DOI: 10.7860/JCDR/2021/48547.14983

Original Article

Microbiology Section

Characterisation of *Candida* Colonisation in Neonates

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ABSTRACT

Introduction: The occurrence of invasive fungal infections has increased significantly worldwide, premature infants in Neonatal Intensive Care Unit (NICU) along with other risk factors are at particular risk of these invasive fungal infections which lead to fungal septicaemia in newborns. Candidaemia is the most common form of invasive candidiasis associated with an unacceptably high mortality rates. *Candida* colonisation in neonates is considered the first step towards developing neonatal sepsis.

Aim: To determine the prevalence of *Candida* colonisation and its characterisation among neonates admitted in NICU.

Materials and Methods: The present study was a prospective cross-sectional study with 150 neonates included in the study. Swabs were taken from four different sites of each neonate and inoculated on Chocolate agar and Sabouraud's Dextrose Agar (SDA) and incubated at 37°C for seven days. *Candida* species isolated were confirmed by gram stain, germ tube test, growth on Chromogenic (CHROM) agar and cornmeal agar. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 11.0.

Results: A total of 32 (21.3%) neonates had *Candida* colonisation. Twenty two (68.7%) were low birth weight and 24 (75%) were born premature. Perineum was the most common (56%) site of colonisation. Among *Candida* isolates, *Candida tropicalis* (63%) was the commonest followed by *Candida parapsilosis* (25%) and *Candida glabrata* and *Candida albicans* (6%). The risk factors identified were low birth weight, premature birth, use of antibiotics.

Conclusion: Colonisation of preterm and low birth weight neonates by *Candida* species is a major risk factor and needs attention to avoid dissemination and life threatening infection.

Keywords: Candidaemia, Low birth weight, Neonatal intensive care units, Non-*albicans Candida*, Preterm neonate

INTRODUCTION

In the past two decades, there has been an increased incidence and prevalence of invasive fungal infections especially in immunocompromised and those requiring prolonged hospitalisation [1]. Among the long list of opportunistic fungi causing serious, life threatening infections, *Candida* species remain single most important cause of opportunistic mycosis worldwide, there was a 400% increase in candidaemia since the 1980s [2]. Since 2013, the Leading International Fungal Education (LIFE) portal has facilitated the estimation of the burden of serious fungal infections country by country for over 5.7 billion people (>80% of the world's population) among which the global estimate for invasive candidiasis was ~700,000 cases globally [3]. Also, *Candida* species have been identified as the third most common cause of late-onset sepsis in NICU patients [4].

In humans, *Candida* species colonises regions including skin, oropharynx, lower respiratory tract, gastrointestinal tract, and genitourinary system. Colonisation with *Candida* species occurs early in life of a neonate due to transmission from mother by vaginal delivery or by health care worker and hospital environment [5]. *Candida* colonisation is an important risk factor for systemic infection in neonates. Multiple risk factors have been identified for making a neonate susceptible to systemic candidiasis, these factors are low birth weight, gestation <25 weeks, prior *Candida* colonisation, prolonged treatment in intensive care units, invasive monitoring techniques, systemic antibiotics and parenteral nutrition [4,6]. It is important to understand factors affecting normal colonisation of the neonate, as well as those factors that predispose to fungal invasiveness. Candidiasis covers a wide range of diseases from more superficial and milder clinical manifestations such as oesophageal or oropharyngeal candidiasis to serious infections including Blood Stream Infections (BSIs) and disseminated candidiasis spreading to multiple organs, which is associated with high mortality rate. Early initiation of aggressive therapy, with careful monitoring, can lead to a successful outcome.

As many studies suggest [7-10], colonisation is the primary step for candidaemia. Such a type of study to look for *Candida* colonisation prevalence and characterisation in NICU had not been carried out before in our institute so this study was undertaken.

