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EVALUATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDEMIA IN INTENSIVECARE UNITS

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ABSTRACT

Background and objectives:Although Candida albicans continues to be the most frequently isolated species in candidemia, recent years have shown a clear shift in the pattern of bloodstream infections, with non-albicans Candida (NAC) species increasingly emerging as important causative agents.This study aims to identify the Candidaspecies responsible for candidemia, and to evaluate their antifungal susceptibility patternsin ICU settings.

Methods:A cross-sectional study was carried out on 75 patients with clinically suspected candidemia admitted to intensive care units. Speciation and Antifungal susceptibility testing of Candidaspecies were performed using the VITEK® 2 Compact system (BioMérieux, France).

Results:The study majorly included male neonates, and confirmed NAC causing candidemia in 24 cases (18.66%), with a median age of 8 days and majority of cases were reported from the NICU(86.66%). Among the organisms detected, non-albicans candida was the most prevalent with 14 (18.66%) isolates. Antifungal testing showed highest resistance with fluconazole and flucytosine.

Interpretations&conclusions:This study underscores the increasing impact of candidemia, marked by the predominance of NAC species and a growing concern of antifungal resistance, especially in the NICU. Voriconazole and amphotericin B demonstrated the greatest effectiveness, whereas reduced sensitivity to fluconazole and flucytosine questions the reliability of current empirical treatment choices. These findings emphasize the importance of routine species identification and antifungal susceptibility testing to ensure timely and targeted management of resistant Candidaspecies..

KEYWORDS: Antifungal susceptibility testing – Candidemia - Fluconazole - Non-albicans Candidaspecies - VITEK® 2 Compact system (BioMérieux, France)

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INTRODUCTION

Candida species are responsible for most opportunistic fungal infections worldwide. Factors such as extremes of age, pregnancy, diabetes mellitus, prolonged use of corticosteroids, extended courses of broad-spectrum antibiotics, and immunocompromised states including AIDS significantly increase susceptibility to Candida infections [1].

Candidemia refers to the detection of Candida species in the bloodstream and is specifically defined as the isolation of Candida species from at least one positive blood culture obtained from either a peripheral vein or a central venous catheter [2].

An increasing proportion of cases are now attributed to Non-albicans Candida (NAC) species, particularly Candida glabrata, Candida parapsilosis, and Candida tropicalis [3].
Identification of Candida species and assessment of their antifungal susceptibility profiles play a crucial role in guiding appropriate therapy and limiting the development of antifungal resistance. In vitro antifungal susceptibility testing (AFST) has gained importance as a valuable tool for clinical decision-making, antifungal drug development, and surveillance of emerging resistance patterns in epidemiological studies [4].

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology of a tertiary care hospital. The study was approved by the Institutional Ethics Committee, and patient confidentiality was maintained throughout.A total of 75 patients of all age groups and both sexes admitted to the ICUs over a period of 12-month, who showed clinical features suggestive of candidemia, were included in the study, and blood samples were collected for fungal culture to identify Candida species and to assess their antifungal resistance patterns.

Isolation and Identification of NAC species: Positive blood culture bottles were detected using the automated BacT/ALERT 3D blood culture system (BioMérieux, France).Specimens were cultured on Sabouraud dextrose agar and incubated until growth appeared, followed by microscopic examination using Gram staining. Gram-positive, oval, budding yeast-like cellssuggestive of Candida were screened with the germ tube test toprovisionally differentiateCandida albicansfrom NAC species. These isolates were then identified within 4-6 hours to the species level using VITEK 2 YST cardin the automated VITEK® 2 Compact system(BioMérieux, France).

