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Species Distribution and Antifungal Susceptibilities of Candida Isolates Recovered from High Risk Neonates and Infants from Tertiary Care Hospital in Northern India

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A B S T R A C T

In recent years, fungal infections have risen exponentially and are a cause of significant morbidity and mortality especially in high risk babies. Although *Candida albicans* remains the most common fungal isolate from neonatal candidemia, longitudinal studies have detected a shift towards non-albicans *Candida* (NAC) species. To study the species distribution and antifungal susceptibility pattern of candidiasis among high risk neonates and infants. Samples were collected aseptically from 128 high risk neonates and infants admitted in the NICU and HDU at JNMCH, Aligarh from February 2013 to October 2014. They were cultured and identified by standard microbiological techniques. Antifungal susceptibility testing (Disc diffusion and broth micro dilution-minimum inhibitory concentration (BMD-MIC) was performed and interpreted as per NCCLS (M27-A2) and CLSI guidelines. Of the 128 neonates and infants studied 89 (69.5 %) had septicaemia, 14(10.9 %) had oral thrush and 12 (9.4 %) had urinary tract infections. In our study we found 39 cases from which 49 isolates of *Candida* were isolated from different specimen. Of the 39 candidiasis cases *Candida albicans* (59.2%) was the most common species isolated while non albicans *Candida* (NAC) were 40.8% (*C. tropicalis* 14.3%, *C. parapsilosis* 12.2%, *C. guilliermondii* 6.2%, *C. glabrata* 4%, *C. krusei* 2% and *C. dubliniensis* 2%). Resistance to fluconazole, ketoconazole, clotrimazole was observed in 10.3%, 10.3%, 6.8% isolates of *C. albicans* respectively. Resistance to fluconazole, clotrimazole and amphotericin B was observed in 15%, 20%, 16.7% isolates of NAC respectively. No resistance was observed against itraconazole and nystatin. The maximum mortality was found in patients with NAC infections (52.9%) in comparison to *C. albicans* infection (31.8%). There is a considerable increase in *Candida* infections especially with NAC in neonates and infants with more resistance towards antifungal drugs.

Introduction

Candida is capable of causing infections in both immunocompetent as well as immunocompromised hosts but the incidence of candidiasis is more in immune-compromised individuals especially high risk babies neonates and infants (1,2). The incidence of candidiasis has increased dramatically over the past three decades (3). In hospitalized neonates and infants, Candida has evolved as an important cause of life threatening invasive infections, particularly in very low birth weight (VLBW) infants (2). These babies regardless of birth weight, size or gestational age, have a greater than average chance of mortality or morbidity, especially up to one year of life. Importance of *Candida* species in nursery and intensive care units (ICUs) is increasingly being recognized (2,4). Correct identification of clinical yeast isolates has become essential for optimal clinical management. Ever-increasing numbers of immunosuppressed patients, a widening range of recognized pathogens, and the discovery of resistance to antifungal drugs are contributing factors to this necessity (5).

Antifungal susceptibility testing is still in its infancy as compared to antibacterial susceptibility testing. Various methods like disc diffusion, agar dilution, broth dilution etc are widely used (6). The development of the National Committee for Clinical Laboratory Standards (NCCLS) reference method (M-27A) for broth dilution method for antifungal susceptibility testing has been an important tool in standardizing susceptibility testing. More recently, addition of colorimetric dyes like Alamar blue to the microdilution procedure and use of E test has resulted in increased consistency and reliability of results.

Infection due to NAC spp. is clinically indistinguishable from that caused by *C. albicans,* but are more resistant to routinely used antifungal drugs (7). Therefore prompt identification of infecting species along with in vitro antifungal susceptibility testing is very important for prevention of emergence and spread of drug resistant Candida spp (8). It has been suggested that emergence of Candida spp. other than *C.albicans* is mostly due to the selection of less susceptible species by the pressure of antifungal agents such as fluconazole.

The increased isolation rates of nonalbicans Candida species and a gradual shift in the antifungal susceptibility profile have underlined the need to monitor laboratory data for possible emergence of resistance and to select most appropriate antifungal agent for therapy.

Taking into consideration the above mentioned facts, the present study was undertaken to isolate and characterize the Candida species from the infections of neonates and infants and to study the antifungal susceptibility pattern of the isolates.

