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# CHARACTERISATION AND SPECIATION OF CANDIDA SPECIES FROM NEONATAL SCNU AT A TERTIARY CARE HOSPITAL IN NORTHEASTERN INDIA

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## ABSTRACT

**Introduction:** In the neonatal intensive care unit, Candida infections are a major source of morbidity and mortality. The incidence of neonatal candidiasis is rising, primarily due to improved survival rates for low-birth-weight newborns, preterm births, medical advancements, life support systems, relative immunodeficiency, and widespread use of broad-spectrum antibiotics. Candida albicans' supremacy has gradually given way to nonalbicans Candida species over the last few decades.

**Aim and Objective:** To determine the spectrum of Candida infections and to characterize the isolated species of Candida in neonatal blood samples.

**Materials and methodology:** It was a hospital based cross sectional study carried out in the Department of Microbiology, Jorhat Medical College [JMC], for a period of one year from July 2015 - June 2016. Blood culture bottles of Neonates received in the lab were tested.

**Results:** Out of 542 samples, 123 [22.6%] samples showed neonatal candidemia. Out of 123 isolates, 100 were identified as *C.utilis* [81.3%], sixteen isolates were identified as *C.tropicalis*[13%],five isolates were identified as *C.glabrata* [4%] *and* two isolates were identified as *C.famata*[1.7%].

**Conclusion:** Neonatal candidemia is a significant condition in our area. The current study emphasises nonalbicans Candida species as an emerging threat in neonatal candidemia.

**Keywords:** Neonatal intensive care unit, Neonatal candidemia, Blood culture bottles, Blood samples, Emerging threat

## INTRODUCTION

One of the main causes of newborn sepsis is Candida blood stream infection [BSI][1]. Fungal pathogens such as Candida are very opportunistic and can present with a variety of symptoms, from invasive infections to mucocutaneous lesions [2]. The third most frequent cause of late-onset sepsis in newborns is Candidemia. It is defined as growth of Candida species from at least one blood culture. It was attributed to the mortality if the neonate expired within three days of a positive blood culture or evidence of disseminated candidiasis in the autopsy. Given the high death rate of 35–60%, particularly in severely unwell newborns, invasive candidiasis, also known as candidemia, warrants more attention [3,4]. The rising prevalence of invasive candidiasis may be explained by the extensive use of broad-spectrum antibiotics and the higher survival rate of extremely preterm infants

who require prolonged hospitalization [5]. Fungal colonization has been related to inadequate nurse-to-patient ratios, poor cleanliness, and NICU congestion. Ten percent of babies colonise within the first week of life, and by the time they stay in the hospital for four weeks, up to 64 percent have colonized [1]. Healthcare workers' [HCW] hands can transmit Candida species either horizontally or vertically through the mother's flora [6]. Growth of Candida species from at least one blood culture was referred to as candidemia[7]. If the neonate passed away within three days of a positive blood culture or an autopsy showing widespread candidiasis, the cause of death was considered to be candidemia[8]. While *Candida albicans* cause 45–55% of Candida blood stream infection in babies, current research has shown a shift towards *non-Albicans Candida [NAC]* species, which are frequently linked to high mortality and inadequate sensitivity to antifungals[1,2]. A thorough comprehension of the regional epidemiology of Candida infection is essential to assess the disease burden and develop an early and successful therapeutic plan [1].

## MATERIAL AND METHODOLOGY

The present study was a hospital based cross sectional study carried out in the Department of Microbiology, Jorhat Medical College (JMC), for a period of one year from July 2015 - June 2016.

**Inclusion criteria-** Blood samples of Neonates [0-28days] admitted in the Special care newborn unit of JMC, during the study period.

**Exclusion criteria-** Neonates with an already established invasive fungal infection and who were put on prophylactic antifungal therapy were excluded.

**Ethical clearance-** The study was duly permitted by Institutional Ethical Committee (Human), Jorhat Medical College, Jorhat, by No.SMEJ/JMCH/MEU/841/2011/1002

**Sample collection and transportation-** The blood culture bottle was labelled with patient information and transported to the Microbiology laboratory within 2 hours for culture and sensitivity. On receiving in the laboratory, it was incubated at $37^0$C in upright position for 24 hrs.

