Document heading doi: 10.21276/apjhs.2018.5.3.13 Original Article

Distribution and susceptibility to various antifungal agents among blood stream *Candida* isolates from Neonatal intensive care unit

Dhirendra Kumar[1], Deepak Kumar[2*], Priyadarshini[1],Sriparna Basu[3], Ragini Tilak[4]

[1]Junior resident Doctor, Department of Microbiology IMS BHU Varanasi, India
[2]Assistant Professor, Department of Microbiology IMS BHU Varanasi, India
[3]Associate Professor Department of Paediatrics IMS BHU Varanasi, India
[4]Professor Department of Microbiology IMS BHU Varanasi, India

Received: 20-06-2018 / Revised: 30-06-2018 / Accepted: 06-08-2018

ABSTRACT

Background: During the past several decades, there has been a steady increase in the frequency of isolation of *Candida* spp. from blood stream infections from NICU worldwide. Furthermore, monitoring programs have detected an increase in the prevalence of infections caused by non albicans Candida (NAC). NAC are reported to be intrinsically resistant and less susceptible to empirically used azoles like Fluconazole. Thus speciation and antifungal susceptibility testing become imperative for these isolates. **Objective**: To study the trend in species distribution and antifungal susceptibility pattern among blood stream *Candida* strains isolated from neonatal intensive care patients was the aim of the study. **Method**: Susceptibility testing of clinically significant *Candida* isolates to various antifungal was performed by E-test in accordance with manufacturer instructions. The results obtained were analyzed and compared. **Results**: The most frequently isolated species was *Candida tropicalis* (52.83%) followed by *Candida parapsilosis* (16.98%), *Candida albicans* (9.4%), *Candida glabrata* (9.4%), *Candida krusei* (7.5%) *and Candida guilliermondii* (3.77%). Overall sensitivity of 85%, 81%, 26%, and 98% respectively to Amphotericin B, Fluconazole, Itraconazole and Voriconazole was found. **Conclusion**: The study shows the clinical significance and mycological shift of Candida species in blood culture of the neonatal population with a predominance of NAC species. Voriconazole showed an excellent activity and can be used in empirical treatment for candidemia rather than Fluconazole.

Key Words: *Candida***,** non albicans Candida, candidaemia, antifungal susceptibility, E test

Introduction

Over the past few decades, there has been a significant increase in blood stream Candida isolates particularly in patients admitted to ICU [1,2].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[3]. The reported mortality rates for infants with neonatal candidiasis lies between 25 and 50% compared to an overall mortality of 4.7% for all NICU admissions [4]. Infants in NICU are at risk of candidemia and development of various other new infections.

**Correspondence*
Dr. Deepak Kumar
Assistant Professor, Department of Microbiology IMS BHU Varanasi, India.
E Mail: deepak.mln30@gmail.com

The risk factors for candidemia are low gestational age, low APGAR scores, gestational diabetes, human immunodeficiency virus infection/acquired immune deficiency syndrome (HIV/AIDS) and congenital malformations [4,5].

Further, the association of colonization with Candida due to intravenous or urinary catheterization in this vulnerable group has been identified as an important risk factor with high predictive value for the development of invasive disease. Until recently, *C. albicans* was by far the predominant species in most countries, causing up to two thirds of all cases of invasive candidiasis. Furthermore, monitoring programs have detected an increase in the prevalence of infections caused by NAC (especially *C. parapsilosis, C. glabrata, and C. krusei*) and other yeast genera [1].This shift is relevant because some of these species have reduced susceptibility or intrinsic

resistance to fluconazole, as is the case for *C. glabrata* and *C. krusei*, respectively. A worrisome finding is the increasing incidence of cross-resistance to newer triazoles among fluconazole resistance species [6]. Thus speciation and antifungal susceptibility (AFS) testing become imperative for these isolates. Unfortunately, data about prevalence or antifungal susceptibility patterns in NICU patients with candidaemia are scarce, and empirical treatment in neonates with suspicion of *Candida* infection frequently has to be instituted by extrapolating information from the adult. The aim of this study was to study the species distribution and antifungal susceptibility pattern among blood stream *Candida* strains isolated from infants from neonatal intensive care unit at a tertiary care centre in Eastern Uttar Pradesh.

Material and methods

The proposed study is a cross-sectional study was carried out in the Department of Microbiology and Paediatrics of S. S. Hospital BHU Varanasi from June 2013 to June 2014. The study population consisted of 499 neonates admitted to the paediatrics department with complaints of fever, low birth weight, and prematurity. Prior consent was taken from every baby's guardian before sample collection and the study was approved by the ethical committee.