Materials and Methods

The present study was carried out in the Department of Microbiology J. N. Medical College, AMU, on 128 high risk neonates and infants admitted in the NICU and in the HDU of Department of Paediatrics, during the period of one and half years from 2013 to 2014. Various clinical specimens including blood, tracheobronchial aspirate, oral swab, ear swab, CSF and urine were collected.

Specimens like endotracheal aspirate, urine, oral swab etc., were subjected to direct microscopy by making a lactophenol cotton blue(LCB) mount and /or a Gram stained smear. The samples were inoculated on to Sabouraud's dextrose agar as the main isolation medium. For blood samples, Approximately 1 to 2 ml of blood was

collected under aseptic precautions and inoculated in biphasic brain heart infusion medium. The culture medium was incubated at 37°C for a week or longer if required. Subculture was done on third, fifth, and seventh day. All the *Candida* isolates were subjected to germ tube test using normal human serum. Colonies were identified up to the species level on the basis of colony characteristics, morphology on Corn meal agar, growth on Hi- CHROME *Candida* agar, carbohydrate fermentation, and assimilation patterns (9,10).

The procedure followed was in accordance with the ethical standards of the responsible committee and informed written consent was taken prior to every procedure.

All the isolates were screened for antifungal susceptibility testing by the Disk Diffusion method modified by Chakrabarti *et al.*, (11) using yeast nitrogen base-glucose (YNBG) agar. The antifungal agents tested were Amphotericin B, Nystatin, Ketoconazole, Clotrimazole, Fluconazole and Itraconazole (HiMedia Laboratories, Mumbai, India). The broth micro dilution-minimum inhibitory concentration (BMD-MIC) of the isolates was performed for the fluconazole, ketoconazole and amphotericin B using RPMI medium and MOPS buffer. MIC results were interpreted as per NCCLS (M27-A2) (12) guidelines. Isolates showing fluconazole MIC ≤ 8 µg/ml were regarded as susceptible, 16-32 µg/ ml as dose-dependent susceptible and ≥ 64 µg/ml as resistant. The quality control test was performed by using the strains of *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028).

Statistical Methods

The 'chi-square' test and the Student's 't' test were used to compare the data. A 'p' value of< 0.05 was taken as indicative of statistical significance, and a 'p' value of < 0.01 was considered highly significant.

Results and Discussion

One hundred and twenty eight high risk neonates and infants were included in the study.

The maximum number of patients included had septicaemia in 89 (69.5 %) cases followed by oral thrush in 14(10.9 %) cases and urinary tract infections in 12 (9.4 %) cases. Other presentation included in the study were chronic suppurative otitis media (CSOM) (3.9%), pneumonia (3.1%) and meningitis (3.1%) (Fig 1).

It can be seen that out of a total of 128 patients, Candida could be isolated in 39 (30.5%) cases (Table 1).

The maximum number of Candida isolates were found from patients with septiceamia (59.2%) followed by cases of oral thrush (16.3%) and cases of urinary tract infections (14.3%) (Table 2).

Candida albicans (59.2%) was the most common species isolated from neonates and infants suffering from candidiasis while non albicans *Candida* were 40.8%. In non albicans *Candida*, *Candida tropicalis* (14.3%) was most frequently isolated spp. followed by *Candida parapsilosis*(12.2%),*Candida guilliermondii* (6.2%), *Candida glabrata*(4%), *Candida krusei* (2%) and *Candida dubliniensis* (2%) (Table 3).

The most common specimen from which Candida was isolated included blood 29 (59.2%) and oral swab 8 (16.3%). The most common species of Candida isolated from almost all the specimen was *Candida albicans* followed by *Candida tropicalis* from the non

albicans *Candida* group (Table 4). 41.4% *C.albicans* and 58.6% non albicans *Candida* was isolated from blood sample.(Fig 2)

Table 5 shows that all the isolates were susceptible to nystatin and Itraconazole. Resistance was observed in 12.2% isolates to fluconazole and clotrimazole, 6.1% isolates to ketoconazole and 2% isolates to amphotericin B.