**Processing of Specimen-** First subculture was done on 5% Sheep blood agar, Mac Conkey agar and Sabouraud Dextrose agar and incubated for 24 hours in $37^0$ C. If no growth was observed on thefirstsubculture, then blood culture bottle was further incubated at $37^o$ C and subculture was done in every alternate day. When no growth was observed on plates after 7th day, the sample was reported as negative. When growth was obtained in the subculture it was identified by doing Gram staining. Suspected colonies of yeast were observed for presence of gram positive oval budding yeast like fungus and psuedohyphae. When budding yeast cells were seen it was reported as Candida species. Blood cultures yielding growth of Gram positive and Gram negative bacteria were not included.

**Identification of Candida species-** Germ Tube Test was done to differentiate *Candida Species* from *Non Candidal species*. Further speciation was done by panel of tests like detection of chlamydospore by growth morphology on Corn Meal Agar [CMA], colour on HiCrome Candida Differential Agar [Difco company], Sugar fermentation and Assimilation tests.

**Speciation using the Vitek 2Compact system with YST card-** Speciation with Vitek 2 Compact system using YST card was done using manufacture’s protocol to compare the conventional method of species identification with this automated identification system.

## RESULTS

In the study, blood samples of a total of five hundred and forty-two neonates admitted to SCNU were screened during a period of one year from July 2015 to June 2016 in the Department of Microbiology, Jorhat Medical College. 74(60.1%) of the neonates belonged to four to six days of age followed by 18 (15%) neonates of one to three days, 16 (13%) belonged to seven to nine days of age while only 15 (12.1%) were above nine days of age as shown in Figure 1.

**Fig 1: Age and sex distribution of study population**

Out of the 542 cases in our study, bacterial growth was found in 268 (49.44%) of the cases, fungal growth in 123 (22.69%). No growth was seen in rest 151(27.85%) of the sample (Figure 2).

**Fig 2: Distribution of isolates from the blood culture of the neonates.**

Out of 123 isolates, 100 were identified as *C.utilis*[81.3%], sixteen isolates were identified as *C.tropicalis*[13%],five isolates were identified as *C.glabrata*[4%] *and* two isolates were identified as*C.famata*[1.7%] by Conventional methods as shown in figure 3.

**Fig 3: Distribution of Candida species by conventional method**

Out of 123 isolates, 98 were identified as *C.utilis*[80%], Sixteen isolates were identified as *C.tropicalis*[13%] and 9 isolates were identified as *C.famata*[7%] by VITEK 2 Compact as shown in figure 4.

**Fig 4: Distribution of Candida species by VITEK method**

**Gram stain of Candida species-**Under the oil immersion objective of the light microscope, *Candida* spp appear as Gram positive, oval budding yeast cells [4-8 μm]with or without formation of pseudohyphae as shown in Figure 5.

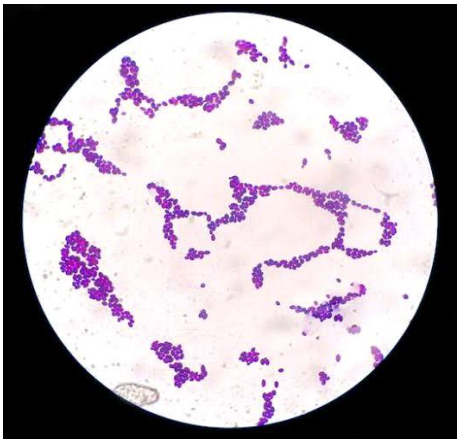

**Fig 5: Appearance of *Candida* species in Gram stain**

Germ Tube test was negative and was considered positive when 30% of yeast cells produced germ tubes as shown in Figure 6.

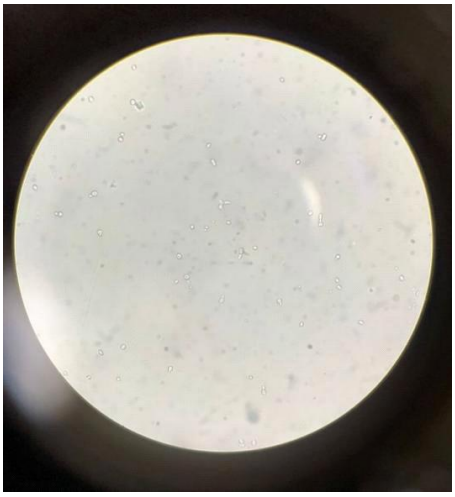

**Fig 6: Appearance of Non albican Candidashowing no germ tubes**

**Fig 7: Growth of Candida species on SDA**

*Candida dubliniensis* showed dark green coloured smooth colonies, *Candida tropicalis* showed blue to purple coloured raised colonies, *Candida glabrata* showed pink to cream coloured smooth colonies *Candida utilis* showed pink colour smooth shiny colonies in CHROMagar as shown figure 8.