Inclusion &exclusion criteria: Eligible for participation in the study were neonates who had been hospitalized for >48 h and had developed candidemia during their ICU stay. Neonates receiving or who had received prior antifungal therapy the last month before the ICU admission and patients with a confirmed systemic fungal infection prior to the diagnosis of candidemia also were excluded from the study.

Candidaemia definition: Candidaemia or invasive candidiasis was defined by a positive culture for yeast obtained from blood drawn from peripheral vein and presence of relevant clinical signs and symptoms. Blood samples (n=499) were obtained aseptically in BHI broth. It was incubated at 370 C. After 48 hours incubation subculture was done on two Sabouraud's Dextrose Agar slope containing chloramphenicol and incubated at 250 C and at 370 C for 7days. The culture tubes were examined daily for 1 week. Similarly, the second subculture was done after 7-day incubation of BHI broth and final subculture (for those samples which showed no growth after 7 days) on 28 days incubation. Colonies suggestive of Candida in SDA tube were further identified and speculated by morphological and biochemical tests like germ-tube test, chlamydospores formation, sugar fermentation test, and sugar assimilation test. [7]Anti-fungal susceptibility was done on supplemented Muller Hinton agar with 2% glucose and methylene blue (0.5µg/ml) by E-test as per manufacturer's instructions using gradient strips of Amphotericin B, Fluconazole, Itraconazole, and Voriconazole obtained from HiMedia Mumbai India. The concentration gradient for fluconazole ranged from 256 to 0.016 µg/ml while for other drugs was 32 to 0.002 µg/ml.

Analysis of the results

The MICs of four antifungal agents: Amphotericin B, Fluconazole, Voriconazole, and Itraconazole were analyzed. Breakpoints chosen for Fluconazole ($\leq$8 µg/ml, susceptible S ; 16–32 µg/ml, susceptible-dose dependent SDD ; $\geq$ 64 µg/ml, resistant R), Voriconazole ($\leq$1 µg/ml, S; 2 µg/ml, SDD; $\geq$ 4 µg/ml, R)were as per the Clinical and Laboratory Standards Institute (CLSI M27 S3**)** [8] . Since no breakpoints have been published for Amphotericin B and Itraconazole, breakpoints chosen were Amphotericin B ($\leq$ 1 µg/ml, S; 2 µg/ml, I; $\geq$ 4 µg/ml, R), and Itraconazole ($\leq$ 0.125 µg/ml, S; 0.25-0.5µg/ml, I; $\geq$ 1µg/ml, R) as in a study by Nguyen et al and Rex et al respectively [9,10]

Results

A total of 53(10.62%) out of 499 samples were culture positive for Candida species. *Candida tropicalis no*(52.83%), *Candida parapsilosis* (16.98%), *Candida albicans* (9.4%), *Candida glabrata* (9.4%), *Candida krusei* (7.5%) *and Candida guilliermondii* (3.77%) were the species causing candidemia , in order of frequency of isolation shown in figure 1. No mixed infection was found. In vitro susceptibility testing results of 53 Candida isolates against Amphotericin B, Fluconazole, Voriconazole, and Itraconazole by E-test method is shown in table 1. Voriconazole showed excellent activity against the isolates, with a susceptibility of 98% (MIC $\leq$ 1 µg/ml). The proportion of isolates for which Amphotericin B MIC was $\leq$1 µg/ml were 84.9% (45). Eight isolates (15%) had MIC $\geq$ 4µg/ml. For Fluconazole the percentages of isolates in each category (S, SDD, and R) were 81%, 0%, 19%. Itraconazole exhibited the least activity against Candida isolates, showing a resistance of 25%. Seven isolates of *C. tropicalis*, two isolates of each of *C. krusei* and *C. parapsilosis* one isolate each of *C. albicans* and *C. glabrata* were found resistant to Itraconazole (MIC $\geq$ 64 µg/ml).