Table 6 shows that out of a total of 49 isolates, 87.8% isolates were sensitive to clotrimazole and fluconazole, 93.9% to ketoconazole, 98% to amphotericin B and 100% each to nystatin and itraconazole. Resistance to fluconazole was observed in 3(10.3%) isolates of *C. albicans* and 3(15%) of NAC isolates. Among NAC 14.3% of *C. tropicalis* and 100% of *C. dubliniensis* and 100% of *C. krusei* were fluconazole resistant. Resistance to clotrimazole was seen in 6.8% isolates of *C. albicans* and 20% of NAC. Among NAC 28.5% of *C. tropicalis*, 33.3% of *C. guilliermondii*, 100% of *C. krusei* were resistant to clotrimazole. Resistance to ketoconazole was observed in 10.3% isolates of *C.albicans* and none of the NAC. Resistance to amphotericin B was observed in 16.7% isolates of *C. parapsilosis*. No resistance was observed against itraconazole and nystatin.

It was observed that 3.4% isolates of *C. albicans* had MIC value of 32µg/ ml. 6.9% of *C. albicans* and 14.3% of *C. tropicalis* had a MIC value of >32 µg/ ml and 100% of *C.dubliniensis* had a MIC value of 64 µg/ ml. This showed that 6.9% isolates of *Candida albicans* were resistant to fluconazole and 1(3.4%) isolate was dose dependent sensitive, while 1(16.7%) isolate of *C. tropicalis* and 1(100%) isolate of *C.dubliniensis* were resistant to fluconazole. Overall 10.2% *Candida* spp. were resistant to fluconazole (Table 7). It was observed that 2 (6.9%) isolates of *C.albicans* and 1(100%) isolate of *C.dubliniensis* had MIC value of> 0.125 µg/ ml which showed that they were resistant to ketoconazole.6.1% of Candida isolates were resistant to ketoconazole.(Table 8)

It was observed that only 1(16.7%) isolate of amphotericin B had MIC >1µg/ml i.e. it was resistant while rest of 83.3% isolates were sensitive to amphotericin B.(Table 9)

A total of 128 children suffering from various clinical diseases, categorized into different predefined high risk groups were included in the study to determine the profile of Candida infections with respect to the predominant species, pathogenic characteristics and antifungal susceptibility analysis of the isolates in high risk neonates and infants.

The most commonly identified patient group in this study were neonates and infants with septicaemia (69.6%), followed by patients with oral thrush (10.9%), urinary tract infection (9.4%).The other group included patient with pneumonia (3.1%), meningitis (3.1%) and ear infection (3.9%). Thus, the study group compromised of a varied patient population with different clinical diagnosis.

Overall the rate of Candida isolation from various specimens in our study group was 30.5%. *C.albicans* formed the largest group (59.2%) of Candida species isolated in this study. Pfaller *et al.*, (13) had reported 66%, Altuncu E (14) 66%, Belet N (15) 65.7%, Ariff S (16) 55% of *Candida albicans* isolation in their respective studies. Indian studies which reported almost similar findings were S Narain (53.3%)(17), Kaur R (50%)(18). However, Kotwal A *et al.*, noted a much higher prevalence of *C.albicans* (78.1%)(19). However certain other Indian studies showed non albicans Candida species as the most frequently isolated species. S Shivprakashan (20), Goel N (21), R.J.

Kothavade (22), Deorukhkar SC *et al.,* (23), Deepak J. *et al.,* (24) they showed *C.tropicalis* as the most frequently isolated species. This species variation may be due to the differences in empiric or prophylaxis practices.

The spectrum of candidiasis varies from country to country. Although *C.albicans* remains the most common isolated spp. from cases of candidemia in USA, Europe, and South America (Brazil), its prevalence is decreasing over the time and non albicans Candida spp. are increasing. The ARTEMIS Surveillance Study which was carried out over a period of 6.5 years (1997–2003) in 127 medical centers in 39 countries has shown an increase in the prevalence of Candida species like *C. tropicalis* (4.6% in 1997 to 7.5% in 2003) and *C. parapsilosis* (4.2% in 1997 to 7.3% in 2003) (13,25). This particular surveillance study showed a 2 to 10-fold increase in the isolation rates of rare species like *C. guillermondii*, *C. kefyr and C. rugosa.*

Although *C. albicans* was the most commonly isolated species (59.2%) in our study non albicans Candida (NAC) also substantially caused candidiasis. The next most common isolate, *C. tropicalis* formed 14.3% of the total isolates. Kontoyiannis *et al.,* (26) and Ariff S (16) also observed *C. tropicalis* as the second most common Candida species after *Candida albicans* to cause candidiasis. S Narain (17), Gelotar P *et al.,* (27), Kaur R (18), from India reported 23.3%, 36% and 40% of isolation rate of *C. tropicalis* respectively. *C. tropicalis* is becoming an increasingly frequent pathogen in NICU.

