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CANDIDA SPECIES PREVALENCE AND ITSANTI-FUNGAL SUSCEPTIBILITY PATTERN IN NICU PATIENTS DIFFERENT FROM POSITIVE BLOOD CULTURE

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Abstract
Introduction-

Recent population-based surveillance studies have shown an increasing incidence of invasive candidiasis in the neonatal ICU during the past decade.Of all blood stream infections (BSI), candidaemia poses the greatest threat with a high fatality rate among neonates. There has been an increase in the number of reports of non-C. albicans species and antifungal resistance has progressively emerge.

Aim-

To isolate and identify Candida species from NICU patients and determine the antifungal drugs susceptibility pattern of Candida species.

Methodology-

Prospective Study on 270 NICU at GMC Kota, Rajasthan over a period of 18 months from December 2022 to June 2024. During this period blood samples were collected into peadiatric Bactec Blood culture bottles. Choosing samples from appropriate patient was done on the basis of inclusion criteria

Result-

The prevalence of candidemia is 11.85% as out of 270 patients 32 have candidemia. Out of 32 Candida isolates, 15 were identified as C. tropicalis, 10 as C. albicans, 3 as C. parapsilosis, 3 as C. glabrata and 1 as C. krusei. Out of 32 Candida isolates, all (100%) were sensitive to amphotericin B followed by 93.8% were sensitive to nystatin, 68.8% to clotrimazole, 59.4% to itraconazole and 40.6% Candida spp. were sensitive to fluconazole. The most common pre-disposing factor was LBW (in 8.1%), followed by Length of stay in ICU≥ 7 Days (in 4.1% cases) and Preterm Neonate (in 4.1% cases). The other Pre-disposing condition seen in NICU for candidemia were neonate of Diabetic Mother(in 1.5%) , neonate on Prolong broad spectrum Antibiotics(in 1.5%), neonate on Prolonged Steroid.

Conclusion-

The worldwide progressive shift towards non-albicans candidemia necessitates regular surveillance and monitoring of laboratory data.

Key words- Candidemia, Neonates, Non albicans candida.

INTRODUCTION

The incidence and prevalence of candidemia are on a rise in many countries worldwide. According to National Nosocomial Infection Surveillance, USA in the 1990s, Candida species remained the fourth most common bloodstream pathogen accounting for 8% of all

hospital-acquired bloodstream infections and is a cause of significant morbidity and mortality.[1,2] The Asian scenario regarding the incidence of candidemia is, however, not very clear due to a lack of multicentric studies. A 13-year long study on candidemia from a tertiary care hospital in Thailand showed a prevalence of 6.14% for Candida species among blood culture isolates.[3] In India, there are a few studies indicating the increasing trend of candidemia in some tertiary care hospitals.[4]The mortality rate associated with candidemia worldwide is also high ranging from 10% to 49%.[5]

Importance of Candida species in Neonatal Intensive Care Units (NICUs) is increasingly being recognized. Candida species accounts for 9%–13% of all blood isolates in NICUs.[6] C. albicans is the most commonly isolated species and accounts for 50%–70% of cases of invasive candidiasis.[2,7] However, the recent studies suggest that, with the introduction of fluconazole and itraconazole, there is an increase in the prevalence of nonalbicans candidial septicemia.[8] Candida tropicalis, Candida glabrata, and Candida parapsilosis are being increasingly isolated in patients of neonatal septicemia.[9] Candida species can spread through vertical transmission from maternal flora or through horizontal transmission from hands of health-care workers. The risk factors associated with candidemia include the use of broad-spectrum antibiotics, low birth weight (LBW), prematurity, and intravenous catheter.[10]

Over the past year, we noticed an increase in the isolation rate of nonalbicans Candida species from cases of neonatal septicemia, which prompted us 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. Further speciation and susceptibility testing of Candida sp. is still not routinely being done at most of the centers and as such no reliable data are available from our region regarding the estimation of antifungal use in hospitals. Furthermore, because of considerable regional variability, the local epidemiological knowledge is critical in terms of prevention and management of invasive Candida infections and can guide to initiate empirical antibiotic treatment which is essential for the management of the neonatal sepsis.

The emergence of antifungal resistance among *Candida* species is considered a leading cause of therapeutic failure and the high mortality rate. The present work aimed to calculate the prevalence of candidaemia among pediatric patients, identify the risk factors, characterize the involved species and determine the susceptibility of the isolated strains to antifungal agents, specifically Amphotericin B, Itraconazole, Fluconazole, Clotrimazole, Nystatin.