Discussion

Neonatal candidemia has gained significant importance and has increased the concern in past two decades. In our study, out of 499 clinical samples a total of 53(10.6%) *Candida* isolates were obtained. This finding was in accordance with an Indian study involving 660 neonates admitted to NICU of a teaching hospital where the culture positivity for *Candida* was found to be 13.6% [11]. Comparing the results of the present study with previous year's record from our center, a shift in terms of increase in the number of NAC species from bloodstream was found. This finding is consistent with other studies where non-albicans *Candida* species predominate in the Indian subcontinent, Asia, and Europe [12-14]. Many neonatal ICU studies in the past have reported *C. parapsilosis* as the predominant pathogen causing candidemia in neonates [15, 16]. Overall, the predominant Candida species isolated in this study was *C. tropicalis* (53%). A similar study in India conducted by Narang et al also showed that the isolation of *C. tropicalis* was highest (41%) [17].The reasons for this change may be either of the two. First, there has been an increase in the number of high-risk patients, namely, immune suppressed patients due to diverse causes and types of medication, and patients requiring invasive devices and antibiotics [18]. Second, however, as suggested by a majority of studies, the selection of NAC strains in this cohort of patients is due to the use of Fluconazole for long-term prophylaxis which is common practice in our setup. [19, 20].In this study, we have found eight *Candida* isolates less susceptible and probably resistant to Amphotericin B (MIC $\geq$ 4µg/ml). Despite more than five decades of use of the polyene antifungals, AMB resistance in *Candida* spp. is rare, but it is slowly increasing, probably in correlation with the increased consumption of Amphotericin B [21]. Previous Studies have shown that *C. glabrata, C. guilliermondii, C. krusei*, and *C. lusitaniae* have a higher propensity than other *Candida* spp. to possess or develop resistance to Amphotericin B. The findings in our study differ here as reduced susceptibility to Amphotericin B was found in six C.*tropicalis* and two C. parapsilosis isolates [22]. This is probably because *Candida tropicalis* and *C. parapsilosis* are among the predominant species of *Candida* isolated from BSI in our study. The activity of fluconazole against *Candida* species remains low comment (resistance of 19 %) 81% were sensitive as indicated by our study. Resistance to Fluconazole was found among four isolates each of C. tropicalis and *C. krusei*, and two *C. glabrata*. Fluconazole resistance has always been a subject of concern in the predominantly BSI isolates of *Candida* like C. glabrata and Candida tropicalis [23]. Increase isolation of blood stream *C. krusei*, known to be intrinsically resistant to Fluconazole has worsened the situation [24]. Different rates of resistance to Fluconazole were detected in Candida strains from different parts of the country, but the finding of Kumar CP *et al* was consistent with our study [25-27].Cross-resistance between Fluconazole and Voriconazole has also been frequently reported in many species, nevertheless, Voriconazole exhibited excellent in vitro antifungal activity and no resistance was found against it in our study [28] Itraconazole showed least in vitro antifungal activity, against the isolates in this study. The resistant rate of the isolates to Itraconazole in this study was 25% which is consistent with the findings of Kothari *et al* [25]. A majority of the isolates (74 %) had a MIC of >0.12 µg/ml. It seems that long-term Fluconazole prophylaxis may have a role in partially selecting less-susceptible isolates to Itraconazole.

Conclusion

The study shows the clinical significance and mycological shift of Candida species in blood culture of the neonatal population with a predominance of NAC species. The high usage of Fluconazole as prophylactic agent appeared to have played a role in this shift, however, it may be recognized that other events like patient specific risk factors might have also contributed to the selection of different species. The results of the present studies on azole susceptibility testing of Candida species have shown that Voriconazole should be used in empirical treatment for candidemia rather fluconazole. Furthermore, species-level identification of Candida isolates and continuous surveillance of antifungal susceptibility is necessary to monitor trends and to choose the correct empiric therapy.