MATERIALS AND METHODS

The present prospective cross-sectional study was conducted in NICU of a tertiary care teaching hospital at Mysore between August 2018-December 2018 after taking approval from Institutional Research and Ethics Committee (EC REG: ECR/134/Inst/KA/2013/RR-16).

Inclusion criteria: The subjects included for the study were neonates admitted in NICU for more than 48 hours.

Exclusion criteria: Neonates with an already established invasive fungal infection were excluded.

Sample size calculation: Sample size was calculated using the below formula:

$$n=Z^2P(1-P)/d^2$$

Where n is the sample size, Z is the statistic corresponding to level of confidence, Z value for 95% confidence is 1.96. P is expected prevalence (taken as 32.5% from the previous study done by Nazir A and Masoodi T [7]), and d is precision, taken as 5%.

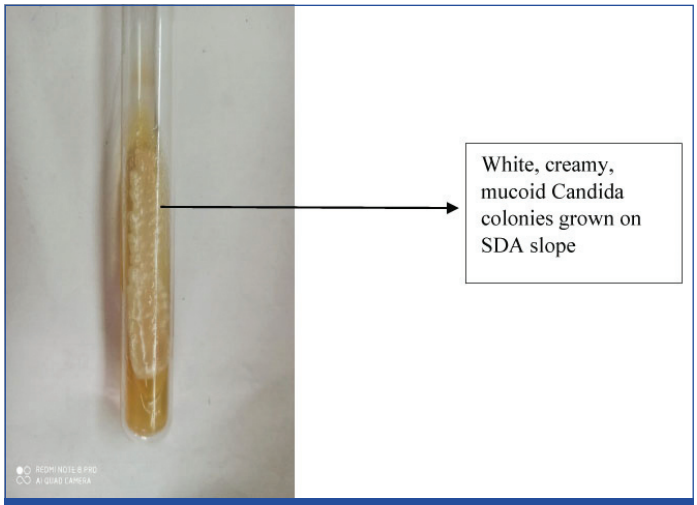
$$n=1.96\times1.96\times0.325(1-0.325)/0.05\times0.05$$

Sample size was calculated as 100 (rounded-off), 150 subjects were included.

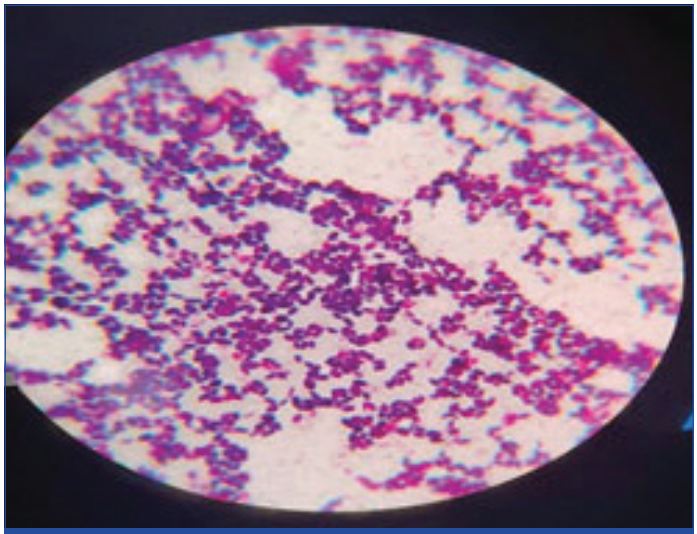
The detailed history was noted down on the proforma after taking consent from the parent. The samples (swabs) were collected from 150 neonates from different sites like perineal area, ear canal, throat and umbilicus with the help of sterile cotton tipped swabs pre-moistened with sterile saline. Different swabs were used for collection from different sites. The swabs were rubbed on the selected site with gentle pressure.

All swabs were inoculated immediately on chocolate agar and SDA, incubated at 37°C for seven days. Culture tubes and plates were

examined daily for their morphological and colonial characters. Growth observed on chocolate agar and SDA was creamy, smooth, pasty colonies after 24-48 hours or at the end of seven days [Table/Fig-1] which was confirmed morphologically, gram positive budding yeast cells by gram's stain were seen [Table/Fig-2]. In addition, blood culture records were looked if any blood sample from the study subjects i.e., of the neonates were received and checked for *Candida* growth and correlated with *Candida* colonisation.



