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Profile and Assessment of Potential Risk Factors of Neonatal Candidemia Cases in a Tertiary-care Hospital in North India

Safiya Firoze[1], Fatima Khan, Shariq Ahmed[1], Asfia Sultan[1], Anees Akhtar[1], Edison Winsent[1], Uzma Firdaus[2], Zeeshan Ahmed[1], Bhanu Chaudhary[3]*

1. Department of Microbiology, Jawaharlal Nehru Medical College (JNMC), Aligarh, Uttar Pradesh, 202001, India.
2. Department of Paediatrics, Jawaharlal Nehru Medical College (JNMC), Aligarh, Uttar Pradesh, 202001, India.
3. MBBBS, Jawaharlal Nehru Medical College (JNMC), Aligarh, Uttar Pradesh, 202001, India.

*** Corresponding author:** Dr. Fatima Khan, Professor, Department of Microbiology, Jawaharlal Nehru Medical College (JNMC), Aligarh, Uttar Pradesh, 202001, India.
Email: fatimasalmanshah@gmail.com

ABSTRACT

Introduction: *Candida* blood stream infections (BSIs) are a major contributing factor to neonatal sepsis and sepsis-related morbidities. Despite advances in newborn care, there remains a considerable morbidity, a trend towards neonatal candidemia, and occasional cases of antifungal resistance.

Aim: Our study aimed to identify the prevalent *Candida* species, antifungal susceptibility (AFS) patterns, and to ascertain risk factors associated with candida BSIs in neonates.

Materials and Methods: This was an eighteen-month retrospective study of candidemia in neonates. Positive aerobic BactT-Alert blood cultures having yeast were speciated (HiMedia Chrom-Agar and Vitek2®). AFS of isolates was performed using VITEK2® and disc diffusion.

Results: 303 (11.1%) out of 2748 positive blood cultures revealed *Candida* species, from which 84 (27.7%) were confirmed as newborn (0 to 28 days) BSI cases. *Candida tropicalis* (29.8%) was the most common isolate, followed by *Candida albicans* (26.2%) and *C. glabrata* (25%). AFS of these isolates revealed overall resistance of amphotericin B as 2.4% and of fluconazole as 13.1%. 60 isolates tested by Vitek 2 were sensitive to voriconazole; there was 5% caspofungin, 1.7% micafungin, and 1.7% flucytosine resistance. Risk factor assessment included NICU admission (60.7%), broad-spectrum antibiotic usage (71.4%), low birth weight (65.5%), prematurity (65.5%,), ventilator support (44%) and mortality (23.8%).

Conclusion: The rise in resistance and neonatal candidemia emphasises the necessity of reevaluating the application of stringent infection control methods, suitable prophylactic antifungals, and a limited antibiotic-use strategy, among other preventative, as well as therapeutic approaches.

Keywords: Candidemia; Neonates; Antifungal; Resistance; Risk factors

INTRODUCTION

Newborn survival has significantly increased as a result of advancements in neonatal care. Nonetheless, both bacterial and mycological systemic infections with early (within 72 hours) and late (post 72 hours) onset still pose a serious risk and are a major contributor to neonatal morbidity[1]. There is growing

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recognition of the role of *Candida* species in neonatal intensive care units (NICUs). It is responsible for 9 to 13% of neonatal blood-stream infections (BSI) and is the third most frequent cause of late-onset sepsis in NICU patients[2].

Although *Candida albicans* has traditionally been the most frequently isolated species, non-albicans *Candida* (NAC), including *C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis,* have recently emerged as important opportunistic pathogens[3]. This shift in epidemiology is concerning, as NAC resistance to azoles is increasingly recognized and appears to be linked to the widespread use of azole agents[4]. Moreover, several NAC species demonstrate cross-resistance to newer triazoles, while others exhibit intrinsic resistance to fluconazole (FLZ). Hence, it is essential that all yeast isolates, whether from blood or other clinical specimens, undergo both antifungal susceptibility testing and species-level identification [3,5].