C. parapsilosis was the third common species isolated (12.2%). In contrast to our study *C. parapsilosis* has been reported as the second most common spp. in neonates in many western studies. (15, 28, 29). Fairchild (30) and Kaufmann (1) have found *C. glabrata* as the most common emerging spp. Occurrence of *C. glabrata* sepsis was noted commonly in patients with significant higher gestational age and birth weight compared to sepsis with non glabrata spp. *C. glabrata* was the second most common NAC spp. isolated in the study conducted by Deorukhkar SC *et al.,* (23). However, we found a much lower incidence of infection by *C. glabrata* (4.1%). According to several investigators, the increase in the frequency of infections has paralleled the increased use of fluconazole in some hospitals. In a more recent study, however, investigators described the association between *C. glabrata* infection and amphotericin B use rather than fluconazole (31). Other less commonly isolated Candida spp., in order of frequency included *C. guilliermondii* (6.2%), *C. dubliniensis* (2%) and *C. krusei* (2%).

Among the patients in whom Candida was isolated, the most common group was neonates and infants with septicaemia (59.2%). The other major patients group with candidiasis included those with oral thrush (16.3%), UTI (14.3%), meningitis (4.1%), ear infection (4.1%), pneumonia (2%). This is in agreement with the findings of Altuncu E *et al.,* (14).

In the present work we studied the susceptibility pattern of various Candida isolates to six antifungal agents, which included Fluconazole, Ketoconazole, Clotrimazole, Itraconazole, Amphotericin B and Nystatin by disk diffusion method. Further, the MIC values of the Candida isolates were evaluated for fluconazole, ketoconazole and amphotericin B by microbroth dilution method. The susceptibility pattern of *Candida* isolates shows that 87.8% isolates were susceptible to fluconazole and clotrimazole, 93.9% isolates were susceptible to ketoconazole, 98% to amphotericin B and all the isolates (100%) were susceptible to each nystatin and itraconazole. Resistance was

observed in 12.2% isolates to fluconazole and clotrimazole, 6.1% isolates to ketoconazole and 2% isolates to amphotericin B. These findings are in agreement with a study conducted Xess *et al.,* (32) who reported 11.7% resistance to fluconazole and Belet N *et al.,* (15) (8.5%). In contrast to our study Narang *et al.,* (33) and Kotwal *et al.,* (19) found a higher rate of fluconazole resistance (24% and 26% respectively).

In this study we found more resistance to azole group of antifungal agents as compared to amphotericin B in Candida isolates similar to the study by Changdeo S. Aher (34). Azole resistance in Candida spp. is of concern because these drugs are frequently used as therapeutic alternatives to amphotericin B. Azole group of antifungal agents are preferred because they are easy to administer and are less nephrotoxic. In our study resistance to fluconazole was observed in 10.3% isolates of *C. albicans*. Similar susceptibility of *C.albicans* isolates was also reported by Mokaddas *et al.,* (35), Fadda *et al.,* (36) and M.W. Rizvi *et al.,* (37). In India, there is a lack of multicentric studies regarding antifungal susceptibility pattern. However, there are few studies from different parts of the country which give some idea regarding the epidemiology of antifungal resistance among candidemia isolates. Recently azole resistance was seen more common in NAC spp. as compared to *C. albicans*, we also found a higher rate of fluconazole resistance among NAC (15%) as compared to *C. albicans* (10%). Deorukhkar *et al.,* (23) also found a higher drug resistance among NAC isolates. Among *C. tropicalis* 1 (14.2%) and 2 (28.6%) isolates were fluconazole and clotrimazole resistant respectively. Fluconazole resistance was observed in 27.3% of NAC spp. 14.2% of *C. tropicalis* and 100% of *C. dubliniensis* and 100% of *C. krusei*.