[Table/Fig-1]: Growth on SDA- *Candida* spp grow as white creamy mucoid colonies.



[Table/Fig-2]: Gram stain of *Candida albicans* showing typical oval budding yeast cells (100x oil immersion).

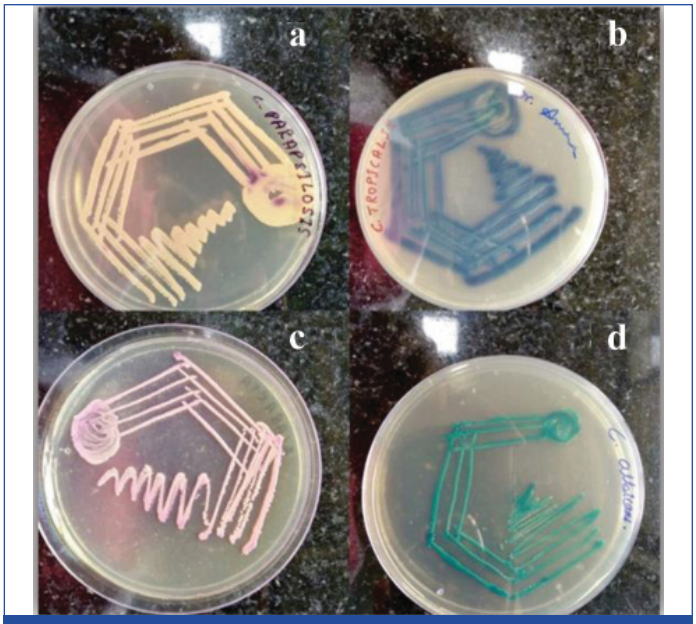
Identification of *Candida* species was done by its ability to form germ tube by Reynolds Braude phenomenon, hyphal, chlamydospore and blastospore formation by inoculating on to cornmeal agar and incubating for 24-48 hours and also growth on CHROM agar. Confirmed *Candida* colonies were inoculated on CHROM agar plates, incubated at 37ºC and the plates were examined at 24 hours, 48 hours and 72 hours after incubation. After 24 hours of incubation majority of the *Candida* colonies had grown well but the accurate colour of the different species developed only after 48 hours of incubation [Table/Fig-3].

STATISTICAL ANALYSIS

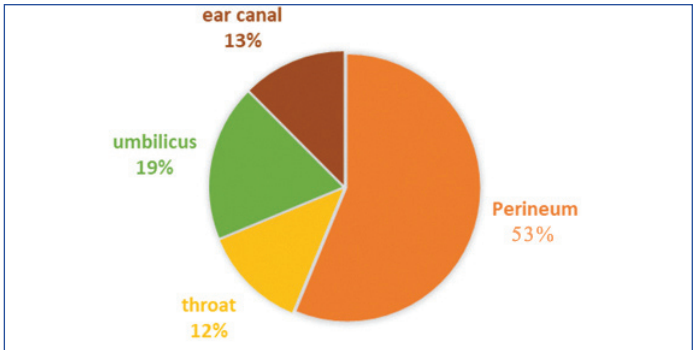
Statistical analysis was done using SPSS version 11.0 and the prevalence of organisms was determined and expressed in percentage.

RESULTS

In the present study *Candida* colonisation was observed in 32 (21.3%) out of 150 neonates. Colonisation was found predominantly in perineal region in 17 (53%) out of 32 isolates, the colonisation in other sites is shown in [Table/Fig-4]. Among the various species isolated in colonised neonates *Candida tropicalis* was the common species followed by *C.parapsilosis C.glabrata* and *C.albicans* [Table/Fig-5].