In neonatal intensive care units (NICUs), candidiasis is believed to arise primarily from endogenous colonization, given the high prevalence of fungal acquisition in this population. Within the first week of life, approximately 10% of neonates become colonized, a figure that may rise to nearly 64% after a four-week hospital stay[6,7]. Outbreaks in NICUs have also been attributed to contaminated injectable preparations, such as lipid emulsions used for total parenteral nutrition, or via transmission from colonized healthcare personnel and patient-to-patient spread, particularly in the context of inadequate hand hygiene[8-10]. Other recognized risk factors include mechanical ventilation, broad-spectrum antibiotic therapy, prior gastrointestinal surgery, indwelling devices, low birth weight (LBW), and prematurity[11,12].

Clinically, *Candida* bloodstream infection often resembles bacterial sepsis, making diagnosis challenging. Mycotic sepsis may present with nonspecific features such as lethargy, glycemic instability, neutropenia, and respiratory distress. Disseminated fungal disease can result in extensive end-organ involvement, affecting the kidneys, liver, lungs, brain, eyes, bones, spleen, and joints. Notably, invasive infection may occur even in the absence of positive culture results[13]. Renal involvement may manifest as acute dysfunction, hypertension, or flank masses, while ocular dissemination can present as endophthalmitis, a severe complication requiring immediate medical intervention[14,15].

The crude death rate linked to candidemia is around 60%, whereas the attributable mortality rate may reach 49%. The at-risk group involved, the quality of medical care, and the resistance or susceptibilities of different *Candida* species and their strains to certain antifungal drugs are some of the variables that can affect the incidence and related mortality of *Candida* BSI[16]. Owing to significant regional variations, local epidemiology expertise is essential for managing and preventing invasive infections caused by *Candida*[17].

This study aimed to identify the various *Candida* pathogens, examine their sensitivity patterns, and assess risk factors associated with neonatal candidemia.

MATERIALS AND METHODS

Study Design and Setting: This (January 2023 to June 2024), retrospective-observational research was conducted at the Microbiology unit of a tertiary-care hospital located in a city of northern India. The study was approved by the Institutional Ethics Committee of Jawaharlal Nehru Medical College, Aligarh, India (IEC Approval No: IECJNMC/1078). Candida species were discovered in 303 (11.1%) of the 2748 blood culture bottles that were labelled positive during the study period (Figure 1). Of these, 90 were isolated from neonate's blood samples that ranged in age from 0 to 28 days. 6 samples were excluded because they lacked corroborated clinical characteristics or because they revealed yeast as a contamination or commensal. This research took into account 84 (27.7%) neonates who showed signs and symptoms of infection such as lethargy, feed intolerance, failure to thrive or sepsis combined with at least one positive blood culture for *Candida* species.

Laboratory Procedures: Blood specimens were obtained for culture, while adhering to aseptic procedures after obtaining informed consents from the guardians of the patients. Automated BacTAlert3D (Biomerieux, France) was used to perform the blood culture. A paired blood culture was taken. Upon flagging of the blood culture bottle, direct microscopy was done using Gram's stain followed by inoculation onto 5% sheep blood agar incubated at 37°C, along with two plates of Sabouraud dextrose agar incubated at 25°C and 37°C. Growth of colonies was visible in 24 hours for all cases. A number of phenotypic assays were run to determine the isolates of *Candida* species. These comprised of CHROMagar Candida (CHROMagar, Paris, France), chlamydospore

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formation on cornmeal agar (HiMedia, India), Gram's staining, and germ tube visualization.

Antifungal Susceptibility Testing: The Clinical and Laboratory Standards Institute (CLSI) standards[18] were followed in the disk diffusion method (Voriconazole - 1µg, Fluconazole - 25µg, Amphotericin B - 10µg, Caspofungin - 5µg, Itraconazole - 10 µg) used to determine the antifungal susceptibility of 24 isolates. Because the hospital serves a highly impoverished community, financial constraints prevented Vitek from being used on all samples. Vitek 2 Compact (Biomerieux, France) was used to identify 60 samples through the use of ID-YST and YS08 (Voriconazole - 0.12-8 µg/mL, Fluconazole - 1-64 µg/mL, Amphotericin B - 0.25-16 µg/mL and 5-Flucytosine - 1-64 µg/mL, Caspofungin – 0.125 -8 µg/mL, Micafungin – 0.06-8 µg/mL) cards for AFS testing.