Fluconazole (or Azole) resistance is predominantly the consequence of previous exposure to fluconazole (or other azoles), particularly repeated and long-term exposure (38).

Roildes *et al.,* (28), from Greece reported that all the isolates were susceptible to AMB, and 97.5% were susceptible to azoles. However, one of the three *C. tropicalis* isolates was found resistant to azoles. Rowen *et al.,*(39) reported that 7.5% of non-albicans Candida blood isolates were non susceptible to fluconazole. The very low rate of azole resistance in their study may be related to the treatment policy in use at their center i.e. no systemic or topical antifungal agents are used prophylactically, and their empiric use is restricted only to few cases.

Overall, 97.3% of the Candida BSIs isolates tested were susceptible to fluconazole, which further confirms the infrequent fluconazole resistance among *C. albicans*, *C. parapsilosis*, and *C. tropicalis* reported in a recent global survey (40). The increase in the rate of fluconazole resistance in *C.tropicalis* is of concern because this species is one of the most commonly isolated NAC spp. and fluconazole is the most common antifungal agent used for the treatment of various types of candidiasis (8). Oberoi J K *et al.,* (41), found *C. tropicalis*, as the most common species isolated and was 90.5 per cent susceptible to fluconazole, whereas *C. parapsilosis* and *C. glabrata* showed lower sensitivity rates of 66.1 and 60.8 per cent, respectively. Deepak J. *et al.,* (24) found a significant proportion of *C. tropicalis* isolates were resistant to azoles especially fluconazole.

Table.1 Prevalence of Candida infection among neonates and infants in the study group

(n =128)

Isolate	No. of cases	%
Candida spp. isolated	39	30.5
No Candida spp. isolated	89	69.5
Total	128	100

Table.2 Distribution of Candida isolates in relation to clinical diagnosis in neonates and infants with candidiasis.

Clinical diagnosis	No. of patients	Sample	No. of isolates	%
Septicaemia	25	Blood(23)	29	59.2
		blood (3)+urine(3)*		
		blood(3)+oral swab(3)*		
Oral thrush	5	Oral swab	5+3*=8	16.3
UTI	4	Urine	4+3*=7	14.3
CSOM	2	Ear swab	2	4.1
Meningitis	2	CSF	2	4.1
Pneumonia	1	Endotracheal aspirate	1	2
Total	**39**		49	100

**3 urine and 3 oral swab Candida isolates were from septicaemia patients.*

Table.3 Candida spp. isolated from patients in the study group

(n=49).

Candida spp.		No. of isolates	%	
***Candida albicans* (29)**	***C.albicans***	29	59.2	
Nonalbicans *Candida* (20)	***C.tropicalis***	7	14.3	40.8
	C.parapsilosis	6	12.2	
	C.guilliermondii	3	6.2	
	C.glabrata	2	4.1	
	C.dubliniensis	1	2	
	C.krusei	1	2	
Total		49	100	

Table.4 Isolation of different Candida spp. from various specimens.

Specimen	Species of Candida							Total
	C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. guilliermondii*	*C. glabrata*	*C. dubliniensis*	*C. krusei*	
Blood	12(41.4)	5(17.2)	5(17.2)	3(10.3)	2(6.9)	1(3.4)	1(3.4)	29(59.2)
Oral swab	8(100)	-	-	-	-	-	-	8(16.3)
Urine	5(71.4)	1(14.3)	1(14.3)	-	-	-	-	7(14.3)
Ear swab	1(50)	1(50)	-	-	-	-	-	2(4.1)
CSF	2(50)	-	-	-	-	-	-	2(4.1)
Endotracheal aspirate	1(100)	-	-	-	-	-	-	1(2.0)
Total	29	7	6	3	2	1	1	49

Figures in parenthesis indicate percentage

Table.5 Susceptibility pattern of Candida isolates to various antifungal agents by disc diffusion method

Antifungal agent	Sensitive	Resistant
Clotrimazole	43(87.8)	6(12.2)
Fluconazole	43(87.8)	6(12.2)
Amphotericin B	48(98)	1(2)
Nystatin	49(100)	0
Ketoconazole	46(93.9)	3(6.1)
Itraconazole	49(100)	0

Figures in parenthesis indicate percentage

Fig.1 Distribution of patients in relation to the clinical diagnosis.