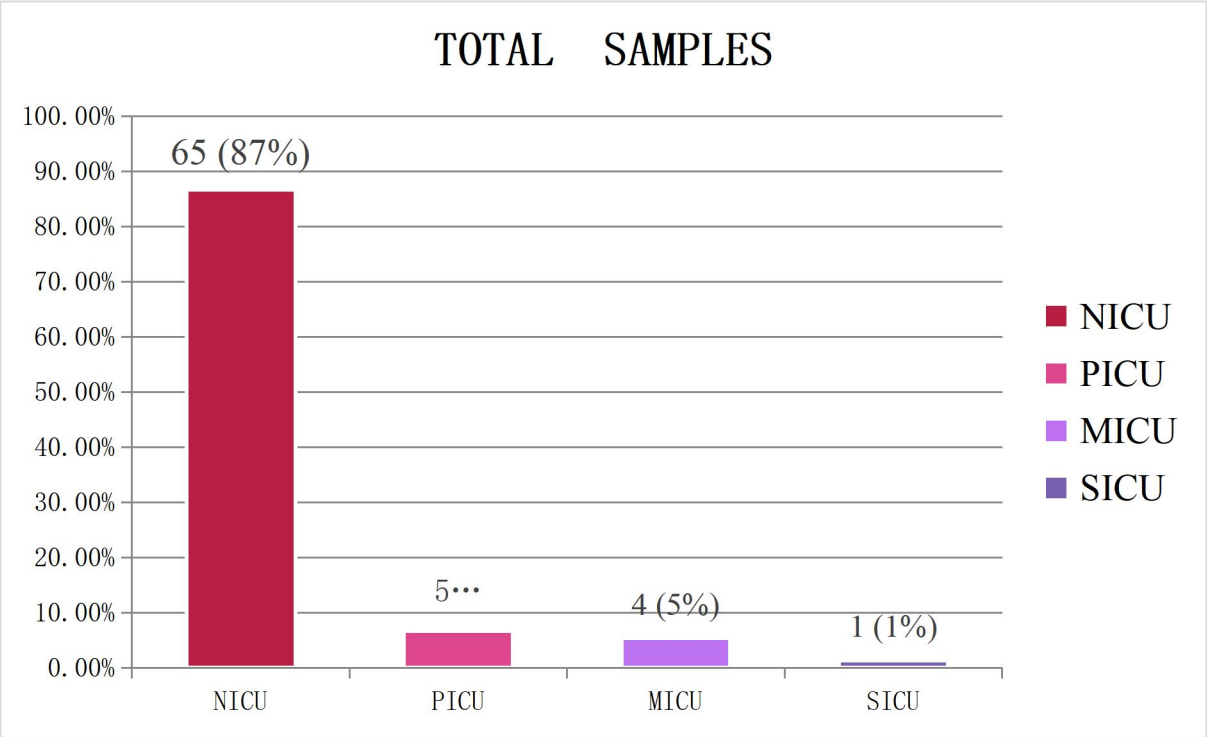
Antifungal susceptibility testing: Antifungal susceptibility profiling was carried out using the automated VITEK® 2 Compact system (BioMérieux, France). The VITEK 2 AST-YS08 card was employed to assess the activity of amphotericin B, fluconazole, voriconazole, caspofungin, micafungin, and 5-flucytosine (5-FC). The susceptibility results were available approximately within 16–18 hours.

RESULTS

Demographic characteristics of the study population: A total of 75 patients were analysed. Among them, 52(69.33%) were male and 23(30.66%) were female, giving a male-to-female ratio of 2:1. Among 75 samples, significant growth in fungal culture was observed in 27/75 (36%) cases and 24/75 (32%) were identified as cases of candidemia. The present study also showed that the candidemia in males was seen in 15/24(62.5%) patients in contrast with that in females[9/24(37.5%)], indicating male predominancewith a median age of 8 days.

Distribution of patients across different Intensive Care Units (ICUs):As demonstrated in Figure 1, majority were admitted to the Neonatal Intensive Care Unit (NICU), accounting for 65(86.66%) cases, with Candida isolates detected in 23 (35.38%) of them.The Paediatric Intensive Care Unit (PICU) accounted for 5(6.66%) cases, followed byMedical Intensive Care Unit (MICU) with 4(5.33%) cases, followed by Surgical Intensive Care Unit (SICU) with 1 (1.33%) case.