A review of the available literature has revealed a dearth of information regarding the epidemiology, pathogenesis, virulence factors, and antifungal susceptibility patterns of *Candida species*..

The knowledge of possible source of such fungimea is imperative for future preventive strategies. As most of the surveillance studies report a rising trend in neonatal candidemia over the years, the authors could possibly have done a trend analysis to evaluate any such inclination. This is equally essential for scrutinizing surveillance programs and policy formulation. To conclude, candidemia in the neonates is a challenging condition not only for the clinicians but also for the microbiologists. Risk factor identification along with speciation and drug susceptibility testing is crucial for timely management of such neonates.

The research paper by Basu, et al.[11] published in this issue of Indian Pediatrics evaluates the

local epidemiology of neonatal candidemia in and around city of Varanasi in India. As their study came across nonalbicans Candida species in nearly double the number of C. albicans candidemia, the authors have very nicely estimated the predictors of such an outcome. Identification and prevention of such risk factors in susceptible neonates can significantly improve outcome of neonates with fungal sepsis. The authors have been able to characterize phenotypically 82 out of 114 isolates of Candida to the species level. Although phenotypic or commercially available rapid detection systems may be useful for clinicians, they often lack reproducibility. MALDI-TOF biotyper is an exception, which, as well as DNA sequencing correctly identifies Candida up to the species level. Hence, molecular methods though expensive, but reproducible and reliable, should be adopted by tertiary care hospitals for monitoring and surveillance of important hospital-associated bugs.

AIMS AND OBJECTIVES

1. To estimate the incidence of candida BSI in NICU of our hospital.
2. To identify the candida subspecies in blood culture and its antifungal sensitivity.
3. To findout the commonest agent responsible for candedimia in NICU patients
4. To study sex distribution of the patients with candedima.

MATERIALS AND METHODS

From neonates, blood samples were collected into pediatric Bactec blood culture bottles. The bottles were transported to the laboratory and immediately incubated in the blood culture systems (BACTEC™-40). Any growth was sub-cultured onto 5% sheep blood agar, MacConkey's agar, and Sabouraud's dextrose agar(SDA) with chloramphenicol(0.05%) and then incubated at 37°C. The diagnosis of candidemia was settled by the presence of positive blood culture showing pure growth of Candida spp. together with supportive clinical findings. The isolated Candida spp. were identified by colony morphology on SDA, germ tube test, and chromogenic media.

RESULT

Out of 270 samples 63.7% were male and 36.3% were female , 61.1% were of rural background and 38.9% were of urban background, 65.6% were of middle income group and 34.4% were of urban lower income group. Out of 32 Candida isolates, 15 were identified as C. tropicalis, 10 as C. albicans, 3 as C. parapsilosis, 3 as C. glabrata and 1 as C. krusei. In present study the Pre-disposing factors were present in 57 subjects . The most common pre-disposing factor was LBW (in 8.1%), followed by Length of stay in ICU≥ 7 Days (in 4.1% cases) and Preterm Neonate (in 4.1% cases). The other Pre-disposing condition seen in NICU for candidemia were neonate of Diabetic Mother(in 1.5%) , neonate on Prolong broad spectrum Antibiotics(in 1.5%), neonate on Prolonged Steroid (in 1.1%) , and presence of CVC(in 0.7%).These 32 Candida isolates when subjected to Germ Tube Test (GTT), GTT was positive in 10 (3.7%) and negative in remaining 22 (8.1%), All the 32 Candida isolates grew at 37 ^{0}C, only 10 of them grew at 45 ^{0}C also. out of 32 Candida isolates, all (100%) were sensitive to amphotericin B followed by 93.8% were sensitive to nystatin, 68.8% to clotrimazole, 59.4% to itraconazole and 40.6% Candida spp. were sensitive to fluconazole. Out of 15 C. tropicalis all (100%) were sensitive to amphotericin B followed by 14 (93.3%) to nystatin, 11 (73.3%) to itraconazole ,10 (66.7%) to clotrimazole and 8 (53.3%) C.

tropicalis were sensitive to fluconazole. out of 10 C. albicans, all (100%) were sensitive to amphotericin B followed by 90% to nystatin, 60% to clotrimazole, 50% to itraconazole and 40% were sensitive to fluconazole. Out of 3 isolates of C. parapsilosis, all (100%) were sensitive to amphotericin B and nystatin followed by 66.7% to clotrimazole, 66.7% itraconazole and 33.3% were sensitive to fluconazole.In our study, 3 isolates of C. glabrata all (100%) were sensitive to amphotericin B and nystatin and clotrimazole, 33.3% to itraconazole and all (100%) were resistant to fluconazole. 1 isolates of C. krusei, all (100%) were sensitive to amphotericin B, nystatin & clotimazole and all (100%) were resistant to itraconazole and fluconazole due to intrinsic resistance.