Statistics: Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20 and the prevalence of organisms was determined and expressed in percentage. Categorical variables were summarized with frequency and continuous variables were summarized with mean and standard deviation. For categorical outcomes, comparison between groups were performed using the Chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

Neonates accounted for 90 of the 303 *Candida* isolates, of which 84 (27.7%) were proven to originate from cases of neonatal bloodstream infection. Likewise, among the 2445 aerobic bacterial isolates, 241 were from neonates, with 234 (9.6%) confirmed as being from neonate BSI cases (Figure 1 & Table 1).

Figure 1. Flagged Blood Culture Bottles and *Candida* in Blood Cultures from Neonates (0 to 28 days)

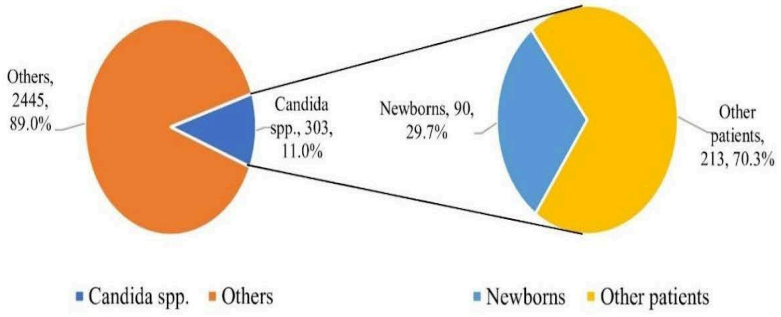

Table 1. Distribution of pure isolates from neonatal septicemia cases (n = 318)

	Organism	No. of isolates	Percentage
Candida species (n = 84)	*Candida albicans*	22	6.9%
	C. glabrata	21	6.6%
	C. krusei	4	1.3%
	C. parapsilosis	9	2.8%
	C. pelliculosa	3	0.9%
	C. tropicalis	25	7.9%
Bacterial isolates (n = 234)	Enterobacteriaceae (n = 84)		
	Citrobacter sp.	6	1.9%
	Escherichia coli	14	4.4%
	Enterobacter sp.	2	0.6%
	Klebsiella	59	18.6%
	Serratia sp.	3	0.9%
	Gram positive cocci (n = 106)		
	Enterococci	19	6%
	Other Staphylococcus sp.	62	19.5%
	Staphylococcus aureus	24	7.5%
	Streptococci	1	0.3%
	Nil-fermenters (n = 44)		
	Acinetobacter	21	6.6%
	Pseudomonas	21	6.6%
	Stenotrophomonas sp.	2	0.6%

The most common pathogens isolated were other *Staphylococcus* species (n= 62, 19.5%), *Klebsiella* species (n= 59, 18.6%), *Candida tropicalis* (n= 25, 7.9%), *Staphylococcus aureus* (n= 24, 7.5%), *C. albicans* (n= 22, 6.9%), and *C. glabrata*, *Acinetobacter* species, and *Pseudomonas* species (n= 21 each, 6.6%).

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Non-albicans *Candida* species were responsible for 73.8% of the 84 newborn *Candida* BSI cases. *C. tropicalis* accounted for 25 (29.8%) of the total species (Table 2).

Table 2. Distribution of *Candida* species isolated from blood of neonates (n = 84)

Organism		No of isolates	Percentage	
Candida albicans		22	26.2%	
Non-albicans Candida (NAC)	*C. glabrata*	21	25%	Total NAC = 73.8%
	C. krusei	4	4.8%	
	C. parapsilosis	9	10.7%	
	C. pelliculosa	3	3.6%	
	C. tropicalis	25	29.8%	

The distribution of the other isolated species was: *Candida albicans,* 22 (26.2%); *C. glabrata,* 21 (25%); *C. parapsilosis,* 9 (10.7%); *C. krusei,* 4 (4.8%); and *Candida pelliculosa,* 3 (3.6%).

