

Original Article

Neonatal Candidaemia — A Retrospective, Observational Study with Comparison between Albicans and Non-albicans Candida among Low Birth Weight and Normal Birth Weight Babies

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Abstract

Background : This study is done to determine the occurrence of Candidemia among the neonatal patients during 3 years of observation alongwith to analyze the trend in species distribution and to examine in vitro susceptibility to common antifungal drugs.

Materials and Methods : A retrospective review of 2312 blood samples collected from the neonates admitted in Neonatal Intensive Care Unit and Sick Newborn Care Unit in 2019, 2020, 2021 and 2022 were done. Neo-nates who had a positive blood culture for fungal element, were enrolled. Incidence of albicans and non-albicans candidemia were evaluated. Drug sensitivity test was employed to determine the sensitivity of Voriconazole, Itraconazole, Fluconazole and Amphotericin B.

Results : Out of blood samples collected from 2312 neonates with suspected Blood stream infection, 568 (24.6%) samples were detected with blood stream infection. Among which 89 samples were identified as fungal isolates and out of those samples candidemia afflicted 83 samples. Among the positive isolates predominant isolate was *Candida albicans* 47.0% (39/83) followed by *Candida tropicalis* 26.5% (22/83), *Candida glabrata* 13.3% (11/83), *Candida parapsilosis* 7.2% (6/83) and *Candida guilliermondii* 6.0% (5/83). Majority of candidemia were due to Non-albicans *Candida* ie, 53.0% of total positive cases. Among the Low Birth Weight (LBW) babies majority were due to *C. albicans*. Susceptibility testing revealed that 92.8% of the retrieved *Candida* isolates were sensitive to voriconazole, 48.2% to Fluconazole while only 31.3% to Itraconazole and 26.5% to Amphotericin B. *Albicans candida* showed comparatively higher resistance to Fluconazole (58.8% against 45.4%) & Itraconazole (71.8% against 65.9%); whereas Non-albicans *Candida* showed comparatively higher resistance to Voriconazole (9.1% against 5.1%) and Amphotericin B (88.6% against 56.4%).

Conclusion : *Candida* spp are assuming an increasing role in nosocomial infections in neonates. The World-wide progressive shift towards Non-albicans Candidemia and increasing resistant pattern to many regularly used antifungals necessitates regular surveillance and monitoring of laboratory data.

Key words : Neonatal Candidemia, Low Birth Weight, Albicans Candidemia, Non-albicans Candidemia.

Invasive candidiasis in neonates is a serious and common cause of late onset sepsis and has a high mortality (25 to 35%)¹. The incidence of such fungal infections has increased 11 fold over the past 15 years. *Candida* species are the 3rd most frequent organism (after coagulase negative Staph and *Staph aureus*) isolated in late onset sepsis in Very Low Birth Weight (VLBW) infants (ie, <1,500 g). Preterm infants are predisposed to *Candida* infections because of immaturity of their immune system and invasive interventions. Transmission of *Candida* may be vertical (from maternal vaginal infection) or nosocomial. Colonization of health workers is as high as 30%¹. Initial site of colonization is usually the gastrointestinal tract. Risk factors for candidiasis include: low birth weight

Editor's Comment :

- The study gives insight into the occurrence of Candidaemia among the neonatal patients and their susceptibility to common antifungal drugs, isolated from blood samples collected in a tertiary care hospital.
- Overall, majority of candidemia were due to non-albicans *Candida*, although *Candida albicans* is the major single species isolated as causative agent, but among the low birth weight babies *Candida albicans* isolates are dominated than non-albicans isolates.
- It is also observed from the study that both albicans and non-albicans isolates show moderate to high levels of resistance to different commonly used antifungals.

(<1,500 g); use of broad spectrum and/or multiple antibiotics; central venous catheters; parenteral alimentation and intravenous fat emulsion; colonization with *Candida* and/or previous episode of mucocutaneous candidiasis; prolonged urinary catheterization. Although initial reports indicated most cases were due to *Candida albicans*, more recent studies show emergence on non-albicans species including *C. parapsilosis*, *C. glabrata* and *C. tropicalis*. Immunologic immaturity and altered cutaneous barriers play some role in the vulnerability of neonates to nosocomial infections. The better prognosis of the patient is associated with the early diagnosis and

