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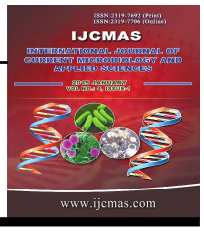

Original Research Article

Antifungal Susceptibility of Bloodstream Candida Isolates in Pediatric Patients

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K e y w o r d s

Candidemia, *Candida spp*., antifungal susceptibility

A B S T R A C T

Candidemia causes considerable attributable mortality and morbidity in children. However, data about susceptibility of *Candida* spp. isolated from pediatric blood samples are scant, and empirical therapy is often erroneously based on susceptibility pattern in adults. Hence we studied the species distribution and antifungal susceptibility of pediatric bloodstream *Candida* spp. isolates. *Candida* non-albicans were predominantly found to cause candidemia in our study.

Introduction

The incidence of bloodstream *Candida* infection, in adults and pediatric patients, has risen in the last decade (1, 2, 3, 4, 5, 6). Currently, *Candida* spp. have become the fourth most frequent causal microorganisms of nosocomial sepsis following infection due to coagulase-negative staphylococci, *Staphylococcus aureus*, and *Enterococcus* species (3, 6). Furthermore, monitoring programs have detected an increase in the prevalence of infections caused by non-*Candida albicans* (essentially *Candida parapsilosis*, *Candida glabrata*, and *Candida krusei*) and other yeast genera (7, 8, 9).

Unfortunately, data about antifungal susceptibility pattern in pediatric patients with candidemia are scarce, and empirical treatment in children with suspicion of invasive Candidiasis frequently has to be instituted by extrapolating information from adult patients. The need to update the mycological profiles in pediatric patients with candidemia was one of the aims of this study.

Materials and methods

Study design: The study was a hospital-based prospective observational study. The study population consisted of 150 patients from ICU's of Gandhi Memorial and Associated Hospitals Lucknow, with risk factors for invasive fungal infection.

Period of study: The study was carried out over a 13-month period, from August 2011 to July 2012.

Case definitions: An episode of candidemia was defined as the isolation of a *Candida* species from blood culture in a patient with temporally related clinical signs and symptoms. Neonates were defined as those <1 month of age, infants were defined as those 1 to 12 months of age, and children were defined as those 1 to 15 years old.

Sample processing: The biphasic Brain Heart Infusion (BHI, Oxoid)fungal blood culture bottle inoculated with blood sample was incubated at 37^0 C aerobically and subcultured on 2^{nd} and 7^{th} days on Sabouraud's dextrose agar (SDA) media (Emmon's modification), pH 7.0 with chloramphenicol to look for fungal isolation.

Identification of organisms and antifungal susceptibility study

Candida isolated from blood cultures were identified according to standard microbiological procedures (10).Speciation of Candida isolates were carried out by using germ tube test, cornmeal agar with Tween 20, carbohydrate assimilation and fermentation tests(10). *Candida albicans* was defined to be positive for germ tube production and terminal chlamydospore and pseudohyphae formation by Dalmau technique. *Candia tropicalis* produced no chlamydospores while *C. glabrata* showed no pseudohyphae using Dalmau technique.(10). Antifungal susceptibility testing was performed, by the Broth microdilution method in RPMI 1640 medium. The MICs of four antifungal agents: amphotericin B, fluconazole, voriconazole, and caspofungin were analysed. Breakpoints applied were as per the Clinical and Laboratory Standards Institute (CLSI) (11). Since no breakpoints have been published for amphotericin B, isolates inhibited by >1 mg/liter, were considered resistant to the drug.

Result and Discussion

A total of 44 episodes of candidemia in 44 patients ≤15 years of age were identified during the study period. *C. tropicalis* (55.9%), *C. albicans* (14.7%), *C. glabrata* (11.8%), *C. kefyr* (8.8 %), *Candida lusitaniae* (5.9 %), *C. guilliermondii* (2.9%), were the predominant species causing candidemia during the study period, in order of frequency of isolation. No mixed infection was found during the entire study period. The etiological agents of candidemia varied according to age, gender, or patients' location in the hospital (Table 1)

Most episodes of candidemia occurred in males (65.9%) and patients less than 1 month old (77.3%), and more cases of candidemia occurred among those hospitalized in neonatal ICUs (NICUs) (75.0%) than in general wards (18.2%) or Pediatrics intensive care units (PICUs) (6.8%). (Table 1)

Antifungal susceptibility pattern of blood stream *Candida* isolates by BMD method

In vitro susceptibility testing of the 44 BSI of *Candida* species against amphotericin B, fluconazole, caspofungin, and voriconazole is shown in table 2. Overall, the rate of resistance was 4% for amphotericin B and 17% for fluconazole. *C. glabrata* was the species most resistant to antifungals; all 5 isolates were found resistant to fluconazole, while 2 isolates were resistant to amphotericin B.