Table 3. Combined Antifungal Susceptibility Profile of *Candida*.

Organism	FLZ (n=84)		AMB (n=84)		VOR n=(60)		CSF n=(60)		MYC n=(60)		5FC n=(60)	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	R
C. albicans (22)	20/22 (90.9%)	**2/19 (9.1%)**	22/22 (100%)	0 (0)	19/19 (100%)	0 (0)	18/19 (94.7%)	**1/19 (5.3%)**	18/19 (94.7%)	**1/19 (5.3%)**	18/19 (94.7%)	1/19 (5.3%)
C. glabrata (21)	21/21 (100%)	0 (0)	21/21 (100%)	0 (0)	18/18 (100%)	0 (0)	18/18 (100%)	0 (0)	18/18 (100%)	0 (0)	18/18 (100%)	0 (0)
C. krusei 4)	0/4 (0)	**4/4 (100%)**	3/4 (75%)	**1/4 (25%)**	2/2 (100%)	0 (0)	2/2 (100%)	0 (0)	2/2 (100%)	0 (0)	2/2 (100%)	0 (0)
C. parapsilosis (9)	8/9 (88.8%)	1/9 (11.2%)	9/9 (100%)	0 (0)	5/5 (100%)	0 (0)	5/5 (100%)	0 (0)	5/5 (100%)	0 (0)	5/5 (100%)	0 (0)
C. pelliculosa (3)	3/3 (100%)	0 (0)	2/3 (66.7%)	**1/3 (33.3%)**	3/3 (100%)	0 (0)	3/3 (100%)	0 (0)	3/3 (100%)	0 (0)	3/3 (100%)	0 (0)
C. tropicalis (25)	21/25 (84%)	**4/25 (16%)**	13/13 (100%)	0 (0)	13/13 (100%)	0 (0)	11/13 (84.6%)	**2/13 (15.4%)**	13/13 (100%)	0 (0)	13/13 (100%)	0 (0)
	73 (86.9%)	11 (13.1%)	82 (97.6%)	2 (2.4%)	60 (100%)	0 (0)	57 (95%)	3 (5%)	59 (98.3%)	1 (1.7%)	59 (98.3%)	1 (1.7%)

Among the 24 *Candida* isolates that were tested for antifungal susceptibility by disk diffusion method (n= 24), resistance to fluconazole, amphotericin B, was 20.8% and 0%, respectively. All of the *C. albicans* isolates were sensitive to fluconazole and amphotericin B. No resistance was seen in *C. glabrata,* to fluconazole and amphotericin B. All of the 2 isolates of *C. krusei* were resistant to fluconazole (100%); no resistance was seen with amphotericin B. All the *C. parapsilosis* isolates were sensitive to all antifungals, except for 1 isolate showing FLZ resistance (25%). Out of 12 *C. tropicalis* isolates tested, resistance was seen with FLZ (2; 16.7%).

Among the 60 *Candida* isolates that were tested for antifungal susceptibility by Vitek 2 (Table 3), (n = 60), resistance to fluconazole (FLZ), amphotericin B (AMB), voriconazole (VOR), caspofungin (CSF), micafungin (MYC), and flucytosine (5FC), was 10%, 3.3%, 0%, 5%, 1.7%, and 1.7%, respectively. All of the *C. albicans* isolates were sensitive to amphotericin B and voriconazole. 2 (10.5%) isolates were resistant to FLZ, while 1 (5.3%) isolate of *C. albicans* was resistant to CSF, MYC, 5FC, each. All the isolates of *Candida glabrata* and *Candida parapsilosis* were sensitive to FLZ, AMB, VOR, CSF, MYC, and 5FC. Both isolates of *C. krusei* were resistant to fluconazole, and 1 (50%) was resistant to amphotericin B. No resistance was seen with VOR, CSF, MYC, and 5FC. Only 1 (33.3%) isolate of *C. pelliculosa* was resistant to amphotericin B, while 2 (15.4%) isolates of *C. tropicalis* were resistant to fluconazole and caspofungin, each. All 3 *C. pelliculosa* isolates were sensitive to FLZ, VOR, CSF, MYC, and 5FC. All *C. tropicalis* isolates were sensitive to AMB, VOR, MYC, and 5FC.

