Original Article

# Emergence of Non-Albicans *Candida* Species in Neonatal Candidemia

**Deepak Juyal, Munesh Sharma, Shekhar Pal, Vyas Kumar Rathaur[1], Neelam Sharma**

*Departments of Microbiology and Immunology, and [1]Pediatrics, Veer Chandra Singh Garhwali Government Medical Sciences and Research Institute, Srinagar Garhwal, Uttarakhand, India*

## Abstract

**Background:** *Candida* species are one of the most common causes of blood stream infections among neonates and account for 9-13% of such infections. Although *Candida albicans* remains the most common fungal isolate from neonatal candidemia, longitudinal studies have detected a shift towards non-albicans *Candida* (NAC) species. **Aim:** To examine the prevalence and epidemiology of candidemia among infants admitted to our hospital. **Materials and Methods:** Blood samples were collected from 548 neonates and only those which yielded pure growth of *Candida* spp. were included in the study. The isolates were identified as per standard mycological techniques and antifungal susceptibility (AFS) was done by disc diffusion method. **Results:** Of the total 132 neonates included in the study, NAC species were responsible for 80.30% cases with *C. parapsilosis* (25.0%) and *C. tropicalis* (21.97%) as the most predominant species; whereas 19.70% of cases were caused by *C. albicans*. AFS results revealed that 65.91, 73.49, and 96.21% isolates were sensitive to fluconazole (FLK), itraconazole (ITR), and amphotericin B (AMB), respectively. **Conclusion:** Candidemia in neonates is an ominous prognostic sign and is an important entity in our hospital. Strict infection control strategies, appropriate preventive and therapeutic measures such as prophylactic antifungal use and a restrictive policy of antibiotic use should be implemented.

**Keywords:** Amphotericin B, *Candida parapsilosis*, *Candida tropicalis*, Low birth weight, Neonates, Neonatal intensive care units (NICU)

**Address for correspondence:** Mr. Deepak Juyal, Department of Microbiology and Immunology, Veer Chandra Singh Garhwali Government Medical Sciences and Research Institute, Srinagar Garhwal - 246 174, Uttarakhand, India. E-mail: deepakk787@gmail.com

## Introduction

Significance of *Candida* spp. in neonatal intensive care units (NICU) is increasingly being recognized. It is the third most common cause of late onset sepsis in NICU patients and accounts for 9-13% of blood stream infections (BSI) in neonates.[1] Although *Candida albicans* has historically been the most frequently isolated species, recently non-albicans *Candida* (NAC) have emerged as important opportunistic pathogen, notably *Candida tropicalis*, *C. parapsilosis*, *C. krusei,* and *C. glabrata*.[2,3] There is growing evidence suggesting a role of increasing use of azole agents in this epidemiological shift. Several of these NAC species exhibit intrinsic resistance to traditional triazoles like fluconazole (FLK) and may also demonstrate cross resistance to newer triazoles.[4] This makes it imperative to perform both speciation and antifungal susceptibility (AFS) of all the yeast isolates from blood or otherwise. Number of factors including the use of indwelling devices, broad spectrum antibiotics, low birth weight (LBW), prematurity, total parenteral nutrition (TPN), gastrointestinal surgery, artificial ventilation, and/or history of fungal colonization contribute to the risk.[5] Preterm, very low birth weight (VLBW): ≤1,500 g; extremely low birth weight (ELBW): ≤1,000 g; and critically ill infants are at highest risk of invasive *Candida* infections[6] *Candida* spp. can also spread through vertical transmission from maternal flora or via horizontal transmission from hands of healthcare workers (HCW).[7,8]

Access this article online

Quick Response Code:

Website: www.najms.org

DOI: 10.4103/1947-2714.118919

Clinical presentation of candidemia resembles sepsis syndrome and to establish a clinical diagnosis is difficult.[7,9] Respiratory insufficiency, feeding intolerance, abdominal distention, temperature instability, lethargy,

and decreased perfusion are the various clinical manifestations associated. *Candida* BSIs are associated with very high crude mortality of over 60%, while attributable mortality may be as high as 49%.[3,10] The incidence and associated mortality due to candidemia can be influenced by several factors including the population at risk, healthcare facility standards, *Candida* spp. involved, and antifungal resistance.[2] Due to considerable regional variability, the local epidemiological knowledge is critical in terms of prevention and management of invasive *Candida* infections.

We were noticing an increase in the isolation rate of NAC species over last few months from cases of neonatal septicemia, which prompted us to undertake the present study, to examine the prevalence and epidemiology of neonatal candidemia at our hospital. We also correlated our results with the associated risk factors and the clinical presentation of the neonates.

