Section: Microbiology

Original Research Article

ASSESSMENT OF NEONATAL CANDIDEMIA IN PRETERM AND LOW-BIRTH-WEIGHT NEONATES AND MOLECULAR DETECTION OF ERG11 GENE IN THE FLUCONAZOLE RESISTANT ISOLATES AT A TERTIARY CARE CENTRE, THANJAVUR

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Received *: 15/01/2026*
Received in revised form *: 20/02/2026*
Accepted *: 11/03/2026*

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DOI: 10.70034/ijmedph.2026.2.482

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (2); 2919-2924

ABSTRACT

Background: With advances in neonatal management, incidence of Invasive candidiasis has decreased in Neonatal Intensive Care Units (NICUs) over the years. Despite the decrease in incidence of Invasive Candidiasis in neonates, the associated mortality rate is high in preterm and very Low Birth Weight (LBW) infants.

Materials and Methods: A total of 200 neonatal cases satisfying the inclusion criteria, admitted with signs and symptoms of neonatal candidemia were included in the study. Fungal colonies were isolated by conventional culture method, identification by microscopic and macroscopic observation of growth. Speciation of Candida was done by germ tube test, corn meal agar morphology, CHROME agar colour, urea hydrolysis, sugar fermentation and sugar assimilation tests. Antifungal susceptibility testing was done by disc diffusion methods and perform molecular characterization of ERG11 gene in fluconazole resistant Candida isolates.

Results: From 200 samples, 14 candida growth were isolated and thus the incidence of fungal sepsis was 7% (14/200). In this study, the fungal sepsis was distributed more or less equally among both sexes and there were 8 cases (57%) in male neonates and 6 cases (43%) in female neonates. Among the study population,7% of the patients showed candidal growth. Among the,14 isolates majority of them were *Candida albicans* (64.3%) followed by *Candida tropicalis* (28.6%) and *Candida krusei* (7%). Antifungal susceptibility testing was performed for Candida isolates by Disk diffusion method. Fluconazole resistance was observed among *C. albicans* and *C. tropicalis* with 33%(3/9) and 50% (2/4) respectively. Fluconazole resistance among the Candida species tested by PCR showed the presence of ERG11 gene in two isolates of *C. albicans*.

Conclusion: The incidence of fungal sepsis in NICU was increased. Candida species were the common cause for fungal infection in neonates. Preterm and low birth weight, prolong hospital stay, administration of broad-spectrum antibiotics and mechanical ventilation were the major risk factors. Nosocomial infection was considered as a major contributor. Routine screening and evaluation can be recommended for prevention and control of fungal sepsis in NICU

Keywords: Fungal Sepsis, Candidemia, Nosocomial, erg11.

INTRODUCTION

Neonatal candidemia is a clinical entity defined as the presence of at least one positive blood culture containing pure growth of Candida species with supportive clinical features in the first 4 weeks of life. Clinical manifestation associated with candidemia is respiratory distress, hemodynamically instability, apnea, hyperglycemia, abdominal distention, feeding intolerance, temperature instability, lethargy, decreased perfusion, and thrombocytopenia. Neonatal mortality rate is one of the indicators measuring the health status of a country. There could be various reasons for neonatal mortality but

septicemia continues to be a major cause of neonatal mortality and morbidity worldwide.[1]

Last three decades have witnessed a significant rise in the incidence of infections due to fungal pathogens. Neonatal sepsis although has classically been attributed to bacterial organisms, prevalence of fungal sepsis among the neonates is on a rise in the present era of antibiotics. Candida spp. account for 9-13% of all blood isolates in NICU.[2] Candida is a ubiquitous fungus and is one of the major causes of increasing fungal infections in neonatal intensive care units. Candidemia, i.e., the presence of Candida species in the blood, is the leading cause of invasive fungal infections in hospitalized neonates, and the fourth most common nosocomial bloodstream infection in worldwide. It has been studied that about 10% babies in NICU get colonized in the first week of life and 64% babies get colonized by 4 weeks of hospital stay. The gastrointestinal tract is the first to become colonized though multiple sites may be involved.[3] Candida species form part of normal commensals of human body. These organisms inhabit various parts of body such as skin and the mucosal surfaces viz the gastrointestinal tract and female genito-urinary tract specially vagina. There are 163 known species of Candida and only 20 of these are significant pathogens implicated in different infections.[4]

The genus Candida comprises about 200 species, of which nearly twenty are considered to be significant pathogens. Some of the commonly isolated species of medical interest are C.albicans (50-60%),C.glabrata(15-60%), C.parapsilosis(10-20%), C.tropicalis(6- 12%), C.dubliniensis, C.krusei (1-3%) and C.guilliermondii(<5%) and C.lusitaniae(<5%).