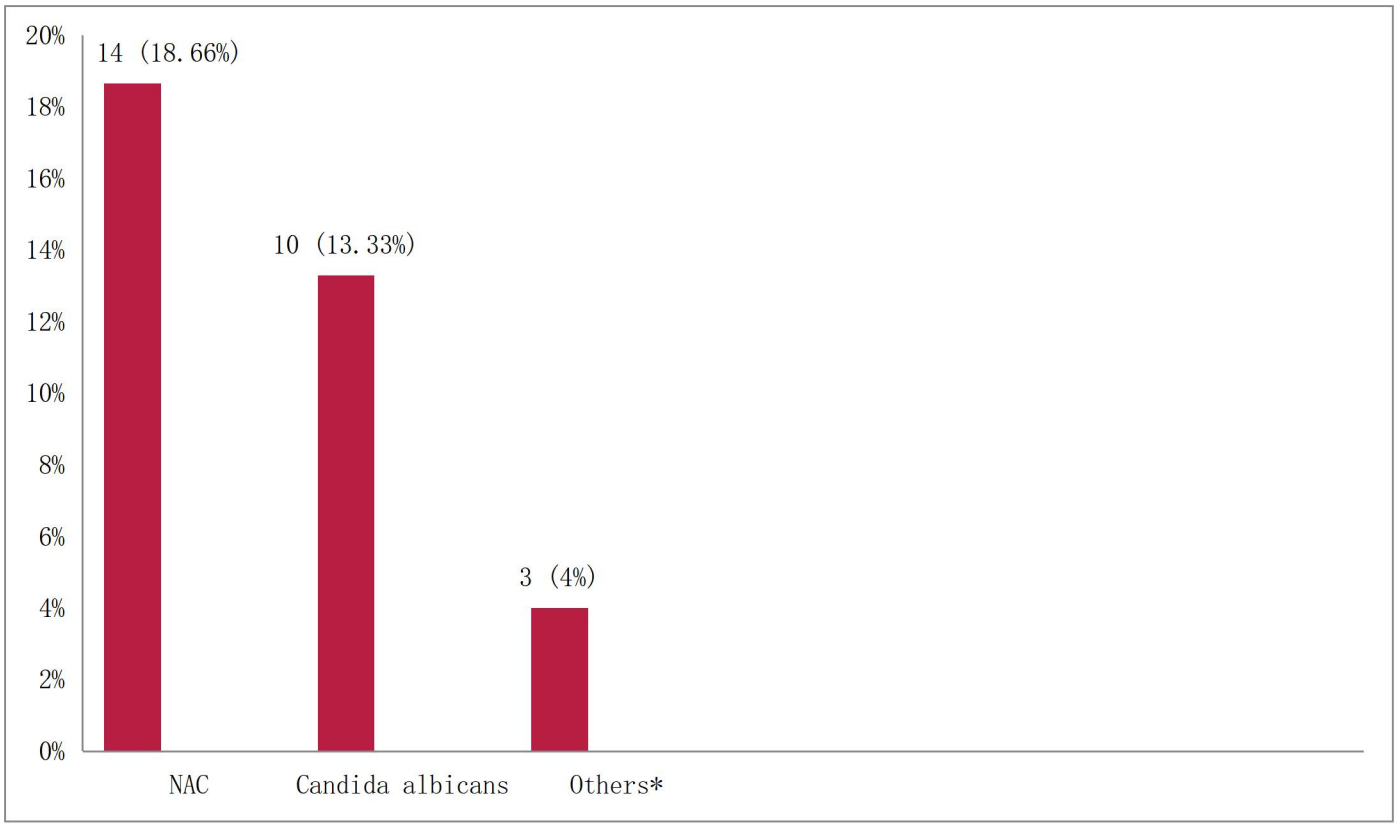
FIGURE 1: ICU wise distribution of patients (n=75)



NICU:Neonatal Intensive Care Unit; PICU: Paediatric Intensive Care Unit; MICU: Medical Intensive Care Unit; SICU: Surgical Intensive Care Unit.