## Materials and Methods

This prospective study was conducted between January 2012 and December 2012 in the Departments of Microbiology and Pediatrics of our rural tertiary care and referral center in Uttarakhand state, India. Average referrals from outside sources are approximately 200-250/year. Premature infants comprise 15-20% of total number of deliveries in the hospital.

Prior to the sample collection, approval from institutional ethical committee and written informed consent from patient's guardian were obtained. From a total of 548 neonates (age <28 days, admitted to NICU with clinical suspicion of septicemia) blood samples were collected into Bactec Peds plus/F culture vials of an automated blood culture system (Bactec 9120, Becton Dickinson, USA) and only those which yielded pure growth of *Candida* spp. were included in the study. Candidemia was defined as the presence of at least one positive blood culture containing pure growth of *Candida* spp. with supportive clinical features. Candidemia was attributed to the mortality of the neonate expired within 3 days of a positive blood culture.

Any growth indicated was subcultured on 5% sheep blood agar, MacConkey's agar, and Sabouraud's dextrose agar (SDA) with chloramphenicol (0.05%) and incubated at 37°C. The *Candida* spp. isolated was identified as per standard mycological techniques.[11] Preliminary identification was done by colony morphology on SDA, chromogenic media (Hichrome, Himedia Pvt. Ltd.), growth at 45°C, germ tube test, chlamydospore formation, and was confirmed by carbohydrate fermentation and assimilation tests.[11]

AFS was performed for FLK (25 µg), itraconazole (ITR, 10 µg), and amphotericin B (AMB, 100 units) using disc diffusion method[12,13] on Muller-Hinton agar supplemented with 2% glucose and methylene blue (5 µg/ml). Zone diameters were interpreted as per the approved National Committee for Clinical Laboratory Standards (NCCLS) guidelines[14] Quality control for AFS was performed using *C. albicans*-ATCC 90028 and *C. parapsilosis*-ATCC 22019.

### Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 11 and the prevalence of organisms was determined and expressed in percentage.

## Results

A total of 381/548 (69.53%) cases were blood culture positive. Pure growth of *Candida* spp. was isolated from 132/381 (34.65%) cases. Pure growth of bacteria and mixed growth of bacteria and yeast were isolated from 207/381 (54.33%) and 42/381 (11.02%) of the cases, respectively.

Of the total 132 neonates included in the study 73 (55.30%) were females and 59 (44.70%) were males. The average gestational age was 32.2 weeks (30-39 weeks) and average birth weight was 1.2 kg (0.93-2.21 kg).

NAC spp. were responsible for 80.30%, whereas 19.70% of the cases were caused due to *C. albicans*. *C. parapsilosis* (25.0%), and *C. tropicalis* (21.97%) were the predominant NAC species isolated, followed by *C. glabrata* (14.39%) and *C. krusei* (10.61%) [Table 1]. AFS results showed 65.91% isolates sensitive to FLK, 73.49 % to ITR, and 96.21% to AMB. Five isolates (one *C. tropicalis*, two *C. krusei*, and two other *Candida* spp.) were found resistant to AMB. NAC species, especially *C. glabrata* and *C. krusei* were more resistant to azoles, particularly FLK, than *C. albicans* [Table 2].

Among the risk factors observed for candidemia [Table 3], prematurity (73.49%) and LBW (67.42%) were the commonest followed by indwelling catheters

**Table 1: Characterization of various *Candida* species isolated from blood (*n* = 132)**

| Organism | No. of isolates (%) |
|---|---|
| *Candida parapsilosis* | 33 (25.0) |
| *C. tropicalis* | 29 (21.97) |
| *C. albicans* | 26 (19.70) |
| *C. glabrata* | 19 (14.39) |
| *C. krusei* | 14 (10.61) |
| *Candida* spp.* | 11 (8.33) |

**Candida* species not otherwise identified

**Table 2: Antifungal susceptibility profile of *Candida* isolates (*n* = 132)**

| Organism | Antifungals tested | | | Total no. of isolates |
|---|---|---|---|---|
| | FLK *n* (%) | ITR *n* (%) | AMB *n* (%) | |
| *Candida parapsilosis* | 27 (81.82) | 29 (87.88) | 33 (100) | 33 |
| *C. tropicalis* | 23 (79.31) | 26 (89.66) | 28 (96.55) | 29 |
| *C. albicans* | 24 (92.31) | 24 (92.31) | 26 (100) | 26 |
| *C. glabrata* | 5 (26.32) | 7 (36.84) | 19 (100) | 19 |
| *C. krusei* | 3 (21.43) | 3 (21.43) | 12 (85.71) | 14 |
| Other *Candida* spp.* | 5 (45.46) | 8 (72.73) | 9 (81.82) | 11 |
| Total | 87 (65.91) | 97 (73.49) | 127 (96.21) | 132 |