Table 4. Demographic Data and Potential Risk Factors in Neonates

	Candidemia (n = 84)				Bacteraemia (n = 234)				P Value	
	No.		%		No.		%			
Admission										
NICU	51		60.7%		87		37.2%		<0.001	
Non-NICU	33		39.3%		147		62.8%		<0.001	
Antibiotics Usage										
< 7 days (d)	18	60	21.4%	71.4%	27	150	11.5%	64.1%	0.013*	
7 - 14 d	32		38.1%		40		17.1%		<0.001	
> 14 d	10		11.9%		83		35.5%		<0.001	
Not used	24		28.6%		84		35.9%		0.112	
Birth Weight										
< 1 kg [ELBW]	14	55	16.7%	65.5%	21	147	9%	62.8%	0.027*	
1 - 1.5 kg [VLBW]	16		19%		47		20.1%		0.419	
1.6 - 2.5 kg [LBW]	25		29.8%		79		33.8%		0.251	
> 2.5 kg	29		34.5%		87		37.2%		0.332	
Gender										
Males	52		61.9%		142		60.7%		0.422	
Females	32		38.1%		92		39.3%		0.422	
Gestational Age										
< 37 weeks (w)	55		65.5%		110		47%		0.002*	
≥ 37 w	29		34.5%		124		53%		0.002*	
Normal Vaginal Delivery	72		85.7%		210		89.7%		0.159	
Outcome										
Alive	64		76.2%		207		88.5%		0.003*	
Death	20		23.8%		27		11.5%		0.003*	
Ventilator Support										
< 7 d	7	37	8.3%	44%	57	104	24.4%	44.4%	0.001*	
7 -14 d	19		22.6%		31		13.2%		0.021*	
> 14 d	11		13.1%		16		6.8%		0.039*	
Not used	47		56%		130		55.6%		0.475	

ELBW= extremely low birth weight; VLBW= very low birth weight; LBW= low birth weight [* indicates P value <0.05]

A total of 318 neonates with bloodstream infection were analyzed, including 84 with candidemia and 234 with bacteremia (Table 4). NICU admission was significantly higher among neonates with candidemia compared to those with bacteremia (60.7% vs. 37.2%, p<0.001). Preterm birth (<37 weeks) was more frequent in the candidemia group (65.5% vs. 47%, p=0.002), with a significantly greater proportion of extremely low birth weight infants (<1 kg) (16.7% vs. 9%, p=0.027). Shorter prior antibiotic exposure (<7 days) was more common in candidemia (p=0.013), whereas prolonged mechanical ventilation (>7 days) was significantly associated with candidemia (p=0.021). Mortality was significantly higher in the candidemia cohort compared with bacteremia (23.8% vs. 11.5%, p=0.003). No statistically significant differences were observed with respect to gender, mode of delivery, or normal birth weight categories.

DISCUSSION

Pathogens isolated in our positive neonatal BSI samples included *Staphylococcus* species (19.5%), *Klebsiella* species (18.6%), *Candida tropicalis* (7.9%), *Staphylococcus aureus* (7.5%), *Candida albicans*

(6.9%), *Candida glabrata, Acinetobacter* species, and *Pseudomonas* species (6.6% each). These findings closely parallel the observations of Nazir and Masoodi, who reported *Staphylococcus* species (16.6%), *Klebsiella* species (16.2%), *Candida tropicalis* (13.8%), *Acinetobacter* species (11.3%), *Staphylococcus aureus* (7.7%), and *Candida albicans* (5.6%) as the predominant isolates[17]. In the current research, 73.8% of the cases of newborn candidemia were caused by non-albicans *Candida* species, while 26.2% of cases were caused by *C. albicans.*