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fast treatment. The current antifungal agents that are available to treat fungemia among newborns and children are based on clinical trials in adults, since there are few comparative studies of antifungal agents in infants. The most commonly used drugs for the treatment of invasive fungal infections in neonates are classified in four different classes: polyene, azoles, analogs of pyrimidines and echinocandins. Estimates suggest that >1.4 million neonatal deaths worldwide annually are due to invasive infections^{2,3}. The incidence of bloodstream infections due to *Candida* species in the overall population ranges from 1.7 to 10 episodes per 1,00,000 inhabitants. An estimated 33–55% of all episodes of Candidemia occur in intensive care units and are associated with mortality rates ranging from 5 to 71%³. In the NICU in the 1990s, the overall incidence of Candidemia increased because of the increased survival and intensive care of extremely preterm infants. During that time period, the proportion of candidemia decreased because of *C. albicans*, whereas increased because of *C. parapsilosis*^{4,5}. Although Blood Stream Infection (BSI) due to *Candida* species (spp) in the Neonatal Intensive Care Unit (NICU) is less frequent than that due to Gram-positive or Gram-negative bacteria, it has higher morbidity and mortality rates. Risk factors for neonatal Candidemia include prematurity, use of central venous lines, endotracheal tubes, parenteral nutrition, broad-spectrum antibiotic administration (especially third-generation cephalosporins), prolonged hospitalization, abdominal surgery, exposure to H2 blockers, and *Candida* colonization. Although *Candida albicans* is the most prevalent yeast pathogen, BSIs caused by *Candida non-albicans*, particularly *Candida parapsilosis* complex and *Candida glabrata* complex, have increased in recent years. India contributes to one-fifth of global live births and more than a quarter of neonatal deaths. About 0.75 million neonates die every year in India, the highest for any country in the world⁶. Neonatal sepsis is the second leading cause of death in this population in India and is responsible for almost a quarter of total neonatal deaths⁷. *Candida parapsilosis* and *Candida tropicalis* are other species getting notorious in Neonatal Intensive Care Unit (NICU) outbreaks. The signs and symptoms are nonspecific and include temperature instability, refusal of feeds, respiratory distress, abdominal distension, apnea, lethargy, bradycardia, decreased perfusion, or seizures. Systemic Candidiasis lead more frequently to end-organ damage than other newborn infections and can involve kidneys, brain, lungs, eyes, liver spleen, bones and joints⁸.

Purpose of the Study :

This study aimed to determine the occurrence of Candidaemia in the NICU of a Tertiary Care Hospital in West Bengal during 4 years of observation; to analyse the trend in species distribution; and to examine in vitro susceptibility to common antifungal drugs.

MATERIALS AND METHODS

A retrospective review of 2312 blood samples collected from the neonates with suspected blood stream infection admitted in Neonatal Intensive Care Unit and Sick Newborn Care Unit in 2019, 2020, 2021 and 2022 were done. Blood samples were collected into Glucose and Bile broths, which were incubated at 37°C for 7 days. Subcultures were made on blood and MacConkey's agar. *Candida* spp. isolated were confirmed by: (a) Germ tube production, (b) growth on cornmeal agar (Hi-Media), (c) pigmentation on Hi-Chrome *Candida* differential agar (Hi-Media), (d) sugar assimilation tests as per standard techniques. Candidemia was diagnosed by isolation of *Candida* species from at least one positive blood culture containing pure growth of *Candida* species with supportive clinical features. Neonates who had a positive blood culture for *Candida* species, were enrolled. Incidence of *Albicans* and *Non-albicans* Candidemia were evaluated. Drug Sensitivity Test was employed to determine the sensitivity of Voriconazole, Itraconazole, Fluconazole and Amphotericin B on Mueller Hilton agar with Methylene blue following the CLSI guidelines.

RESULTS

Out of blood samples collected from 2312 neonates with suspected Blood stream infection, 568 (24.6%) samples were detected with Blood stream infection. Among which 89 samples were identified as fungal isolates and out of those samples 83 ie, 93.2% samples were detected as *Candida* isolates. Among the *Candida* isolates majority were belong to *Candida albicans* (39/89, 43.9%) followed by *Candida tropicalis* (22/89, 24.7%), *Candida glabrata* (11/89, 12.4%), *Candida parapsilosis* (6/89, 6.7%) and *Candida guilliermondii* (5/89, 5.6%).

Among the positive *Candida* isolates Share of *Candida albicans* was 47.0% (39/83) followed by *Candida tropicalis* 26.5% (22/83), *Candida glabrata* 13.3% (11/83), *Candida parapsilosis* 7.2% (6/83) and *Candida guilliermondii* 6.0% (5/83).