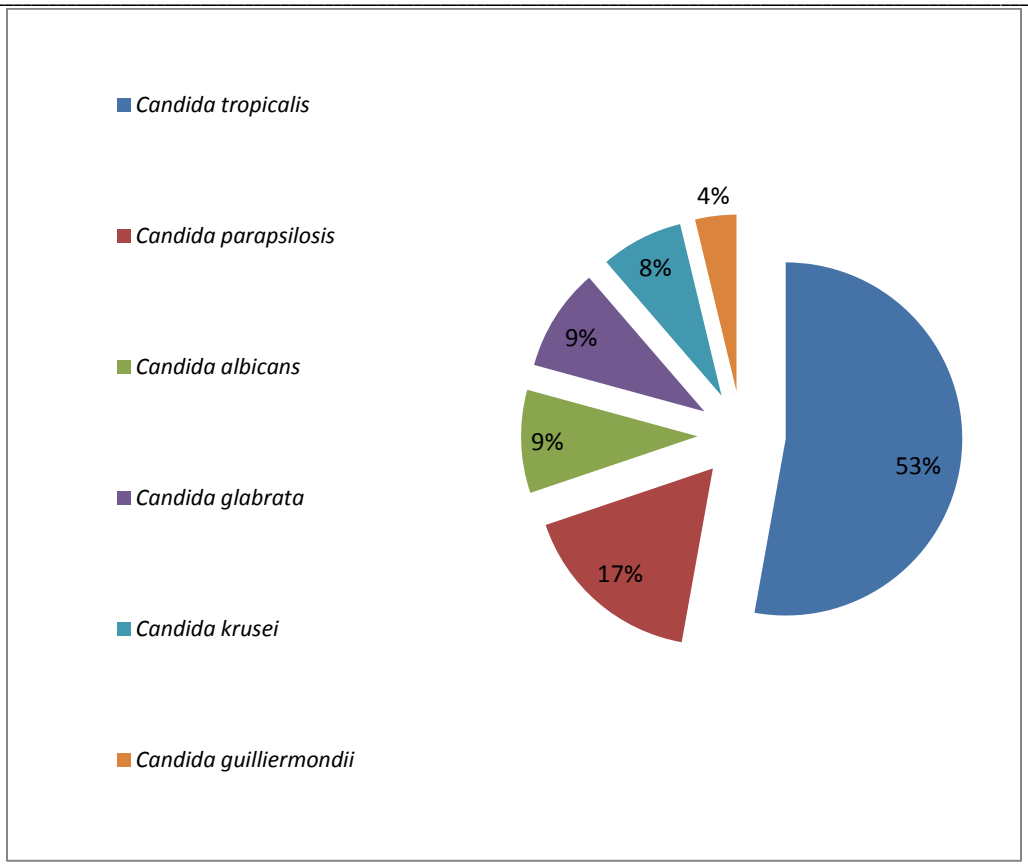

Fig 1: Distribution of blood stream *Candida* spp. isolated from Neonatal Intensive Care Unit

Table 1: Antifungal susceptibility testing results of 53 fungal isolates by E-Test method

Isolate	No	AMB			FLC			ITR			VRC		
		S	I	R	S	SDD	R	S	I	R	S	SDD	R
C .tropicalis	28	2	0	6	24	0	4	5	16	7	27	1	0
C. parapsilosis	9	7	0	2	9	0	0	2	5	2	9	0	0
C. glabrata	5	5	0	0	3	0	2	4	0	1	5	0	0
C. albicans	5	5	0	0	5	0	0	2	2	1	5	0	0
C. krusei	4	4	0	0	0	0	4	1	1	2	4	0	0
C. guilliermondii	2	2	0	0	2	0	0	0	2	0	2	0	0
Total (%)	**53**	**45(85)**	**0**	**8(15)**	**43(81)**	**0**	**10(19)**	**14(26)**	**26(49)**	**13(25)**	**52(98)**	**1(2)**	**0**

AMB=Amphotericin B; FLC=Fluconazole; ITR=Itraconazole; VRC=Voriconazole; S=Sensitive; I= Intermediate; R=Resistance; SDD; Sensitive dose dependent.

Reference

1. Eggimann P, Bille J, Marchetti O. Diagnosis of invasive candidiasis in the ICU. Annals of intensive care. 2011 ;1(1):37.
2. Kumar D, Bhattacharyya S, Gupta P, Banerjee G, Kumar M, Singh M. Antifungal susceptibility of bloodstream Candida Isolates in Pediatric patients. Int. J. Curr. Microbiol. Appl.Sci. 2015;4:716-20.

3. Benjamin D. Neonatal Candidiasis Among Extremely Low Birth Weight Infants: Risk Factors, Mortality Rates, and Neurodevelopmental Outcomes at 18 to 22 Months. PEDIATRICS. 2006;117(1):84-92.

4. Benjamin D, Garges H, Steinbach W. Candida bloodstream infection in neonates. Seminars in Perinatology. 2003;27(5):375-383.

5. Grohskopf LA, Sinkowitz-Cochran RL, Garrett DO, et al: A national point-prevalence survey of pediatric intensive care unit acquired infections in the United States. *J Pediatr* 2002; 140:432–438

6. Johnson E, Warnock D, Luker J, Porter S, Scully C. Emergence of azole drug resistance in Candida species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. Journal of Antimicrobial Chemotherapy. 1995;35(1):103-114.

7. Larone DH. Medically Important Fungi- A guidance to Identification. 2 nd ed. Washington: American Society for Microbiology; 1993.

8. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. CLSI document M27 S3(ISBN 1558-6502)

9. Nguyen M, Clancy C, Yu V, Yu Y, Morris A, Snydman D et al. Do In Vitro Susceptibility Data Predict the Microbiologic Response to Amphotericin B? Results of a Prospective Study of Patients with Candida Fungemia. The Journal of Infectious Diseases. 1998;177(2):425-430.