Distribution of fungal species isolated from ICU patients: As shown in Figure 2, among the 27 fungal isolates, NAC was the most prevalentwith 14 (18.66%) isolates, where the distribution was as follows:*Candida krusei* – 6/14 (42.85%); *Candida parapsilosis*– 4/14 (28.57%); *Candida tropicalis* – 3/14 (21.42%); *Candida ciferrii* – 1/14 (7.14%). This was followed by *Candida albicans* with 10 (13.33%) isolates and*Cryptococcus laurentii* was detected in 3 (4%) isolates.Among the24 cases of candidemia, NAC species accounted for 14/24 (58.33%) isolates, whereas *Candida albicans* comprised 10/24 (41.66%) isolates.

FIGURE2: Overall distribution of fungal pathogens in ICU (n =75)



Others - *Crytococcus laurentii*: 3(4%); NAC: *non-albicans candida

Antifungal susceptibility pattern of NAC species: As shown in Table 1, Voriconazole demonstrated as the most effective agent, with 21 isolates (87.5%) sensitive along with that Amphotericin B demonstrated sensitivity in 20 isolates (83.3%). Micafungin showed good efficacy, with 18 isolates (75%) being sensitive. In contrast, lower susceptibility was observed with Fluconazole (62.5% sensitive) and Caspofungin (58.3% sensitive), while Flucytosine showed equal proportions of sensitive (50%) and resistant (50%) isolates.

TABLE 1: Overall Antifungal susceptibility pattern of *Candida* species (n=24)

ANTIFUNGAL AGENTS	SENSITIVE (%)	INTERMEDIATE(%)	RESISTANT (%)
FLUCONAZOLE	15 (62.5)	0	9 (37.5)
VORICONAZOLE	21 (87.5)	1 (4.1)	2 (8.3)
CASPOFUNGIN	14 (58.3)	3 (12.5)	7 (29.1)
MICAFUNGIN	18 (75)	0	6 (25)
AMPHOTERICIN-B	20 (83.3)	1 (4.1)	3 (12.5)
FLUCYTOSINE	12 (50)	0	12 (50)

Table 2 outlines the antifungal susceptibility patterns of different*Candida* isolates, where S [n (%)] denotes the number and percentage of susceptible isolates. Overall, *C. albicans* showed relatively higher susceptibility to most antifungal agents, with the highest sensitivity observed to Amphotericin B (41.66%) followed by Voriconazole and Micafungin (37.5%). In contrast, NAC isolates exhibited more variable and generally lower susceptibility patterns. *C. parapsilosis* demonstrated moderate sensitivity across all tested drugs (16.66%), whereas *C. krusei* and *C. tropicalis* showed limited response, particularly to Fluconazole and Echinocandins. Notably, the single isolate of *Candida ciferrii* was resistant to all antifungals tested.

Table2: Susceptibility pattern of antifungals in different isolates of *Candida*species(n=24)

Isolates [n (%)]	**Fluconazole S[n (%)]**	**Voriconazole S[n (%)]**	**Caspofungin S[n (%)]**	**Micafungin S[n (%)]**	**Amp-B S[n (%)]**	**Flucytosine S[n (%)]**
C. albicans [10(41.6)]	8 (33.33)	9 (37.5)	8 (33.33)	9 (37.5)	10 (41.66)	7 (29.16)
C. krusei[6(25)]	1 (4.16)	6 (25)	1 (4.16)	4 (16.66)	4 (16.66)	1 (4.16)
C. parapsilosis[4(16.6)]	4 (16.66)	4 (16.66)	4 (16.66)	4 (16.66)	4 (16.66)	4 (16.66)
C. tropicalis[3(12.5)]	2 (8.33)	2 (8.33)	1 (4.16)	1 (4.16)	2 (8.33)	0
C. ciferrii[1(4.1)]	0	0	0	0	0	0