Majority of candidaemia were due to non-*albicans* *Candida* ie, 49.4% of total isolates and 52.8% of total positive isolates, although largest share among all positive isolates were belong to *Candida albicans* ie, 47.0% (39/83) (Tables 1A & 1B).

Among the samples, 59.6% (53/89) were collected from male patient and 40.4% (36/89) from female patients. Male: female ratio was 1.5:1. Among which 50% of *Candida albicans* and *Candida glabrata* isolates, 100% *Candida parapsilosis* isolates and 66.7% *Candida tropicalis* isolates belong to blood sample collected from male babies; 100% of *Candida guilliermondii* isolates were detected from the blood sample collected from female babies (Table 2).

Table 1A — Distribution of isolates among the samples

Name of organism	Number of isolate		Proportion	
Candida albicans	39		43.8%	
Candida glabrata	11	44	12.4%	49.4%
Candida guilliermondii	5		5.6%	
Candida parapsilosis	6		6.7%	
Candida tropicalis	22		24.7%	
NG	6		6.7%	
Grand Total	89		100.00%	

Table 1B — Distribution of isolates among the positive samples

Name of organism	Number of isolate		Proportion	
Candida albicans	39		47.0%	
Candida glabrata	11	44	13.3%	53.0%
Candida guilliermondii	5		6.0%	
Candida parapsilosis	6		7.2%	
Candida tropicalis	22		26.5%	
Grand Total	83		100.00%	

Table 2 — Distribution of sex among individual isolates

Name of organism	Proportion among female baby	Proportion among male baby	Total number of isolates
Candida albicans	50.0%	50.0%	39
Candida glabrata	50.0%	50.0%	11
Candida guilliermondii	100.0%	0.0%	5
Candida parapsilosis	0.0%	100.0%	6
Candida tropicalis	33.3%	66.7%	22
NG	33.3%	66.7%	6

Among the positive isolates 30.1% (25/83) belong to low birth weight babies ie, birth weight <2.5 kg, among which majority were isolated as Candida albicans (22/25, 88.0%) isolates and rests are Candida tropicalis (3/25, 12.0%) isolates. Among the babies with birth weight > 2.5 kg ie, 69.9% of total isolates, 29.3% (17/58) isolates belong to Candida albicans, 32.8% (19/58) Candida tropicalis, 19.0% (11/58) Candida glabrata, 10.3% (6/58) Candida parapsilosis and 8.6% (5/58) belong to Candida guilliermondii (Table 3).

In 37 out of 39 isolates (94.9%), which were later identified as Candida albicans, were germ tube positive. In 2 other isolates, which were later identified as Candida tropicalis, were also germ tube positive. Other isolates were germ tube test negative (Table 4).

Drug susceptibility testing revealed that 7.2% (6/83) of all the retrieved Candida isolates were resistant to voriconazole, 51.8% (43/83) resistant to Fluconazole,

Table 3 — Isolates prevalence according to birth weight (among the positive isolates)

Birth weight	< 2.5 kg		> 2.5 kg		Grand Total
Candida albicans	22	88.0%	17	29.3%	39
Candida glabrata			11	19.0%	11
Candida guilliermondii			5	8.6%	5
Candida parapsilosis			6	10.3%	6
Candida tropicalis	3	12.0%	19	32.8%	22
Grand Total	25	(30.1%)	58	(69.9%)	83

Table 4 — Germ tube test positivity among positive isolates

Name of organisms	Germ Tube Test		Grand Total
	-	+	
Candida albicans	2	37	39
Candida glabrata	7		11
Candida guilliermondii	3		5
Candida parapsilosis	4		6
Candida tropicalis	20	2	22

Table 5 — Resistant pattern of all isolates

Name of drugs	Resistant	Susceptible	Grand Total
Voriconazole	7.2% (6)	92.8% (77)	83
Fluconazole	51.8% (43)	48.2% (40)	83
Itraconazole	68.7% (57)	31.3% (26)	83
Amphotericin B	73.5% (61)	26.5% (22)	83

68.7% (57/83) resistant to Itraconazole and 73.5% (61/83) resistant to Amphotericin B (Table 5).