C.auris is an emerging, multidrug resistant, fungal pathogen that has been detected throughout the world. C.auris was first isolated from an external ear canal from Japanese in the year 2009 but the earliest known infection has been documented in South Korea in 1996.First reports of multidrug resistant C.auris was reported in India in 2013.[5]

The specific epidemiology pertaining to the incidence of Candida infections and distribution of Candida spp. varies from region to region, country to country and even as per health-care setup and patient cohorts within the country.[6] Therefore species identification plays an important role in understanding the epidemiology of candidiasis in a particular health-care setup. The changing trends of candidiasis need for continuous monitoring of species distribution and antifungal susceptibility testing.[7]

Invasive candidiasis can be either acquired in-utero (within 6 days of life) or post- partum (after 6 days of life). If acquired in-utero, it is referred to as congenital candidiasis (CC). CC is usually cutaneous initially and becomes systemic later, known as congenital systemic candidiasis. It is a rare form of invasive candidiasis with very few cases (around 100) reported in medical literature. The other type of infection is acquired after 6 days of life and is referred to as neonatal candidiasis. It is the common form of invasive candidiasis in neonates.

The clinical spectrum of infection ranges from superficial mucocutaneous to deep invasive candidiasis.GI tract is considered as the major route for candidiasis. Perforations in the GI mucosa induced either by chemotherapy/irradiation or major surgery causes the fungus to invade the blood stream,[8] resulting in hematogenous spread into various organs causing deep seated infections.

The pathogenicity of Candida spp. is mediated by a number of virulence factors. Virulence factors are all traits required for establishment and progression of infection. These factors interact directly with host cells causing damage. Important virulence factors of Candida spp. Include adhesion or adherence to host tissues and medical devices, biofilm formation, the secretion of extracellular hydrolytic enzymes, thigmotrophism, polymorphism and phenotypic switching.[9]

The clinical manifestations caused by Candida spp. are extremely varied, ranging from acute, sub acute and chronic to episodic. Involvement may be localized to the mouth, throat, vagina, skin, scalp, fingers, nails, bronchi, lungs or the gastro-intestinal tract or become systemic as in septicemia, endocarditis, and meningitis.[10] Candidal infections can be transmitted to neonates due to due to maternal vaginal infection or it occurs nosocomially i.e. acquired in hospitals, and is transmitted via contaminated medical devices, hands of healthcare workers, catheters, etc. C. parapsilosis is found to be the most common Candida species present on the hands of healthcare workers.[11,12]

Aim

To examine the "Prevalence of Neonatal Candidemia in Preterm & Low birth Weight Babies and their Phenotypic and Antifungal Susceptibility and Molecular Study of Fluconazole resistant Candidia species" at a Tertiary care centre, Thanjavur

Objectives

1. To isolate and identify the Candida isolates in LBW, preterm neonates from blood samples.
2. To study the antifungal susceptibility profile of Candida sp. isolates by disc diffusion method.
3. To perform molecular characterization of ERG11 gene in fluconazole resistant Candida isolates.

MATERIALS AND METHODS

Prospective cross sectional study was conducted in the department of microbiology central microbiology laboratory, Thanjavur medical college and hospital, Thanjavur in collaboration with Department of peadiatrics, Raja Mirasudar Hospital, Thanjavur.

Study population and size: Two hundred neonates admitted to NICU with suspected sepsis were selected for the study.

Inclusion Criteria: Neonates with Birth weight <2.5kg, preterm babies less than 32 weeks**,** neonates with symptoms of Sepsis, and neonates born for immune compromised mother like Diabetic, HIV positive mothers were included in the study.

Collection of specimen: Written consent was obtained from the parent (or) care giver of the neonates after providing full explanation about the current study in their local language. All the data collected were kept confidential. Standard operating procedures are followed during sample Collection.

Ethical considerations: Written informed consent was obtained from parents. Confidentiality was maintained. Institutional ethical approval obtained (add number).

Sample collection and processing: Blood samples were collected aseptically and cultured using standard microbiological methods. Fungal colonies were isolated by conventional culture method, identification by microscopic and macroscopic observation of growth.

Identification of Candida was done using Germ tube test, Cornmeal agar morphology, CHROMagar, Sugar fermentation and assimilation and Urease test

Antifungal susceptibility testing: Antifungal susceptibility was tested using disc diffusion method using fluconazole, amphotericin-B, voriconazole, and nystatin.

Molecular analysis: PCR detection of ERG11 gene in fluconazole-resistant isolates.