Risk Factors: As shown in Table 3, Neonatal sepsis emerged as a major contributor, seen in 14 of 24 candidemia cases (58.33%) whereas adult sepsis and diabetes were the least important risk factors in this study. Multiple *Candida* species were involved, with *C. albicans*being most frequently identified one. One case of respiratory distress showed the growth of*C.ciferrii*, suggesting that antibiotic pressure favours opportunistic fungal infection.Fisher's Exact test analysis demonstrated that although 58.33% of neonatal sepsis were associated with candidemia but it is statistically not significant. Also, adult sepsis and diabetes mellitus were found to be significantly associated with non-candidemia cases as compared to candidemia cases in this study. Other variables including low birth weight, prior exposure to broad-spectrum antibiotics, and respiratory distress did not show a statistically significant association ($p > 0.05$).

TABLE 3: Risk factors among all Candidemia cases (n=24)

RISK FACTORS	Positive [n (%)]	Negative[n (%)]	p value	Candida spp.
NEONATALSEPSIS	14(58.33)	10(41.6)	0.387	• *Candida albicans* (6) • *Candida krusei* (3) • *Candida parapsilosis*(3) • *Candida tropicalis* (2)
SEPSIS (IN ADULTS)	1(4.1)	23(95.8)	<0.001	• *Candida albicans* (1)
DIABETES MELLITUS	1(4.1)	23(95.8)	<0.001	• *Candida albicans* (1)
PRE-TERM BABY	16(66.6)	8(33.3)	0.042	• *Candida albicans* (5) • *Candida krusei* (5) • *Candida parapsilosis*(3) • *Candida tropicalis* (2)
LOW BIRTH WEIGHT	9(37.5)	15(62.5)	0.148	• *Candida albicans* (3) • *Candida krusei* (2) • *Candida parapsilosis*(2) • *Candida tropicalis* (2)
BROAD SPECTRUM ANTIBIOTICS	13(54.1)	11(45.8)	0.773	• *Candida albicans* (5) • *Candida krusei* (3) • *Candidaparapsilosis*(3) • *Candida tropicalis* (1) • *Candida ciferrii* (1)
RESPIRATORY DISTRESS	14(58.3)	10(41.6)	0.387	• *Candida albicans* (7) • *Candida krusei* (3) • *Candida tropicalis* (3) • *Candida ciferrii* (1)

A detailed summary of all 24 candidemia cases is highlighted in table 4 which clearly depicts that *C.tropicalis* and *C.krusei* in this study have shown maximum antifungal resistance.

TABLE 4: SUMMARY OF ALL CANDIDEMIA CASES (n=24)

CASE	A/S	ORGANISM	RISK	DRUG SUSCEPTIBILITY					
				Flu	V	C	M	A	F
1	6D/F	*C. albicans*	Neonatal sepsis; Low birth weight; Broad-spectrum antibiotics use; Respiratory distress	S	S	S	S	S	S
2	8D/F	*C. krusei*	Neonatal sepsis; Preterm baby; Broad-spectrum antibiotics use	S	S	S	S	S	S
3	7D/M	*C. krusei*	Neonatal sepsis; Low birth weight; Respiratory distress	R	S	I	S	R	R