FLK: Fluconazole, ITR: Itraconazole, AMB: Amphotericin-B. **Candida* species not otherwise identified

**Table 3: Potential risk factors for candidemia among neonates (*n* = 132)**

| Risk factors | Number of cases | Percentage (%) |
|---|---|---|
| Prematurity | 97 | 73.49 |
| Low birth weight | 89 | 67.42 |
| Indwelling catheters | 85 | 64.39 |
| Broad spectrum antibiotic use | 81 | 61.36 |
| Total parenteral nutrition | 73 | 55.30 |
| Ventilator support | 65 | 49.24 |
| Prolonged hyperalimentation | 43 | 32.58 |

**Table 4: Various clinical presentations observed in cases of neonatal candidemia (*n* = 132)**

| Sign/symptom | Number of cases | Percentage (%) |
|---|---|---|
| Failure to thrive | 94 | 71.21 |
| Feed intolerance | 87 | 65.91 |
| Respiratory distress | 84 | 63.64 |
| Abdominal distention | 78 | 59.09 |
| Lethargy | 73 | 55.30 |
| Poor perfusion | 38 | 28.79 |
| Convulsions | 27 | 20.45 |

(64.39%) and broad spectrum antibiotic use (61.36%). Failure to thrive (71.21%), feed intolerance (65.91%), and respiratory distress (63.64%) were the most common clinical presentations seen [Table 4]. Crude mortality due to candidemia was 34.85% as 46/132 of neonates died within 72 h of the candidemia diagnosis. Additional 13 (9.85%) neonates died post 72 h of the diagnosis of candidemia, so were not included in crude mortality. All the other patients were treated successfully.

## Discussion

In the present study NAC species accounted for 80.30% of the cases of neonatal candidemia, whereas *C.albicans* was responsible for 19.70% of cases. This corroborates well with the results of other authors.[15-18] Striking feature of the present study was isolation of *C. parapsilosis* (25.0%) and *C. tropicalis* (21.97%) as the most common NAC species.

Although *C. parapsilosis* is less virulent, but under certain conditions (IV catheters, high IV glucose concentrations) virulence may increase many folds and it is relatively difficult to eradicate this organism.[19] This subsequently has pharmacotherapeutic and pharmacoeconomic implication as to treat such infections is quite difficult. *C. parapsilosis* is an emerging fungal pathogen and the major threat for neonates in NICU as it frequently colonizes the hands of HCW, has high affinity for intravascular devices, and parenteral nutrition.[20,21] In the present study, 64.39 and 55.30% of patients were on indwelling catheters and TPN respectively, out of these, 14 patients had *C. parapsilosis* candidemia which is 42.42% of all the *C. parapsilosis* isolates. Higher affinity of *C. parapsilosis* to adhere on foreign material and ability to form biofilms are important factors for the development of fungemia.[22] Outbreaks of *C. parapsilosis* BSI in NICUs have been previously reported.[23,24]

*C. tropicalis* causes infections with high mortality in adults and children with hematological malignancies or in immunocompromised individuals.[25] Ability of this organism to produce clusters is one of its major virulence factors. Once introduced into the immunocompromised host, *C. tropicalis* may be more virulent than *C. albicans* and can rapidly progress from colonization to invasion. It is the second leading cause of candidemia in adults, but is quite infrequent among neonates.[25] However, what we speculate is that premature and LBW infants have an immature immune system and may behave like an immunocompromised adult patient in this regard. In the present study, *C. parapsilosis* and *C. tropicalis* have emerged as predominant species accounting for 46.97% of infected neonates.

AFS results showed that 34.09% of *Candida* isolates were resistant to antifungal drugs. High degree of resistance to azoles was seen among *C. krusei* (78.57%) and *C. glabrata* (63.16%). Previous studies have reported that resistance

to azole compounds among *C. glabrata* species can range from 3.6 to 64%.[26,27] A significant proportion of *C. tropicalis* isolates were resistant to azoles especially FLK. Although resistance to AMB was quite low (3.79%), but is a matter of concern as emergence of such isolates may pose serious therapeutic challenges and also increases risk of nosocomial infection. A noteworthy feature observed was that 3/46 neonates who died were infected with these AMB resistant strains.