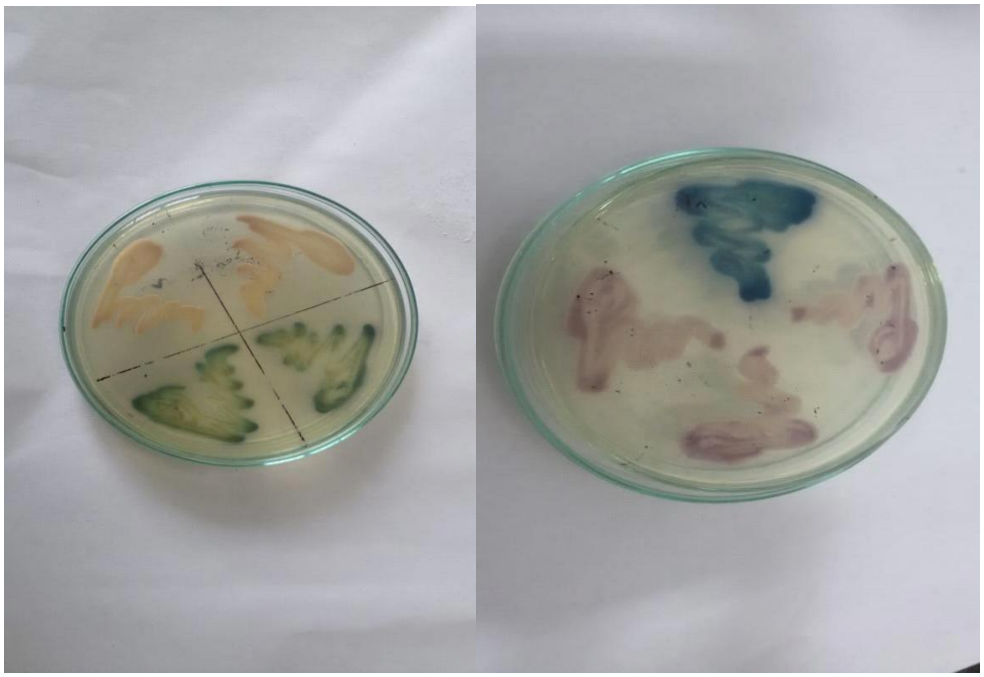

**Fig 8: Growth of Candida species on CHROM agar**

Figure 9 shows the growth of *C. utilis* withonly small blastoconidia and no pseudohyphae on CMA.