4	7D/F	*C. krusei*	Preterm baby; Low birth weight	R	S	I	S	S	R
5	4D/F	*C. krusei*	Preterm baby; Low birth weight	R	S	I	S	R	R
6	20D/M	*C. albicans*	Neonatal Sepsis; Preterm baby	S	S	S	S	S	S
7	4D/F	*C. parapsilosis*	Neonatal Sepsis; Low birth weight; Broad-spectrum antibiotics use	S	S	S	S	S	S
8	12D/M	*C. parapsilosis*	Neonatal Sepsis; Preterm baby; Low birth weight	S	S	S	S	S	S
9	6D/M	*C. parapsilosis*	Preterm baby; Broad-spectrum antibiotics use	S	S	S	S	S	S
10	8D/M	*C. albicans*	Broad-spectrum antibiotics use; Respiratory distress; Oral Candidiasis	S	S	S	S	S	S
11	7D/M	*C. parapsilosis*	Neonatal Sepsis; Preterm baby; Broad-spectrum antibiotics use	S	S	S	S	S	S
12	19D/M	*C. albicans*	Preterm Baby; Respiratory distress; Suspected Meningitis	S	R	R	S	S	S
13	18D/M	*C. albicans*	Respiratory distress	S	S	S	S	S	S
14	7D/M	*C. albicans*	Respiratory distress	R	S	R	R	S	R
15	9D/F	*C. albicans*	Neonatal sepsis; Preterm baby; Broad-spectrum antibiotics use;Respiratory distress	S	S	S	S	S	S
16	5D/M	*C. tropicalis*	Neonatal sepsis; Preterm baby; Low birth weight; Broad-spectrum antibiotics use; Respiratory distress	S	R	R	R	R	R
17	8D/M	*C. tropicalis*	Neonatal Sepsis; Preterm Baby; Low birth weight; Respiratory distress	S	S	S	S	S	R
18	10D/M	*C. tropicalis*	Respiratory distress	R	S	R	R	S	R
19	10D/M	*C. albicans*	Neonatal sepsis; Preterm Baby; Broad-spectrum antibiotics use	S	S	S	S	S	R
20	74/F	*C. albicans*	Sepsis; Diabetes; Broad-spectrum antibiotics use	R	S	S	S	S	R
21	8D/M	*C. krusei*	Early onset neonatal sepsis; Preterm baby; Broad-spectrum antibiotics use; Respiratory distress; Pneumonia	R	S	R	R	S	R
22	6D/M	*C. krusei*	Preterm baby; Broad-spectrum antibiotics use; Respiratory distress	R	S	R	R	S	R
23	6D/M	*C. albicans*	Neonatal Sepsis; Preterm baby; Low birth weight; Respiratory distress	S	S	S	S	S	S
24	10D/M	*C. ciferrii*	Neonatal sepsis; Preterm baby; Broad-spectrum antibiotics use; Respiratory distress	R	I	R	R	I	R

S: Sensitive; R: Resistant; I: Intermediate

DISCUSSION

In this ICU-based study of 75 patients, predominantly from the NICU, candidemia was identified in 24 cases (32.0%), a higher prevalence than reported by Bansal J et al. (6.2%) [5] and Biswas B et al. (9.65%) [6], though direct comparisons are difficult due to differing denominators (our study reports proportion among symptomatic samples rather than all admissions).A male predominance (62.5%) was observed, with a median age of 8 days, highlighting the greater burden of candidemia in neonateswhich is in accordance with other studies [7,8,9].In contrast, studies by Yılmaz Karadağ F et al. reported a nearly equal gender distribution (52.1% males and 47.9% females) among 165 candidemia patients, representing an older, predominantly adult population[10] whereas Ünal et al.reported a similar male predominance (62.6% males vs. 37.4% females) but in an older cohort, with a median age of 61 years[11].

The predominance of non-albicans Candida (NAC) in our study, accounting for 14 (58.33%) isolates, reflects the ongoing shift away from C. albicans (41.66%) and aligns with reports by Shettigar CG et al. [12] and Bansal J et al. [5], who documented NAC rates of 64.81% and 68% respectively in candidemia. Most cases occurred in the NICU, where NAC accounted for 14/23 (61%) cases whereas Candida albicans, accounted for 9/23 (39%) isolates.Among NAC species,C. krusei was the leading species (42.85%), followed by C. parapsilosis (28.57%), C. tropicalis (21.42%), and C. ciferrii (7.14%). Only a single isolate of C. albicans (4.1%) was detected in the MICU. These findings suggest that neonates are at a higher risk for developing candidemia, indicating increased vulnerability of neonates due to factors such as immature immunity, prematurity, and invasive interventions, as also highlighted by Bhushan S et al. [7].