[Table/Fig-3]: Colony morphology of *Candida* species on CHROMagar: (a) *Candida parapsilosis*: cream to white smooth colonies; (b) *Candida tropicalis*: blue to metallic blue coloured raised colonies; (c) *Candida glabrata*: pink smooth colonies; (d) *Candida albicans*: light green coloured smooth colonies.



[Table/Fig-4]: Distribution of *Candida* colonisation in various sites.

Candida species	No. of isolates (%)
C.tropicalis	20 (63)
C.parapilosis	08 (25)
C.glabrata	02 (06)
C.albicans	02 (06)
Total	32 (100)

[Table/Fig-5]: Different *Candida* species isolated from the colonised subjects.

Age distribution among the colonised neonates, 2-10 days was predominant with 18 (56%) out of 32, total 26 (81%) of the affected neonates were below 30 days of age and 06 (19%) were above 30 days. The low birth weight 22 (55%) was most predominant risk factor, distribution of other risk factors in *Candida* colonised neonates are shown in [Table/Fig-6].

Variables	No. of subjects	Candida colonised n (%)
Place of delivery		
Outborn	60	19 (31.7)
Inborn	90	13 (14.4)
Preterm	87	24 (27.6)
Birth weight		
LBW (2.5 kg-1.5 kg)	40	22 (55)
ELBW (<1.5 kg)	32	4 (12.5)
PROM	56	14 (25)
Antibiotic therapy	136	32 (23.5)

[Table/Fig-6]: Risk factor identified in neonates with candidaemia.
LBW: Low birth weight; ELBW: Extremely low birth weight; PROM: Premature rupture of membranes

Blood culture data of neonates colonised with *Candida* was collected from the departmental blood culture register for correlation. It was found that *Candida* spp was isolated from blood culture among 14 out of the 32 *Candida* colonised neonates and same *Candida* spp was isolated from blood and swab in those neonates.

DISCUSSION

Neonates represent a highly vulnerable patient population. It is estimated that invasive infections cause >1.4 million neonatal deaths worldwide annually [11]. There has been a significant increase in the incidence of invasive fungal infections worldwide especially in patients admitted in intensive care units. Among different fungal pathogens, colonisation by *Candida* species is very frequent in NICU and a necessary first step in the pathogenesis of systemic infection. *Candida* species are a common cause of neonatal nosocomial BSIs in premature infants and are a leading cause of infectious-related mortality in the NICU. The overall prevalence of *Candida* colonisation was 21.3% in neonates admitted to the NICU. Prevalence reported by other studies over the last decade point towards an increasing *Candida* prevalence, findings of these studies have been summarised in [Table/Fig-7] [7-10,12-18].

In this study, most of the neonates had *Candida* colonisation by day ten and maximum colonisation was seen in perineum region. Several risk factors have been associated with *Candida* colonisation, major risk factors identified in this study were low birth weight, prematurity and use of antibiotic therapy. High percentage of the neonates with colonisation with *Candida* in present study had Low Birth Weight (LBW) (68%) and Extremely Low Birth Weight (ELBW) (13%) and 75% of them were born preterm, which is consistent with observations of other studies that the incidence of *Candida* colonisation was inversely proportional to birth weight and gestational age. This association of *Candida* colonisation and lower gestational age can be due to prematurity being associated with compromised immunity such as decreased skin and mucosal integrity, the relative quantitative deficiency of protective maternal IgA to *Candida*, also excessive handling of preterm neonates in neonatal units promotes horizontal acquisition [5]. Prolonged PROM (≥24hrs), especially in vaginally colonised mothers has been associated with neonatal septicaemia [19]. In present study, there was no association between Premature Rupture Of Membranes (PROM) and *Candida* colonisation was found, also the mothers with vaginal colonisation did not checked.

Historically, *Candida albicans* was the most frequently isolated species, but recently non-*albicans Candida* (NAC) species have emerged as important opportunistic pathogens [20]. In this study, the NAC species were the predominant organism responsible for colonisation in neonates and the remaining were of *C. albicans*. *C.tropicalis* was the most common species isolated.