10. Rex J, Pfaller M, Galgiani J, Bartlett M, Espinel-Ingroff A, Ghannoum M et al. Development of Interpretive Breakpoints for Antifungal Susceptibility Testing: Conceptual Framework and Analysis of In Vitro-In Vivo Correlation Data for Fluconazole, Itraconazole, and Candida Infections. Clinical Infectious Diseases. 1997;24(2):235-247.

11. Agarwal J, Bansal S, Malik GK, Jain A. Trends in neonatal septicemia: emergence of non-albicans Candida. Indian pediatrics. 2004;41(7):712-5.

12. Fungal Infections in Intensive Care Unit: Challenges in Diagnosis Kumar D, Purbey M K. 2017;6(2):MR01-MR04.

13. Pfaller M, Diekema D, Rinaldi M, Barnes R, Hu B, Veselov A et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-Year Analysis of Susceptibilities of Candida and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing. Journal of Clinical Microbiology. 2005;43(12):5848-5859.

14. Shivaprakasha S, Radhakrishnan K, Karim PM. *Candida* spp. other than *Candida albicans*: A major cause of fungemia in a tertiary care centre. Indian J Med Microbiol 2007;25:405-7.

15. Huang Y, Lin T, Lien R, Chou Y, Kuo C, Yang P et al. Candidaemia in Special Care Nurseries: Comparison of Albicans and Parapsilosis Infection. Journal of Infection. 2000;40(2):171-175..

16. Kossoff E, Buescher E, Karlowicz M. Candidemia in a neonatal intensive care unit: trends during fifteen years and clinical features of 111 cases. The Pediatric Infectious Disease Journal. 1998;17(6):504-508..

17. Narang A, Agarwal PB, Chakrabarti A, Kumar P. Epidemiology of systemic candidiasis in a tertiary care neonatal unit. J Trop Ped 1998;44:104-8

18. Leroy O, Mira J P, Montravers P, Gangneux J P, Lortholary O. Comparison of albicans vs. non-albicans candidemia in French intensive care units. Crit Care. 2010;14: R98.

19. Chow J, Golan Y, Ruthazer R, Karchmer A, Carmeli Y, Lichtenberg D et al. Factors Associated with Candidemia Caused by Non- albicans Candida Species Versus Candida albicans in the Intensive Care Unit. Clinical Infectious Diseases. 2008;46(8):1206-1213.

20. Chow J, Golan Y, Ruthazer R, Karchmer A, Carmeli Y, Lichtenberg D et al. Risk factors for albicans and non-albicans candidemia in the intensive care unit. Critical Care Medicine. 2008;36(7):1993-1998.

21. Dick J, Merz W, Saral R. Incidence of polyene-resistant yeasts recovered from clinical specimens. Antimicrobial Agents and Chemotherapy. 1980;18(1):158-163.

22. Collin B, Clancy C, Nguyen M. Antifungal resistance in non- albicans Candida species. Drug Resistance Updates. 1999;2(1):9-14.

23. Pfaller M, Diekema D. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of Candida. Clinical Microbiology and Infection. 2004;10(s1):11-23.

24. White TC. Mechanisms of Resistance to Antifungal Agents. In: Murray PK, Baron EJ, Landry ML, Jorgensen JJ, Pfaller MA, editors. Manual of clinical microbiology. 9th ed. Washinton D.C.: ASM Press; 2007. p. 1961-71

25. Kothari A, Sagar V. Epidemiology of *Candida* Bloodstream Infections in a Tertiary Care Institute in India. Indian J Med Microbiol 2008;27:171-2.

26. Kumar CP, Sundararajan T, Menon T, Venkatadesikalu M. Candidiosis in children with onco-hematological studies in Chennai, South India. Jpn J Infect Dis 2005;58:218-21.

27. Kumar D. Comparative Analysis of Disc Diffusion and E-test with Broth Micro-dilution for Susceptibility Testing of Clinical Candida Isolates Against Amphotericin B, Fluconazole, Voriconazole and Caspofungin. JCDR. 2015

;3(2):12.

28. Pfaller M, Diekema D, Gibbs D, Newell V, Ellis D, Tullio V et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-Year Analysis of Susceptibilities of Candida Species to Fluconazole and Voriconazole as Determined by CLSI Standardized Disk Diffusion. Journal of Clinical Microbiology. 2010;48(4):1366-1377.

Conflict of Interest: None
Source of Support: Nil