In our study, *Candida tropicalis* (29.8%) was the predominant species, followed by *C. albicans* (26.2%), *C. glabrata* (25%), *C. parapsilosis* (10.7%), *C. krusei* (4.8%), and *C. pelliculosa* (3.6%). Similar trends of *C. tropicalis* predominance have been reported nationally[16, 19, 20], including by Nazir and Masoodi[17] and Basu et al.[21], whereas Juyal et al. observed *C. parapsilosis* as most common[3]. Contrastingly, Tunc et al. in Turkey reported *C. albicans* predominance[30]. The association of *C. tropicalis* with NICU outbreaks[17, 23] underscores its virulence[3]. Fluconazole resistance (13.1%) was mainly among NAC, consistent with other Indian reports[24-26], though all isolates remained 100% voriconazole susceptible[19].

In contrast to our investigation, where fungal resistance to amphotericin B was found to be 2.4% across all *Candida* isolates, the Central India-based publication by Narain et al., showed that all fungal isolates were susceptible to the antifungals. Biswas et al., however, found comparable outcomes, citing a 3.6% resistance to amphotericin B[19]. Caspofungin, micafungin, and flucytosine had overall resistance of 5%, 1.7%, and 1.7%, respectively.

61.9 % were females and 38.2% were males, and 65.5% had low birth weight (<2.5 kg). Extremely low birth weight (≤1 kg) was significantly associated with candidemia (16.7%) compared to bacteremia (9%) ($p<0.05$). Prematurity was also prominent, with 65.5% of candidemia cases occurring in infants born before 36 weeks versus 47% in bacteremia ($p<0.05$). Notably, late preterm births (33-36 weeks) were significantly higher in candidemia (30.6%) than bacteremia (20.1%). Conversely, term births (>36 weeks) were significantly associated with bacterial BSIs (53% vs. 34.5%). These findings reaffirm preterm and low birth weight neonates as high-risk groups for candidemia[12, 27].In our study, prior antibiotic exposure was documented in 71.4% of neonates with candidemia. Importantly, antibiotic use for less than one week was statistically significant ($p<0.05$) in candidemia (21.4%) compared with bacterial BSIs (11.5%). Our findings corroborate earlier studies by Fu et al.[11, 12] and Benjamin et al., which demonstrated that broad-spectrum antibiotic exposure within seven days before diagnosis markedly increased candidemia risk in low-birth-weight neonates.

Ventilator support was required for 44% of the neonates with Candida BSI. Compared to neonatal bacteremia cases, which showed 13.2% of neonates requiring ventilatory assistance between 7 and 14 days, we found that 22.6% of neonatal candidemia patients required such ventilator support. This difference was statistically significant ($p<0.05$). Furthermore, ventilator support for longer than two weeks was linked to 13.1% of occurrences of newborn candidemia. This finding was statistically significant ($p<0.05$) in comparison to instances of newborn bacteremia, of which 6.8% required continuous ventilator support above 2 weeks.

In the present study, we observed several notable strengths in the form of identification of key risk factors such as low birth weight, prematurity, prior antibiotic exposure, and prolonged ventilator support adds important clinical relevance, further supported by statistical significance. However, certain limitations such as being a likely single-center observational study, the generalizability of the findings may be limited. Additionally, the absence of outcome measures such as mortality and lack of adjustment for potential confounders may influence interpretation.

CONCLUSIONS

This study underscores the significance of neonatal bloodstream infections, with a predominance of non-albicans Candida species, particularly Candida tropicalis, and the emergence of antifungal resistance patterns in NICU settings. Prematurity, low birth weight, prior antibiotic exposure, and prolonged ventilator support were identified as the key risk factors for candidemia.

These findings emphasize the need for strict infection control measures, including adherence to hand hygiene and aseptic practices, along with implementation of robust antimicrobial stewardship programs to limit unnecessary antibiotic use. Routine surveillance of pathogen distribution and antifungal susceptibility, early identification of high-risk neonates with consideration of targeted prophylaxis, strengthening of

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laboratory diagnostics, and regular staff training are recommended to improve neonatal outcomes.

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No potential conflict of interest was reported by the authors.

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