Limitation(s)

The study had certain limitations. Blood samples did not collect from all the subjects to correlate the isolated coloniser with systemic infection. In addition, follow-up of candidemic neonates could not be done.

CONCLUSION(S)

In conclusion, in the present study it was observed that the neonates with risk factors have increased chance of being colonised with *Candida* in turn they may progress to candidaemia, which may increase the hospital stay, add socio-economic burden and increased morbidity and mortality.

Hence, focus should be shifted on prevention of candidaemia in these high-risk neonates by screening them for *Candida* colonisation, selectively keeping under observation and if suspected candidaemia they can start with antifungal agents instead of giving empirical treatment to all. Also, preventive measures like screening of NICU staff for carriers, restricted antibiotic use can decrease *Candida* colonisation and hence its infection.

Study	Year	Place	Study population	Findings
Present study	2021	Karnataka, India	Neonates	Neonates positive for candidaemia-21% with *C.tropicalis* isolated as predominant species
Nazir A and Masoodi T [7]	2018	Kashmir, India	Neonates	Neonates positive for candidaemia-32.5% with *C.tropicalis* isolated as predominant species
Azim A et al., [8]	2018	Lucknow, India	Adults (ICU)	95% unifocal and 81% multifocal *Candida* colonisation with *C. glabrata* 11 species isolated.
Latha GS et al., [9]	2017	Karnataka, India	Neonates	13.8% neonates positive for *Candida* culture. Low birth weight identified as most common risk factor.
Pandita N et al., [10]	2017	Delhi, India	Neonates	Fungal sepsis in 13.6% neonates. *C.glabrata* most common species isolated, LBW and Prematurity most associated risk factors
Bansiwal K et al., [12]	2016	Rajasthan, India	Neonates	*Candida* colonisation rate was 47.31% in neonates with *C.albicans* most common isolated species
Abdallah Y et al., [13]	2015	Uganda	Neonates	*Candida* colonisation seen in 23.5% neonates. *C.albicans* most common isolated species, prematurity most common associated risk factor.
Mahnoudabadi AZ et al., [14]	2015	Iran	Neonates and Children	34% oral swabs and 21% urine samples were positive for *Candida* species with *C.albicans* most common species isolated
Wadile RG and Bhate VM [15]	2015	Maharashtra, India	Neonates	Positive *Candida* culture in 32.26% neonates with *C.albicans* most common isolated species and LBW most common associated risk factor.
Noori Sanami M et al., [16]	2015	Iran	Neonates	31.7% neonates positive for *Candida* culture. Rectum and umbilicus most common site for colonisation.
Tak V et al., [17]	2014	Delhi, India	Adults	14.95% per 1000 ICU patients. *C.tropicalis* most common isolated species.
Sardana V et al., [18]	2012	Meerut, India	Neonates	30.1% of neonates had candidemia with *C.glabrata* was the most common isolated species.

[Table/Fig-7]: *Candida* prevalence reported by other studies [7-10,12-18].

REFERENCES

[1] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev. 2007;20(1):133-63.
[2] Blot S, Dimopoulos G, Rello J, Vogelaers D. Is *Candida* really a threat in the ICU? Curr Opin Crit Care. 2008;14:600-04.
[3] Bongomin F, Gago S, Oladele RO, Denning DW. Global and multinational prevalence of fungal diseases estimate precision. J Fungi (Basel). 2017;3(4):57.
[4] Avila-Aguero ML, Canas-Coto A, Ulloa-Gutierrez R, Caro MA, Alfaro B, Paris MM. Risk factors for *Candida* infections in a neonatal intensive care unit in Costa Rica. Internat J Infectio Dis. 2005;9:90-95.
[5] Ali GY, Algohary EH, Rashed KA, Almoghanum M, Khalifa AA. Prevalence of *Candida* colonisation in preterm newborns and VLBW in neonatal intensive care unit: Role of maternal colonisation as a risk factor in transmission of disease. J Matern Fetal Neonatal Med. 2012;25(6):789-95.
[6] Shettigar CG, Shettigar S. Non *albicans* Candidemia: An emerging menace in neonatal intensive care unit. Int J Contemp Pediatr. 2018;5(2):436-41.
[7] Nazir A, Masoodi T. Spectrum of candidal species isolated from neonates admitted in an Intensive Care Unit of teaching hospital of Kashmir, North India. J Lab Physicians. 2018;10(03):255-59.