Table 1: Species wise distribution of Candida species

Total Candidial Isolates	**32**
Species	**No.**
Candida Albicans	10
Candida Trpoicalis	15
Candida Krusei	1
Candida Parapsilosis	3
Candida Glabrata	3

Table 2: Antifungal susceptibility/resistance (S/R) pattern of Candida species

	Candida Albicans		**Candida Trpoicalis**		**Candida Krusei**		**Candida Parapsilosis**		**Candida Glabrata**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Flucanazole	4	6	8	7	0	1	1	2	0	3
Amphotericin B	10	-	15	-	1	0	3	-	3	-
Itracanazole	5	5	11	4	0	1	2	1	1	2
Nystatin	9	1	14	1	1	0	3	0	3	0
Clotrimazole	6	4	10	5	1	0	2	1	3	0

DISCUSSION

The present prospective observational study was done in Govt. Medical College, Kota, Rajasthan over a period of 18 months from December 2022 to June 2024. During this period blood samples were collected into peadiatric Bactec Blood culture bottles. Choosing samples from appropriate patient was done on the basis of inclusion criteria, implied in proforma. The proforma included detailed history of current and past illness, antimicrobial therapy and catheterization which helped to choose the patients sample for study.

Gender-wise distribution:

In our study, out of 270 patients 172 (63.7%) were male patients and 98 (36.3%) were female patients.

Warris et al.(2020) in their study in NICU found that there was prevalence of male neonates as compared to female patients (57.6% males versus 42.4% females) which is consistent with results of present study. [83]

Residential Profile of the patients

In present study the majority of patients belonged to rural background as compared to urban background (61.1% rural patients versus 38.9% urban patients).

The predominance of rural background patients could be attributed to high propotion of rural population in our country and higher number of rural patients attending government set-up.

Socio-Economic Status Profile of the patients

In present study all the patients belonged to middle and lower income group which could be explained from the fact that majority of patients attending Government Hospital belonged to middle and lower income group and patients from Upper Socio-Economic Status usually attend Private Hospitals.

Pre-disposing condition profile of cases

In present study the Pre-disposing factors were present in 57 subjects . The most common pre-disposing factor was LBW (in 8.1%), followed by Length of stay in ICU≥ 7 Days (in 4.1% cases) and Preterm Neonate (in 4.1% cases). The other Pre-disposing condition seen in NICU for candidemia were neonate of Diabetic Mother, neonate on Prolong broad spectrum Antibiotics, neonate on Prolonged Steroid, and presence of CVC.

Species distribution of Candida:

In present study Candida speciation was done by conventional methods which included GTT, growth at 37 ^{0} C & 45 ^{0} C, Dalmau technique, CHROM agar, Urease test, sugar fermentation and sugar assimilation. A battery of tests was performed because a single method is not 100% sensitive for Candida species identification.

The present study showed that among 32 Candida isolates, there was predominance of non albicans Candida species contributing to 68.8 % of isolates and C. albicans contributing only to 31.2 % of isolates. In present study maximum number of isolates were identified as C. tropicalis 15 (46.9%) followed by C. albicans 10 (31.2%), C. parapsilosis 3 (9.4%), C. glabrata 3 (9.4%) and C. krusei 1 (3.1%).

Similar results were obtained in study conducted by Bansal et al (2019) [84](N=313) most commonly isolated species C. tropicalis (46.33%) followed by C. albicans (34.82%), C. parapsilosis (10.54%), C. glabrata (5.75%) and C. krusei (2.56%).

Table-Species-wisedistribution of*Candida*

StudyGroup	***C. albicans***	***C. tropicalis***	***C. parapsilosis***	***C. glabrata***	***C. krusei***
Presentstudy (N=32)	31.2%	46.9%	9.4%	9.4%	3.1%

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Bansal et al (2019)[84](N=313)	34.82%	46.33%	10.54%	5.75%	2.56%
Goliyaetal (2012)[85](N=108)	45.37%	10.19%	3.70%	7.41%	24.07%
Sidaetal(2015)[86](N=67)	38.80%	56.73%	1.49%	-	-

ANTIFUNGAL SUSCEPTIBILITY TESTING

Resistance to azoles and polyenes continues to increase and is a matter of concern as this is most commonly used empirical therapy for suspected fungal infections.

