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K. non-albicans Candida species in bloodstream infections in a tertiary care hospital at New Delhi, India. The Indian journal of medical research. 2012 Dec;136(6):997.
6. Krcmery V, Barnes AJ. Non-albicans Candida spp. causing fungemia: pathogenicity and antifungal resistance. Journal of hospital infection. 2002 Apr 1;50(4):243-60.
7. Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Gross U, Kuhns M. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Microbiology and Infection. 2011 Sep 1;17(9):1359-65.
8. Valenza G, Strasen J, Schäfer F, Frosch M, Kurzai O, Abele-Horn M. Evaluation of new colorimetric Vitek 2 yeast identification card by use of different source media. Journal of clinical microbiology. 2008 Nov;46(11):3784-7.
9. Bhattacharjee P. Epidemiology and antifungal

susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Current medical mycology. 2016 Jun;2(2):20.
10. Kaur H, Singh S, Rudramurthy SM, Ghosh AK, Jayashree M, Narayana Y, Ray P, Chakrabarti A. Candidaemia in a tertiary care center of a developing country: Monitoring possible change in the spectrum of agents and antifungal susceptibility. Indian journal of medical microbiology. 2020 Jan 1;38(1):109-16.
11. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care center of north India: a 5-year study. Infection. 2007 Aug; 35:256-9.
12. Verma AK, Prasad KN, Singh M, Dixit AK, Ayyagari A. Candidaemia in patients of a tertiary health care hospital from north India. Indian Journal of Medical Research. 2003 Mar 1; 117:122.
13. Kumar CP, Sundararajan T, Menon T, Venkatadesikalu M. Candidosis in children with onco-hematological diseases in Chennai, south India. Japanese journal of infectious diseases. 2005 Aug 1;58(4):218.
14. Sahni V, Agarwal SK, Singh NP, Anuradha S, Sikdar S, Wadhwa A, Kaur R. Candidemia--an under-recognized nosocomial infection in Indian hospitals. JAPI. 2005 Jul;53:607-11.
15. Sridharan S, Gopalakrishnan R, Nambi PS, Kumar S, Sethuraman N, Ramasubramanian V. Clinical profile of Non-neutropenic patients with invasive Candidiasis: A retrospective study in a tertiary care center. Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine. 2021 Mar;25(3):267.
16. Kothari A, Sagar V. Epidemiology of Candida bloodstream infections in a tertiary care institute in India. Indian journal of medical microbiology. 2009 Apr 1;27(2):171-2.
17. Chander J, Singla N, Sidhu SK, Gombar S. Epidemiology of Candida bloodstream infections: experience of a tertiary care center in North India. The Journal of Infection in Developing Countries. 2013 Sep 16;7(09):670-5.
18. Chakrabarti A, Mohan B, Shrivastava SK, Marak RS. Change in distribution & antifungal susceptibility of Candida species isolated from candidaemia cases in a tertiary care center during 1996-2000. Indian Journal of Medical Research. 2002 Jul 1; 116:5.
19. Singh RI, Xess I, Mathur P, Behera B, Gupta B, Misra MC. Epidemiology of candidaemia in critically ill trauma patients: experiences of a level I trauma center in North India. Journal of medical microbiology. 2011 Mar;60(3):342-8.
20. Rani R, Mohapatra NP, Mehta G, Randhawa VS. Changing trends of Candida species in neonatal septicemia in a tertiary North Indian hospital. Indian journal of medical microbiology. 2002 Jan 1;20(1):42-4.
21. Shivaprakasha S, Radhakrishnan K, Karim PM. Candida spp. other than Candida albicans: a major cause of fungemia in a tertiary care center. Indian Journal of Medical Microbiology. 2007 Oct 1;25(4):405-7.
22. Levy I, Rubin LG, Vasishtha S, Tucci V, Sood SK. The emergence of Candida parapsilosis as the predominant species causing candidemia in children. Reviews of Infectious Diseases. 1998 May 1;26(5):1086-8.
23. Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of non-albicans Candida species and antifungal resistance in a tertiary care hospital. Japanese journal of infectious diseases. 2005 Dec 1;58(6):344.
24. Gupta A, Gupta A, Varma A. Candida glabrata candidemia: An emerging threat in critically ill patients. Indian Journal of Critical Care Medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2015 Mar;19(3):151.
25. Tak V, Mathur P, Varghese P, Gunjiyal J, Xess I, Misra MC. The epidemiological profile of candidemia at an Indian trauma care center. Journal of Laboratory Physicians. 2014 Jul;6(02):096-101.
26. Basu S, Kumar R, Tilak R, Kumar A. Candida bloodstream infection in neonates: experience from a tertiary care teaching hospital of Central India. Indian Pediatrics. 2017 Jul; 54:556-9.
27. Gandham NR, Vyawahare CR, Jadhav SV, Misra RN. Candidemia: Speciation and antifungal susceptibility testing from a tertiary care hospital in Maharashtra, India. Medical Journal of Dr. DY Patil University. 2016 Sep 1;9(5):596-9.
28. Bille E, Dauphin B, Leto J, Bougnoux ME, Beretti JL, Lotz A, Suarez S, Meyer J, Join-Lambert O, Descamps P, Grall N. MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, Aspergillus spp. and positive blood cultures. Clinical microbiology and infection. 2012 Nov 1;18(11):1117-25.
29. Yaman G, Akyar I, Can S. Evaluation of the MALDI TOF-MS method for identification of Candida strains isolated from blood cultures. Diagnostic microbiology and infectious disease. 2012 May 1;73(1):65-7.

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