Combinations of various risk factors are known to be strongly associated with development of candidemia, and our results also suggest the same. The major risk factors identified in our study were prematurity, LBW, indwelling catheters, and broad spectrum antibiotic therapy. More than 65% of our cases were either premature or LBW, highlighting the significant burden of this disease among such infants. Use of multiple invasive devices (catheters, endotracheal tubes) or surgery causes break in the skin/mucosal integrity, which predisposes these sites for colonization/infection by *Candida* spp. Broad spectrum antibiotics ranging two to four in number were being administered to most of the neonates in the present study. Antibiotics promote fungal overgrowth at the expense of normal bacterial flora and encourage translocation of yeast across the intact mucosa. The risk of candidemia is known to increase exponentially with each class of antimicrobial used. TPN induces gut mucosal atrophy and has immunosuppressive effects which again predisposes individual for infection. Moreover, certain *Candida* spp. like *C. parapsilosis* has higher affinity towards parenteral nutrition, and can be responsible for outbreaks in NICUs. The hands of HCW and environmental surfaces are newly appreciated potential reservoirs for nosocomial strains of *Candida* spp.

Neonatal candidemia is generally associated with high mortality. In the present study, crude mortality was 34.85%, which was quite high. Even though candidemia is associated with prolonged hospitalization, we found that most of the fatal cases occurred in infants <3 weeks of age and had LBW compared with the ones who survived. Given that infants of this age have decreased immunity, their host response to *Candida* may have contributed to mortality. It has been observed that clearance of fungemia is directly proportional to catheter removal as soon as candidemia is detected and failure to do so is a risk factor for death.[1] Also removal of catheter alone is not sufficient and administration of antifungal agents is equally important as early treatment with antifungals is associated with enhanced survival.[28,29] However in the present study, both these factors were frequent as majority of neonates who died had delay in the initiation of therapy and catheter retention for >48 h after diagnosis of candidemia which may have contributed to the high mortality.

Based on high perinatal risk factors for early onset sepsis, the current hospital antibiotic policy recommends empiric use of ampicillin-gentamicin for neonates born within the facility and cefotaxime-amikacin for neonates reffered from elsewhere. Long-term use of these broad spectrum antibiotics must have created a negative pressure and favorable environment for *Candida* spp. to flourish. This substantiates the need of prophylactic antifungals to be used in a set up where continuous upsurge in the incidence of candidemia is seen.

## Conclusion

Candidemia in neonates is an ominous prognostic sign and is an important entity in our hospital. Preventive measures such as use of filters for parenteral nutrition, prophylactic antifungal use, and a restrictive policy of antibiotic use to decrease *Candida* colonization/infection rates should be implemented to reduce the morbidity and mortality associated with these infections. Reporting of fungal BSI and the spectrum of species involved are essential measures in any ICU in order to implement appropriate preventive and therapeutic strategies. Though powered to detect significant risk factors for fungal sepsis, this is a single center study and our findings may not be generalizable to other institutions. Additional studies are necessary to validate our findings and define more accurately the reservoirs, mode of transmission, emergence of new species, and their sensitivity patterns. Epidemiological data of our study can serve as a template for the development of local guidelines for prevention and appropriate treatment of neonatal candidemia.

## References

1. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, *et al*. National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis among extremely low birth weight infants: Risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics 2006;117:84-92.
2. Goel N, Ranjan PK, Aggarwal R, Chaudhary U, Sanjeev N. Emergence of non *albicans Candida* in neonatal septicemia and antifungal susceptibility: Experience from a tertiary care center. J Lab Physicians 2009;1:53-5.
3. Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K. Non-*albicans Candida* species in blood stream infections in a tertiary care hospital at New Delhi, India. Indian J Med Res 2012;136:997-1003.
4. Magill SS, Shields C, Sears CL, Choti M, Merz WG. Triazole cross-resistance among *Candida* spp.: Case report, occurrence among bloodstream isolates, and implications for antifungal therapy. J Clin Microbiol 2006;44:529-35.
5. Singhi S, Rao DS, Chakrabarti A. *Candida* colonization and candidemia in a pediatric intensive care unit. Pediatr Crit Care Med 2008;9:91-5.
6. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA,

Ehrenkranz RA, *et al*. Late-onset sepsis in very low birth weight neonates: The experience of the NICHD Neonatal Research Network. Pediatrics 2002;110:285-91.