The rising isolation of NAC species in ICUs is clinically significant due to their reduced susceptibility to commonly used antifungals, particularly fluconazole [13]. In this study, 9/24 (37.5%) candidemia cases demonstrated fluconazole resistance; of which C.albicans was isolated in 2/9 (22.2%) cases whereas NAC was isolated in 7/9 (77.8%) cases which is in accordance with other studies that have also shown reduced susceptibility of fluconazole among NAC species [12,13]. Out of the 7 NAC spp. in our study; C.krusei was isolated in 5/7 (71.4%) cases showing maximum fluconazole resistance with C.tropicalis and C.ciferrii being isolated in one case each. This is in accordance with other studies which have shown emerging fluconazole resistance in C.krusei isolates [7,14]. Notably, two C. krusei cases (cases 3 and 5, Table 4) showed concurrent resistance to fluconazole and amphotericin B, restricting effective treatment options to echinocandins and voriconazole.

The antifungal susceptibility pattern observed in this study highlights key clinical concerns. While high sensitivity to voriconazole (87.5%) and amphotericin B (83.3%) supports their use as reliable empiric therapies, the lower susceptibility to fluconazole (62.5%), caspofungin (58.3%), and flucytosine (50%) signals emerging resistance among Candida species, reinforcing the need for routine species identification and susceptibility testing. Similar trends have been reported globally, including a paediatric candidemia study by Ahmad S et al., which documented C. albicans sensitivity to voriconazole (71.4 %) and amphotericin B (62.9 %) but only 57.1 % sensitivity to fluconazole; NAC in that study showed 67.8 % sensitivity to fluconazole and 62.2 % to amphotericin B.[15]. In the present study, echinocandin resistance was noted in 6/24 (25%) cases, with C. krusei, C. tropicalis,C.albicans and C. ciferrii accounting for 2 (33.3%), 2 (33.3%), 1 (16.7%) and 1 (16.7%) isolates respectively, a finding that contrasts sharply with European surveillance data from 2024 reporting minimal echinocandin resistance (median 0.5% in C. glabrata and 0% in other major species) [16].

The reduced antifungal susceptibility of NAC species observed in our study is alarming, as these organisms commonly possess intrinsic or acquired resistance mechanisms such as efflux pumps and ERG11 mutations, particularly in C. tropicalis [17]. The isolation of a pan-resistant C. ciferrii strain highlights the capacity of rare Candida species to cause highly drug-resistant ICU-associated candidemia, emphasizing the need for continuous surveillance; this is supported by Olender A et al., who reported a C. ciferrii isolate with MIC values >32 for fluconazole and posaconazole in a cystic fibrosis patient [18].

In summary, the study highlights a high burden of candidemia in the NICU, with NAC species predominating and showing varied antifungal susceptibility patterns, emphasizing the need for routine species-level identification and susceptibility testing of all bloodstream Candida isolates in ICU settings. Given the favourable susceptibility profile and increasing use of voriconazole in neonates, standardized dosing guidelines are urgently required; as noted by S. Bhushan et al. [7], no universally accepted dosing recommendations currently exist for newborns and young children, although a single-centre study from China suggests an intravenous dose of 5–7 mg/kg every 12 hours may be suitable for children under two years, particularly in Asian populations [19]. Strengthening infection prevention measures, minimizing unnecessary antifungal use, and adopting rapid diagnostic tools and molecular assays may help reduce complications. Overall, the findings stress the importance of localized antifungal stewardship and tailored management strategies, with early diagnosis and targeted therapy being crucial to curb the rising threat of resistant NAC infections in critically ill patients.

DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

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