[8] Azim A, Ahmed A, Baronia A, Yadav R, Sharma P, Marak R. Epidemiology of *Candida* colonisation in medical surgical intensive care unit of a tertiary care teaching hospital of North India. J Microbiol Infectio Dis. 2018;8(4):147-52.
[9] Latha GS, Veeresh Babu DV, Thejraj HK. Clinical profile of candidiasis in neonates. Int J Contemp Pediatr. 2017;4(5):1875-80.
[10] Pandita N, Peshin C, Wasim S, Bhat NK, Gupta A. Profile of fungal septicaemia in new born at a tertiary care hospital in North India. Int J Contemp Pediatr. 2017;4(2):455-59.
[11] Neves RP, de Carvalho Parahym AMR, da Silva CM, Macêdo DPC, Leal AFG, Neves HJ, et al. Fungal infections in neonatal intensive care. Selected Topics in Neonatal Care. 2018;8:109-21.
[12] Bansiwal K, Binnani A, Gupta PS. Study of candida colonisation and candidemia in neonates admitted in neonatal intensive care unit at a tertiary care institute of Bikaner, Rajasthan, India. Int J Curr Microbiol App Sci. 2016;5(10):156-65.
[13] Abdallah Y, Kaddu-Mulindwa D, Nankunda J, Musoke PM. Prevalence and immediate outcome of *Candida* colonised preterm neonates admitted to Special Care Unit of Mulago Hospital, Kampala Uganda. Afr Health Sci. 2015;15(1):197-205.
[14] Mahmoudabadi AZ, Rezaei-Matehkolaei A, Ghanavati F. The susceptibility patterns of *Candida* species isolated from urine samples to posaconazole and caspofungin. Jundishapur J Microbiol. 2015;8(3):e24298.
[15] Wadile RG, Bhate VM. Study of clinical spectrum and risk factors of neonatal candidemia. Indian J Pathol Microbiol. 2015;58:472-74.
[16] Noori Sanami M, Radfar M, Shirvani F, Nabavi M, Gachkar L. *Candida* colonisation in low birth weight and very low birth weight infants in a neonatal intensive care unit. Arch Pediatr Infect Dis. 2015;3(4):e21234.
[17] Tak V, Mathur P, Varghese P, Gunjiyal J, Xess I, Misra MC. The epidemiological profile of candidemia at an Indian trauma care center. J Lab Physicians. 2014;6(2):96-101.
[18] Sardana V, Pandey A, Madan M, Goel SP, Asthana AK. Neonatal candidemia: A changing trend. Indian J Pathol Microbiol. 2012;55(1):132-33.
[19] Santhanam S, Arun S, Rebekah G, Ponmudi NJ, Chandran J, Jose R, et al. Perinatal risk factors for neonatal early-onset group b streptococcal sepsis after initiation of risk-based maternal intrapartum antibiotic prophylaxis—A case control study. J Trop Pediatr. 2018;64(4):312-16.
[20] Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-*albicans Candida* species in neonatal candidemia. N Am J Med Sci. 2013;5(9):541-45.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 25, 2021
- Manual Googling: Apr 22, 2021
- iThenticate Software: May 25, 2021 (10%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 22, 2021**
Date of Peer Review: **Mar 15, 2021**
Date of Acceptance: **Apr 22, 2021**
Date of Publishing: **Jun 01, 2021**