Among the individual isolates, 5.1% (2/39) Candida albicans isolates are resistant to Voriconazole, 58.8% (23/39) resistant to Fluconazole 71.8% (28/39) resistant to Itraconazole and 56.4% (22/39) resistant to Amphotericin B. None of the Candida glabrata isolates resistant to voriconazole, 54.4% (6/11) resistant to Fluconazole, 45.4% (5/11) resistant to Itraconazole and 81.2% (9/11) were resistant to Amphotericin B. All Candida guilliermondii isolates was resistant to itra-conazole and amphotericin B, 40.0% (2/5) resistant to voriconazole and 60.0% (3/5) resistant to Fluconazole. Isolates diagnosed with Candida parapsilosis 83.3% (5/6) were resistant to Fluconazole and itraconazole, none was resistant to voriconazole & all were resistant to Amphotericin B. 9.1% (2/22) Candida tropicalis isolates were resistant to Voriconazole, 27.3% (6/22) to Fluconazole, 63.6% (14/22) to Itraconazole and 86.4% (19/22) resistant to Amphotericin B (Table 6).

Table 6 — Resistance pattern of different isolates with respect to available antifungals

Name of drugs	Candida albicans (n=39)	Candida glabrata (n=11)	Candida guilliermondii (n=5)	Candida parapsilosis (n=6)	Candida tropicalis (n=22)	Grand Total (n=83)
Voriconazole	5.1% (2)	0.0% (0)	40.0% (2)	0.0% (0)	9.1% (2)	7.2% (6)
Fluconazole	58.8% (23)	54.5% (6)	60.0% (3)	83.3% (5)	27.3% (6)	51.8% (43)
Itraconazole	71.8% (28)	45.4% (5)	100.0% (5)	83.3% (5)	63.6% (14)	68.7% (57)
Amphotericin B	56.4% (22)	81.2% (9)	100.0% (5)	100.0% (6)	86.4% (19)	73.5% (61)

On analysis of the resistance pattern of Albicans and Non-albicans Candida, it has shown that Albicans Candida are comparatively more resistance to Fluconazole (58.8% against 45.4%) & Itraconazole (71.8% against 65.9%); whereas Non-albicans Candida are comparatively more resistance to Voriconazole (9.1% against 5.1%) and Amphotericin B (88.6% against 56.4%)(Table 7).

On analysis of the susceptibility of the anti-fungals according to birth weight, it was seen that In LBW, Candida albicans isolates showed higher resistance to Fluconazole (63.6% versus 52.9%) & Amphotericin-B (59.1% versus 52.9%) than normal birth weight babies (Table 8).

DISCUSSION

Candidemia is a significant cause of morbidity and mortality in neonates admitted in the NICU. Although historically Candida albicans was the most frequently isolated Candida spp. from cases of neonatal septicaemia, recently Non-albicans Candidaemia notably Candida tropicalis, Candida glabrata, Candida parapsilosis have emerged as important pathogens. The isolation rate of neonatal candidaemia varies from place to place. Candidaemia was found to be responsible for (53/334) 15.8% cases of neonatal septicaemia in present study, which is consistent with the observations of Diana M Hassan, *et al* (32/214) 15%⁹ and Jain, *et al* reported that Candidemia accounted for 15.8% of the neonatal Blood stream infections¹⁰. Although a higher incidence of 20.4% was reported by Rao, *et al*¹¹. Male female ratio in our study was 1.5:1, which is consistent with the study by Giuseppina Caggiano in ic2017, where this ratio was 1.6:1¹².

In the present study, majority of candidaemia were due to non-albicans Candida ie, 49.4% of total isolates and 52.8% of total positive isolates; although the major single species isolated as causative agent was Candida albicans (47.2%) and majority of the isolates due to Non-albicans candida. Although among the low birth weight babies Candida albicans isolates are dominated than non-albicans isolates (88.0% against 12.0%). Jinjian Fu, *et al* observed 39.6% Candida albicans (19/48) among the isolates found in their study and consistent with our observation¹³ and MS Srinibas Rao, *et al* observed growth of 26.92% Candida albicans, in their study¹⁴, a lower proportion from our study. Both of the study found majority of isolates as Non-albicans candida, Candida spp. proportion in candidemia may be varied because of geographical variation in different regions.