RESULTS

A total of 93/200 (46.5%) cases were positive for bacterial blood culture.

Table 1: Description of type of isolate observed in blood culture in the neonatal septicemia cases in the study

S.No	Isolate in blood culture	No of cases	Percentage %
1.	Klebsiella	47	23.5
2.	E.coli	13	6.5
3.	Methicillin resistant-CONS	13	6.5
4.	Pseudomonas	11	5.5
5.	Acinetobacter	9	4.5
6.	Candidal species	14	7
7.	Negative for bacteria and fungi	93	46.5

Among the 200 samples majority of them Bacterial culture positive 93(46.5%) and candida culture positive 14(7%). Among the bacterial culture Klebsiella 47(23.5%) was the most predominant followed by E.coli 13(6.5%),MRCONS 13(6.5%), Pseudomonas 11(5.5%)and Acinetobacter 9/(4.5%).

Table 2: Incidence of various fungal isolates for neonatal Candidemia observed in the study

S.No	Type of isolate	Number of isolate (x)	Total N	Incidence (x/N*100)
1	Candida total	14	200	7%
2	Candida albicans	9	14	64.3%
3	Candida tropicalis	4	14	28.6%
4	Candida krusei	1	14	7.1%

Among 14 candida isolates majority of them were identified to belong to Candida albicans (64.3%) followed by Candida tropicalis (28.6%) and Candida krusei (7.1%).

Table 3: Biomarker description of Candidal neonatal sepsis observed in the study (N=14)

S.No	Risk factor	No of fungal sepsis cases	Percentag%
1	Positive CRP	10	71.4
2	WBC count >7500 cells/cc	14	100
3	Platelet count <30000	14	100

Table 3 shows increased total leucocyte count and Thrombocytopenia in all neonates. CRP increased in 71% in out of 200 samples

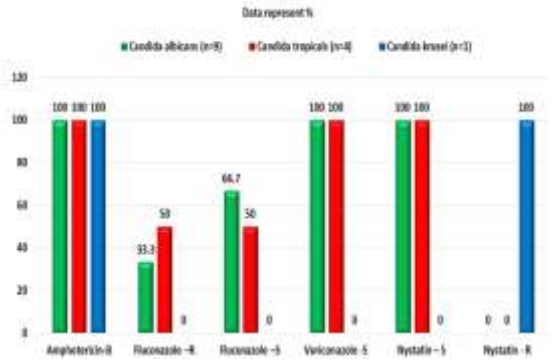
Table 4: Comparison of various risk factors with respect to the Candidal sepsis observed in the study

S. No	Risk factors	C albicans (N=9)		C tropicalis (n=4)		C krusei (n=1)		Chi square value	P value
		n	%	n	%	n	%		
1	Ventilator support	2	22.2	2	50	1	100	2.86	0.238 (NS)
2	Antibiotic use	9	100	4	100	1	100	--	--
3	Mode of delivery LSCS	9	100	3	75	1	100	2.69	0.26 (NS)
	Mode of delivery NVD	0	0	1	25	0	0		
4	Total duration of hospital stay in days	14	2.6	12.7	2.3	10	--	1.280	0.316 (NS)
5	Outcome (recovery)	8	88.9	3	75	0	0	4.26	0.118 (NS)
	Outcome (Death)	1	11.1	1	25	1	100		

Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. NS = Not significant.

In the above table 4. Shows among candida sepsis neonates 28.5% and in Non candida sepsis 10.7% had ventilator support more than 7 days. Neonates with prolonged ventilator support are more prone for candida sepsis. This was statistically significant. Regarding duration of hospital stay among Candida sepsis was 100 % whereas among Non Candida sepsis 82%. Hence prolonged hospital stay found to be potential risk factor for Candida sepsis.

Figure 1: Comparison of antifungal sensitive/resistance pattern between the three *Candida spp.* observed in the study.



Data are expressed as mean with SD. Fisher's exact test was used to compare the proportions between the three groups. *indicates $p<0.05$ and considered statistically significant. NS = Not significant.

The study shows resistance to flucanazole 33% among C.albicans and 50% cases in C.tropicalis. All isolates were susceptible to Voriconazole, Amphotericin-B and Nystatin.

Table 5: Descriptive statistics of PCR-erg11 test results observed for fluconazole resistant Candida species in the study (N=5)

S.No	Type of isolate	PCR-ERG11 gene detected	Total N	Incidence (x/N*100)
1	Candida total	2	5	40%
2	Candida albicans positive for erg-11 (n=3)	2	3	60%
3	Candida tropicalis (n=2)	0	2	0%

Fluconazole resistance among the Candida species tested by PCR showed the presence of ERG11 gene in 2 isolates of C.albicans.