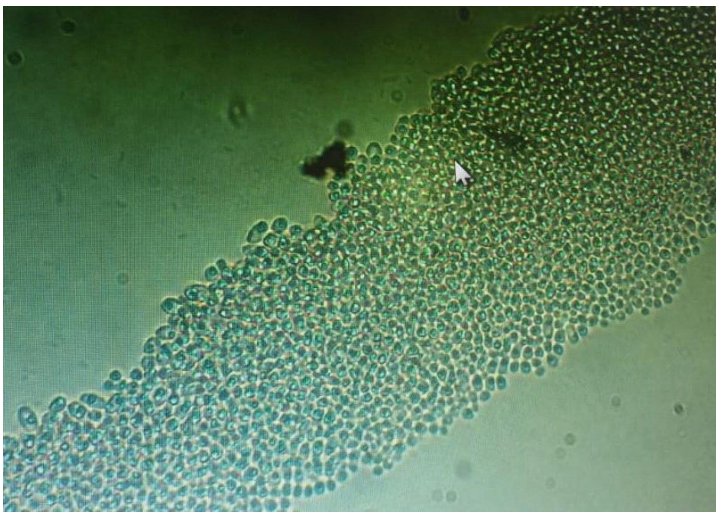

**Fig 9: Growth of Candida species on Cornmeal agar**

**Fig 10: Sugar fermentation test by Candida species**

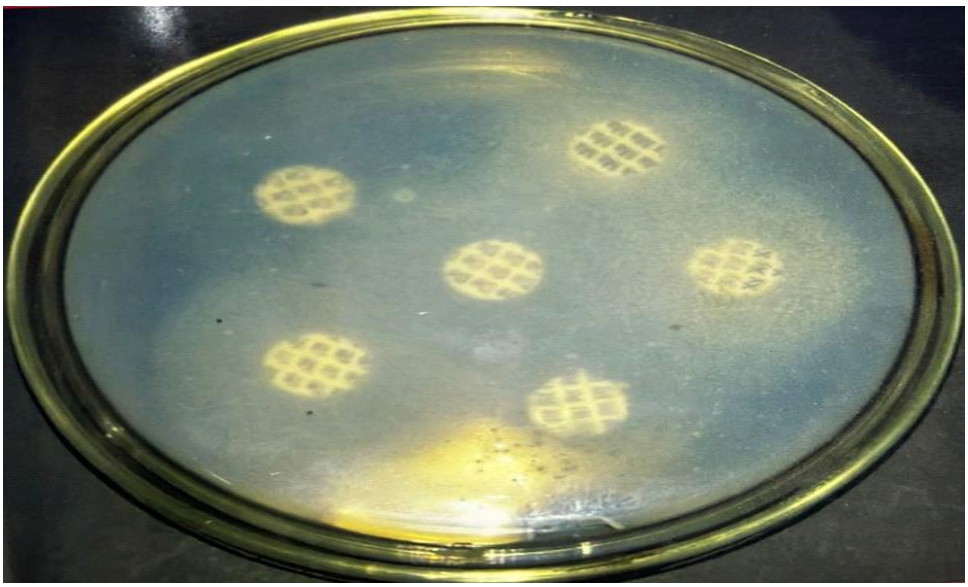

**Fig 11: Sugar assimilation test by Candida species**

Comparison between conventional method and automated method [VITEK 2] in identification was done. Out of 123 isolates, 100 were identified as *C.utilis*by conventional method whereas 98by VITEK-2A Compact. Sixteen isolates were identified as *C.tropicalis*by both conventional method as well as by VITEK 2 Compact. Two isolates were identified as*C.famata* by Conventional methods whereas VITEK-2 identified 9 isolates as *C.famata*. Five isolates were identified as *C.glabrata*byconventional method which was not identified by VITEK -2 Compact System [Table-1].

**TABLE 1: Comparison of fungal growth isolates by Conventional method and vitek-2 system**

| CANDIDA SPECIES | CONVENTIONAL METHOD | | AUTOMATED METHOD [VITEK- 2] | |
|---|---|---|---|---|
| | TOTAL NUMER OF ISOLATES [N = 123] | PERCENTAGE [%] | TOTAL NUMBER OF ISOLATES [N = 123] | PERCENTAGE [%] |
| *Candida utilis [*n=*100]* | 100/123 | 81.30% | 98/123 | 80% |
| *Candida tropicalis* [n= 16] | 16/123 | 13% | 16/123 | 13% |
| *Candida famata* [n=9] | 2/123 | 1.7% | 9/123 | 7% |
| *Candida glabrata*[n=5] | 5/123 | 4.06% | 0/123 | 0% |

## DISCUSSION

The present study was carried out with the objective of isolation, characterization and speciation of Candida species from newborn. A total number of 542 neonates who were admitted in Special Care Newborn Unit of Jorhat Medical College and Hospital, were enrolled in the study. Only 123 cases with fungal culture positive with pure growth of yeast were included

### Age and gender distribution of neonates with Candidemia

It was observed in the study that 60% [n= 74/123] of the neonates belonged from four to six days of age while only 12.1% [n=15/123] were above nine days of age. In the study conducted by Nagaveni P et al [2016][9],46.7% neonates were aged less than or equal to 3 days of age and the rest 53.3% belonged to the age group of greater than 3 days of age which is similar to our study.

Out of 123 cases in our study 64 [52%] patients were males and 59 [47.9.%] patients were females which was similar to Berhane M et al [2021][10]where they have reported male preponderance of 70.5% followed by females [57%] in their study. The prevalence of candidemia in male patients, as observed in the current study, was also observed by Biswas Binita et al [2023][11]where 62.5% male than in female [37.5%]. The male predominance seen can be due to the x-linked immunoregulatory gene making male neonates more susceptible to infection**.** Since males have only one X-chromosome they are immunologically less protected than females against candidemia. Several intrinsic immune receptors in the body including Toll-like receptors [TLR]7 and TLR8, cytokine receptors, like IL12RG and IL13RA2, transcription factors, like forkhead box P3 [FOXP3], and IL-1 receptor-associated kinase 1 [IRAK1], a crucial regulatory molecule in TLR signaling, are encoded on the X chromosome and exhibit sex-specific induction after bacterial or fungal infection[12].