The most frequent Candida spp. isolated from the blood stream (among the positive isolates) in the present study was Candida albicans (47.0%) followed by Candida tropicalis (26.5%), Candida glabrata (13.3%), Candida parapsilosis (7.2%) and Candida guilliermondii (6.0%). Jinjian Fu, *et al* observed 39.6% Candida albicans (19/

Table 7 — Comparative resistance pattern of Albicans & Non-albicans Candida

Name of drugs	Candida albicans (n=39)	Non-albicans candida (n=44)	Grand Total (n=83)
Voriconazole	5.1% (2)	9.1% (4)	7.2% (6)
Fluconazole	58.8% (23)	45.4% (20)	51.8% (43)
Itraconazole	71.8% (28)	65.9% (29)	68.7% (57)
Amphotericin B	56.4% (22)	88.6% (39)	73.5% (61)

Table 8 — Resistance pattern of Candida albicans according to birth weight

Name of drugs	BW <2.5 Kg (n=22)	BW >2.5 Kg (n=17)	Grand Total (n=39)
Voriconazole	0.0% (0)	11.8% (2)	5.1% (2)
Fluconazole	63.6% (14)	52.9% (9)	58.8% (23)
Itraconazole	68.2% (15)	76.5% (13)	71.8% (28)
Amphotericin B	59.1% (13)	52.9% (9)	56.4% (22)

48) followed by Candida glabrata at 33.3% (16/48), and Candida tropicalis at 27.1% (13/48)¹³. MS Srinibas Rao, *et al* observed growth of 26.92% Candida albicans, 36.53% Candida tropicalis, 19.23% Candida glabrata, 7.69% Candida parapsilosis and 3.84% Candida guilliermondii in their study¹⁴.

In our study, 92.8% Candida isolates were found to be susceptible to Voriconazole, 48.2% susceptible to Fluconazole, 31.3% susceptible to Itraconazole and only 26.5% susceptible to Amphotericin B. Study done by Hassan DM, *et al* reported 75% Candida isolates susceptible to Voriconazole, 87.5% to amphotericin B and 81.25% to Fluconazole. In this study, all isolates showed higher sensitivity than our study⁹.

In any case, the variation in the distribution and susceptibility pattern of Albicans or Non-albicans Candida isolates detected in neonatal population admitted in critical care set up may be due to variations in the population studied, pre-dominance of nosocomial pathogens inhabiting in the labour room, operation theatres, NICU or SNCUs, surgical procedures, asepsis maintained during surgical procedure, in house infection control measures taken and infection prevention policies alongwith geographical distribution, resistance patterns of the fungal isolates in question; moreover, contamination due to poor personal hygiene during normal labour or Post-procedural contamination and localized outbreaks may be possible reasons for the differences reported.

In our study, majority of Candida albicans isolates showed sensitivity to Voriconazole (94.9%) followed by Amphotericin B (43.6%), Fluconazole (41.2%) and Itraconazole (28.2%). Among the non-candida isolates, Candida glabrata showed most (100%) sensitivity to Voriconazole and least (18.8%) to Amphotericin B. Candida guilliermondii isolate found in our study showed resistant to Itraconazole & Amphotericin B and sensitive to

Voriconazole (60.0%) and Fluconazole (40.0%); 9.1% *Candida tropicalis* isolates showed resistant to Voriconazole & 27.3% to Fluconazole whereas 63.6% & 86.4% isolates showed resistant to Itraconazole & Amphotericin B respectively. *Candida parapsilosis* isolates showed 100% sensitivity to Voriconazole but major resistant to Fluconazole and Itraconazole (83.3% each) & 100% resistant to Amphotericin B *Candida* isolates showed higher resistant to Fluconazole than non-*Candida* isolates (58.8% versus 45.4%). Whereas study done by MS Srinibas Rao, *et al* showed 91% *Candida tropicalis* isolates, 67.8% *Candida parapsilosis* isolates & 62.5% *Candida glabrata* isolates were sensitive to Fluconazole¹⁴, and study done by Mamta Lamba, *et al* revealed Non-albicans *Candida* (NAC) showed good sensitivity to Fluconazole as compared to *Candida albicans*. But, regarding Fluconazole sensitivity of *Candida tropicalis* (93%), *Candida glabrata* (67%) and *Candida parapsilosis* (100%) and 57% in *Candida albicans* is much higher than our study. The sensitivity to Amphotericin B was 95% among all *Candida* isolates in contrary to 29% in our study¹⁵.

CONCLUSION

Limitations of the present study are mainly related to its retrospective nature with limited follow-up data. Nevertheless, this study shows that *Candida* spp. plays a significant role in neonatal candidaemia and assuming an increasing role in nosocomial infections in neonates with predisposition to Non-albicans Candidemia. This study also shows increasing resistant pattern to many regularly used antifungals which necessitates regular surveillance and monitoring of laboratory data.

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Conflict of Interest : None.

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