DISCUSSION

Invasive candidiasis remains a major cause of nosocomial bloodstream infection in NICUs, particularly among preterm and low-birth-weight (LBW) infants. In the present study, the incidence of neonatal fungal sepsis was 7%, which is comparable with previous reports ranging from 1.4% to 16.5% across different regions and settings. Studies from Pakistan and Australasia have documented incidences between 1.4%–5.9% and 1.6%–5%, respectively, while other reports have noted rates of up to 8.1% and higher in high-risk neonatal populations. Similar to findings in extremely low-birth-weight infants reported by Benjamin et al., our incidence falls within the expected range for high-risk NICU populations.

A slight male predominance (57%) was observed, consistent with earlier studies of neonatal sepsis that report higher susceptibility among male neonates. Biological explanations, including possible X-linked immune-regulatory factors, have been proposed in the literature. Prematurity and low birth weight were strongly associated with candidemia in this study.

Gestational age showed an inverse relationship with infection, with most cases occurring in neonates born at ≤31 weeks. This finding aligns with previous reports demonstrating increased risk of invasive fungal infection with decreasing gestational age and birth weight.

Prolonged hospitalization was identified as a universal risk factor among affected neonates, highlighting the importance of strict infection-control practices in NICUs. Mechanical ventilation was present in 36% of cases, supporting earlier studies that demonstrate increased risk of fungal sepsis in ventilated neonates. Surfactant therapy was administered in the majority of preterm neonates with respiratory distress syndrome and has been reported elsewhere to be associated with invasive fungal infection, likely reflecting underlying prematurity and severity of illness rather than direct causation.

All cases in this study had prior exposure to broad-spectrum antibiotics for more than five days, consistent with other studies reporting >90% association. Prolonged antibiotic use disrupts normal flora and facilitates Candida colonization and invasion, particularly in the presence of indwelling vascular lines. Regular line care and antimicrobial stewardship are therefore critical preventive measures.

Among laboratory markers, thrombocytopenia was the most consistent finding and was present in the majority of cases, supporting its role as a surrogate marker of neonatal candidemia. Elevated C-reactive protein was also frequently observed. Early recognition of these laboratory indicators may help guide timely antifungal therapy in high-risk neonates.

All fungal isolates were Candida species, with Candida albicans being the most common, followed by C. tropicalis and C. krusei. This distribution is consistent with previous neonatal studies, although non-albicans Candida species are increasingly reported and may be associated with antifungal resistance and higher mortality. Fluconazole resistance was observed in a subset of isolates, and ERG11 gene detection in some resistant strains supports previously described molecular mechanisms of azole resistance. All isolates remained susceptible to amphotericin-B and voriconazole, underscoring their continued therapeutic relevance.

The overall mortality rate in this study was 21%, comparable to reported rates of 17%–60% in neonatal candidemia. Mortality was higher among neonates infected with azole-resistant species and non-albicans Candida, emphasizing the need for early species identification and antifungal susceptibility testing. Given the significant morbidity, mortality, and potential neurodevelopmental sequelae associated with neonatal candidemia, preventive strategies such as infection-control measures, judicious antibiotic use, and early diagnostic screening are essential in NICU settings.

Overall, the findings reinforce the continued burden of neonatal candidemia in high-risk NICU populations and highlight the importance of early diagnosis, species-level identification, and targeted antifungal therapy to improve clinical outcomes.

CONCLUSION

Emergence of non albicans candida (NAC) species and their association with higher mortality and longer duration of hospital stay is a cause for concern. Early removal of central line, timely fungal culture with Candida speciation and susceptibility testing are necessary for appropriate institution of treatment and better outcome. Frequent empirical use of fluconazole and amphotericin B may be avoided as it may lead to a shift in species distribution and higher antifungal resistance. An incidence of 7% and a Mortality rate of 21% in this study isists the need for specific strategies to prevent the candida blood stream infections.

Prevention of fungal sepsis starts from antenatal period. Proper screening for genital infection and treatment during ANC period is the firststep. Standard precaution should be followed in every hospital for a clean delivery.

Transit of sick neonates with all precaution will help to avoid exposure to pathogens. Avoidance of unwanted admissions, early discharge of healthy neonates and following universal precaution will reduce the disease burden.

A restrictive policy of antibiotic use should be implemented to decrease Candida colonization/infection rates to reduce the morbidity and mortality associated with these infections. Mortality of candidemia is related to the underlying condition, the susceptibility and virulence of the organism and, particularly, to the delay in the diagnosis and treatment.

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