The percentages of isolates of *Candida* spp. in general in each category (S or susceptible, SDD or susceptible, dose dependent, and R or resistant) were 67%, 0%, and 17% and 100%, 0%, and 0% for fluconazole and voriconazole, respectively according to the breakpoints recommended by CLSI. For

amphotericin B and caspofungin , applying the breakpoints of previous studies the percentages of isolates of *Candida* spp. in each category (S, I, and R) were 57%, 39%, and 4% and 100%, 0%, and 0% respectively.

Studies on susceptibility pattern of Candida spp. causing bloodstream infections are more often to be found from adult populations. Thus, pediatricians often have to rely on data from these studies when applying treatment. To our knowledge, this is one of the unique prospective series of candidemia reported in pediatric patients, having a total of 44 patients from a tertiary hospital of North India. Data were collected for a period of 12 months and incorporated with results of in- vitro susceptibility to four systemic antifungal agents.

As in case of adults, candidemia was found to occur more frequently in males (60.6%) (12, 13,14). Among pediatric patients, candidemia was found to be more frequent in neonates (35.4%) and children over 1 year of age. As in other studies , a higher proportion (75.0%) of candidemia episodes occur in patients admitted to Neonatal Intensive Care Units (NICU) (15,16).

Overall, the predominant fungal pathogen implicated in bloodstream infection in our study was *C. tropicalis* which caused 55.9% of episodes.

In the present study, Non-albicans *Candida* species accounted for 81.8% of cases of neonatal candidemia, whereas *C.albicans* was responsible for only 18.2% of cases. This corroborates well with the results of other authors.[17,18,19,20]. Ther is one study from north India which mentions that *C. tropicalis* was most common in cases of candidemia(21). In their case also, candidemia was more prevalent in meales than females. In another report from Uttarakhand, India , it is mentioned that *C. parapsilosis* was the commonest *Candida* species associated with bloodstream infection.(22).

Table.1 Characteristics of Candidemia episodes and distribution of isolate

Patients characteristic	**No. (%) of episodes**						
	Total	***C. tropicalis***	***C. albicans***	***C. glabrata***	***C. kefyr***	***C. lusitaniae***	***C. guilliermondii***
Age							
<1 month	34(77.3)	19(55.9)	5(14.7)	4(11.8)	3(8.8)	2(5.9)	1(2.9)
1-12 month	0	0	0	0	0	0	0
1-18 years	10(22.7)	6(60.0)	3(30.0)	1(10.0)	0	0	0
Gender							
Male	29(65.9)	18(62.1)	5(17.2)	4(13.8)	0	2(6.9)	0
Female	15(34.1)	7(46.6)	3(20.0)	1(6.7)	3(20.0)	0	1(6.7)
Location at the time of IFI							
NICU	33(75.0)	18(54.5)	5(15.2)	4(12.1)	3(9.1)	2(6.1)	1(308)
PICU	3(6.8)	3(100)	0	0	0	0	0
Ward	8(18.2)	4(22.2)	3(37.5)	1(12.5)	0	0	0
TOTAL	**44**	**25(56.8)**	**8(18.2)**	**5(11.4)**	**3(6.8)**	**2(4.5)**	**1(2.3)**

Table.2 Categorical result of Antifungal Susceptibility Testing of 44 blood stream Candida isolates by BMD Method

Isolate	No	AMB			FLC			CAS			VRC		
		S	I	R	S	SDD	R	S	I	R	S	SDD	R
C .tropicalis	25	14	11	0	25	0	0	25	0	0	25	0	0
C. albicans	8	6	2	0	8	0	0	8	0	0	8	0	0
C. glabrata	5	0	3	2	0	0	5	5	0	0	5	0	0
C. kefyr	3	3	0	0	3	0	0	3	0	0	3	0	0
C. lusitaniae	2	1	1	0	2	0	0	2	0	0	2	0	0
C. guilliermondii	1	1	0	0	1	0	0	1	0	0	1	0	0
Total (%)	**44**	**25(57)**	**17 (39)**	**2 (4)**	**39 (67)**	**0**	**5(17)**	**44 (100)**	**0**	**0**	**44 (100)**	**0**	**0**

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