As the SCNU at JMCH is 35 bedded and the rate of admission is high it leads to overcrowding at times, nursing care by mothers and sharing of non-invasive equipments. It is known that such factors can potentiate person to person transmission of Candida species as horizontal transmission has been shown to be an equally important route of Candida acquisition [13]. The incidence of

Candidemia in Special care newborn hospital in our study is 22.69% which is high. This value is comparable to 21.3% that was observed byDadal A et al in 2021 [14]where as BiswasBinita et al [2023][11] in their study reported 9.65% incidence of candidemia.

**General profile of isolated *Candida* spp-**

In the present study predominance of *Non albicans* candida was found to be predominant in the study. The present study is consistent with studies carried out by Rajni E et al [2021][15]and Dr. Sharada C Metgud[2024][16]who showed *Non albicans* candida to be the predominant isolates. A study from Northeast India demonstrated a prevalence of 6% candidemia with a predominance of*Non-albicans Candida* species of 70%. which is also concordance with our study[17].

In the present study *Candida utilis* was the most common isolate [n=98/123; 80%] followed by *C.tropicalis*[n=16/123; 13%], and *C.famata*[n= 9/123; 7%] which is consistent with the studies obtained by Singh et al [2024][18]showed 32% prevalence of *Candida utilis*. A case series conducted by Dr. Prasanna Gonti et al [2022][19]andSreelekshmi T. S et al [2021][20]showed *C. utilis* cause of septicemia in neonates and children respectively which is not consistent with our study. In our study after *C.utilis*, *C.tropicalis*[13%] and *C. famata*[7%] was found to be predominant. Veronica W[2024][21]and Behera SR et al [2023][22]reported the prevalence of *C. tropicalis as 17.1% and 29.3% respectively and C. famata* as 1.6% and 5.65% *respectively.*

*C.utilis* being regarded as a pathogen with moderate virulence and occasionally isolated from clinical specimens, it is mostly utilised in the food industry for nonethanolic fermentation processes. But these days, reports of C. utilis as an opportunistic pathogen causing newborn septicaemia have begun coming from all over the world [18]. Our study indicates higher incidence of *Candida utilis* than other non albicans candida isolates in the blood samples from the neonates admitted in Special Care Newborn Unit [SCNU]. This could be possibily due to overcrowding in the SCNU which must have led to possible outbreak. Besides this, other contributing risk factors associated were mechanical ventilation, prematurity, low birth weight, respiratory distress, premature rupture of membrane and meconium stained liquor[23]. Most studies reported that the VITEK 2 A Compact YST card system was a reliable method in identification of 93-99% of yeasts [24].Aubertine C.Let al [2000][25] have investigated the concordance between the VITEK2 YST card system and conventional methods. In their study, an unequivocal identification was obtained for 155 [92.8%] isolates and all isolates of *C. albicans, C. glabrata* and *C. krusei*were identified correctly.

One out of 22 *C. Parapsilosis* isolates had a low discrimination result. This is not consistent with our present study where out of 123 isolate, 100 were identified as *C.utilis*by conventional method whereas 98 as *C.utilis*by VITEK-2 Compact. A total of 16 isolates were identified as *C.tropicalis*by both conventional method as well as by VITEK-2. Conventional methods identified 2 isolates as *C.famata*whereas VITEK-2 identified 9 as *C.famata.* 5 isolates were identified as *C.glabrata*byconventional method which was detected as *C* .famata by VITEK -2 Compact. Furthermore, there is a continued need for surveillance of candidemia to monitor changes in the epidemiological features and antifungal susceptibility and also to develop and evaluate prevention strategies. Furthermore, speciation of *Candida* in neonatal candidemia and measures taken to reduce the risk factors wherever possible and can reduce morbidity, mortality, and antifungal drug pressure in the neonates [26, 27]. Over the past years, an increase in the isolation rate of nonalbicans *Candida* species from cases of neonatal septicemia, which prompted to undertake the present study to analyze and evaluate the change in the species distribution of *Candida* species in neonatal septicemia and determine their *in vitro* antifungal susceptibility and the risk factors associated with their acquisition.

## CONCLUSION

Candidemia in hospitalized patients especially in ICU is emerging as a significant problem worldwide.In the present study non albicans candida species was found to be predominant.Our study indicates higher incidence of *Candida utilis* than other non albicans candida isolates in the blood samples from the neonates admitted in Special Care Newborn Unit [SCNU].This could be possibly due to overcrowding in the SCNU which must have led to possible outbreak. The findings of our current study underline the necessity of routine surveillance, infection control methods such as hand and personal hygiene by healthcare providers, correct catheter care, and antibiotic stewardship.

**Declarations:**

**Conflicts of interest:** There is not 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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