In present study, out of 32 *Candida* isolates, all (100%) were sensitive to amphotericin B followed by 93.8% were sensitive to nystatin, 68.8% toclotrimazole, 59.4% to itraconazole and 40.6% *Candida spp.* were sensitive to fluconazole.

While study done by Kamiar Zomorodian et al (2011)[91] (N=206) reported all isolates of *Candida Spp.* were sensitive to amphotericin B, 96.6% were sensitive to fluconazole, 79.1% were sensitive to itraconazole which is consistent with results of present study.

Reena et al (2019)[89] (N=120) observed all *Candida* isolates were sensitive to amphotericin B, 67.5% were sensitive to itraconazole and 60% were sensitive to fluconazole which is consistent with results of present study.

Antifungal susceptibility pattern of *C.tropicalis*:

In present study, out of 15 *C. tropicalis* all (100%) were sensitive to amphotericinBfollowedby14(93.3%)tonystatin,11(73.3%)to itraconazole ,

10 (66.7%) to clotrimazole and 8 (53.3%) *C. tropicalis* were sensitive to fluconazole.

While study done by Kamiar Zomorodian et al (2011)[91] reported all *C. tropicalis* were sensitive to amphotericin B, 96% were sensitive to fluconazole, 64% were sensitive to itraconazole.

Reena et al (2019)[89] observed all *C. tropicalis* were sensitive to amphotericin B, 68.75% were sensitive to itraconazole and 75% were sensitive to fluconazole which is consistent with results of present study.

Antifungal susceptibility pattern of *C.albicans*:

In present study, out of 10 *C. albicans*, all (100%) were sensitive to amphotericin B followed by 90% to nystatin, 60% to clotrimazole, 50% to itraconazole and 40% were sensitive to fluconazole.

While study done by Kamiar Zomorodian et al (2011)[91] reported all *C. albicans* were sensitive to amphotericin B, 100% were sensitive to fluconazole, 86% were sensitive to itraconazole.

Study conducted by Reena et al (2019)[89] observed all *C. albicans* were sensitive to amphotericin B, 60.78% were sensitive to itraconazole and 76.47% were sensitive to fluconazole.

Antifungal susceptibility pattern of *C.parapsilosis*:

In present study, out of 3 isolates of *C. parapsilosis*,all (100%) were sensitive to amphotericin B and nystatinfollowed by 66.7% to clotrimazole, 66.7% itraconazole and 33.3% were sensitive to fluconazole.

While study done by Kamiar Zomorodian et al (2011)[91] reported all *C. parapsilosis* were sensitive to amphotericin B, 96% were sensitive tofluconazole, 92.3% were sensitive to itraconazole.

Study conducted by Reena et al (2019)[89] observed all *C. parapsilosis* were sensitive to amphotericin B, 58.33% were sensitive to itraconazole and50% were sensitive to fluconazole.

Antifungal susceptibility pattern of *C.glabrata*:

In our study, 3 isolates of *C. glabrata* all (100%) were sensitive to amphotericin B and nystatin and clotrimazole, 33.3% to itraconazole and all (100%) were resistant to fluconazole.

While study done by Kamiar Zomorodian et al (2011)[91] reported all *C. glabrata* were sensitive to amphotericin B, 90.5% were sensitive to fluconazole, 59.5% were sensitive to itraconazole.

Study conducted by Reena et al (2019)[89] observed all *C. glabrata* were sensitive to amphotericin B, 25% were sensitive to itraconazole and 25% were sensitive to fluconazole.

Antifungal susceptibility pattern of* C.krusei*:

In present study, 1 isolates of *C. krusei*, all (100%) were sensitive to amphotericin B, nystatin & clotimazole and all (100%) were resistant to itraconazole and fluconazole due to intrinsic resistance.

While study done by Kamiar Zomorodian et al (2011)[91] reported all *C. krusei* were sensitive to amphotericin B, itraconazole and also fluconazole.

CONCLUSION

Neonatal candidemia often carries an ominous prognosis. The worldwide progressive shift towards non-albicans candidemia necessitates regular surveillance and monitoring of laboratory data. An epidemiological knowledge is critical in terms of preemptive management that should encompass disciplined infection control practices and a restrictive policy for antibiotic and antifungal prophylaxis.

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