7. Ariff S, Saleem AF, Soofi SB, Sajjad R. Clinical spectrum and outcomes of neonatal candidiasis in a tertiary care hospital in Karachi, Pakistan. J Infect Dev Ctries 2011;5:216-23.
8. Adib SM, Bared EE, Fanous R, Kyriacos S. Practices of Lebanese gynecologists regarding treatment of recurrent vulvovaginal candidiasis. N Am J Med Sci 2011;3:406-10.
9. Zaoutis TE, Prasad PA, Localio AR, Coffin SE, Bell LM, Walsh TJ, *et al*. Risk factors and predictors for candidemia in pediatric intensive care unit patients: Implications for prevention. Clin Infect Dis 2010;51:e38-45.
10. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, *et al*. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 2003;37:1172-7.
11. McGinnis MR. Yeast Identification. In: Laboratory Handbook of Medical Mycology. New York: Academic Press; 1980. p. 337-73.
12. Barry AL, Brown SD. Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. J Clin Microbiol 1996;34:2154-7.
13. Kirkpatrick WR, Turner TM, Fothergill AW, McCarthy DI, Redding SW, Rinaldi MG, *et al*. Fluconazole disk diffusion susceptibility testing of *Candida* species. J Clin Microbiol 1998;36:3429-32.
14. National Committee for Clinical Laboratory Standards. Methods for antifungal disk diffusion susceptibility testing yeast: Approved guideline M-44A. Wayne, PA: NCCLS; 2004.
15. Sardana V, Pandey A, Madan M, Goel SP, Asthana AK. Neonatal candidemia: A changing trend. Indian J Pathol Microbiol 2012;55:132-3.
16. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care centre of North India: 5-year study. Infection 2007;35:256-9.
17. Baradkar VP, Mathur M, Kumar S, Rathi M. *Candida glabrata* emerging pathogen in neonatal sepsis. Ann Trop Med Pub Health 2008;1:5-8.
18. Kothari A, Sagar V. Epidemiology of *Candida* bloodstream infections in a tertiary care institute in India. Indian J Med Microbiol 2009;27:171-2.
19. Hartung de Capriles C, Mata-Essayag S, Azpiróz A, Ponente A, Magaldi S, Pérez C, *et al*. Neonatal candidiasis in Venezuela: Clinical and epidemiological aspects. Rev Latinoam Microbiol 2005;47:11-20.
20. Trofa D, Gácser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. Clin Microbiol Rev 2008;21:606-25.
21. Kuhn DM, Mikherjee PK, Clark TA, Pujol C, Chandra J, Hajjeh RA, *et al*. *Candida parapsilosis* characterization in an outbreak setting. Emerg Infect Dis 2004;10:1074-81.
22. Bonassoli LA, Bertoli M, Svidzinski TI. High frequency of *Candida parapsilosis* on the hands of healthy hosts. J Hosp Infect 2005;59:159-62.
23. Almirante B, Rodríguez D, Cuenca-Estrella M, Almela M, Sanchez F, Ayats J, *et al*. Epidemiology, risk factors, and prognosis of *Candida parapsilosis* bloodstream infections: Case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2006;44:1681-5.
24. Lupetti A, Tavanti A, Davini P, Ghelardi E, Corsini V, Merusi I, *et al*. Horizontal transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. J Clin Microbiol 2002;40:2363-9.
25. Roilides E, Farmaki E, Evdoridou J, Francesconi A, Kasai M, Filioti J, *et al*. *Candida tropicalis* in a neonatal intensive care unit: Epidemiologic and molecular analysis of an outbreak of infection with an uncommon neonatal pathogen. J Clin Microbiol 2003;41:735-41.
26. Sobel JD, Zervos M, Reed BD, Hooton T, Soper D, Nyirjesy P, *et al*. Fluconazole susceptibility of vaginal isolates obtained from women with complicated *Candida* vaginitis: Clinical implications. Antimicrob Agents Chemother 2003;47:34-8.
27. Tseng Y, Lee W, Kuo T. *In-Vitro* susceptibility of fluconazole and amphotericin B against *Candida* isolates from women with Vaginal Candidiasis in Taiwan. J Food Drug Anal 2005;13:12-6.
28. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, *et al*. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: A multi-institutional study. Clin Infect Dis 2006;43:25-31.
29. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of *Candida* bloodstream infection until positive blood culture results are obtained: A potential risk factor for hospital mortality. Antimicrob Agents Chemother 2005;49:3640-5.

**How to cite this article:** Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-albicans *candida* species in neonatal candidemia. North Am J Med Sci 2013;5:541-5.

**Source of Support:** Nil. **Conflict of Interest:** None declared.