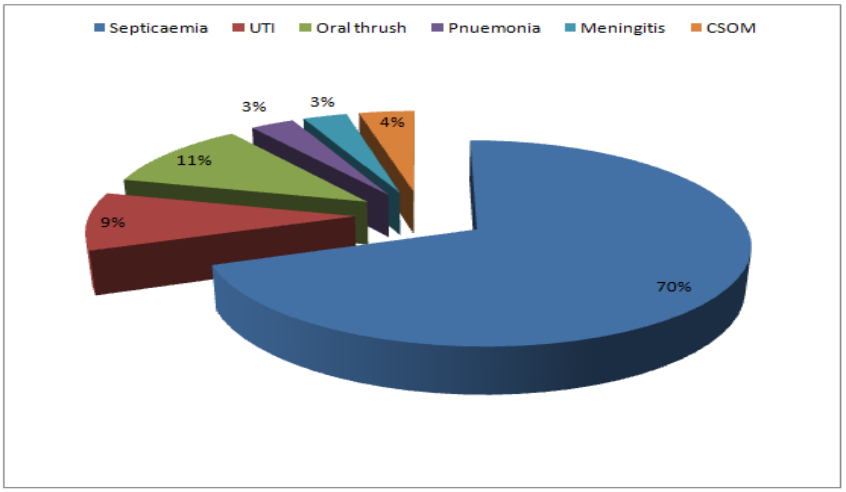


Table.6 Antifungal susceptibility pattern of various Candida spp. by disc diffusion method.

Candida *spp.*	No. of isolates	Clotrimazole		Fluconazole		Ketoconazole		Amphotericin B		Itraconazole		Nystatin	
		S	R	S	R	S	R	S	R	S	R	S	R
C. albicans	29	27 (93.1)	2 (6.8)	26 (89.7)	3 (10.3)	26 (89.7)	3 (10.3)	29 (100)	0	29 (100)	0	29 (100)	0
C. tropicalis	7	5 (71.4)	2 (28.6)	6 (85.7)	1 (14.3)	7 (100)	0	7 (100)	0	7 (100)	0	7 (100)	0
C. parapsilosis	6	6 (100)	0	6 (100)	0	6 (100)	0	5 (83.3)	1 (16.7)	6 (100)	0	6 (100)	0
C. guilliermondii	3	2 (66.7)	1 (33.3)	3 (100)	0	3 (100)	0	3 (100)	0	3 (100)	0	3 (100)	0
C. glabrata	2	2 (100)	0	2 (100)	0	2 (100)	0	2 (100)	0	2 (100)	0	2 (100)	0
C. dubliniensis	1	1 (100)	0	0	1 (100)	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0
C. krusei	1	0	1 (100)	0	1 (100)	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0
Total	49	43 (87.8)	6 (12.2)	43 (47.8)	6 (12.2)	46 (93.9)	3 (8.1)	48 (98)	1 (2)	49 (100)	0	49 (100)	0

Figures in parenthesis indicate percentage

Table.7 MIC values for Fluconazole (Broth microdilution method)

Candida spp.	MIC of fluconazole (µg/ ml)								Total
	0.5	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**	
C. albicans	26	-	-	-	-	-	1(3.4)	2(6.9)	29
C. tropicalis	6	-	-	-	-	-	-	1(14.3)	7
C. parapsilosis	6	-	-	-	-	-	-	-	6
C. guilliermondii	3	-	-	-	-	-	-	-	3
C. glabrata	2	-	-	-	-	-	-	-	2
C. krusei	-	-	-	-	-		-	1(100)	1
C. dubliniensis	-	-	-	-	-	-	-	1(100)	1
Total	**43**	**-**	**-**	**-**	**-**	**-**	**1**	**5**	**49**

Figures in parenthesis indicate percentage
(S,<8 µg/ml; S-DD, >8 µg/ml and ≤32 µg/ml; R, >32 µg/ ml)

Table.8 MIC of ketoconazole (Broth microdilution method)

Candida spp.	MIC of ketoconazole (µg/ ml)									Total
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	
C. albicans	27	-	2(6.9%)	-	-	-	-	-	-	29
C. tropicalis	7	-	-	-	-	-	-	-	-	7
C. parapsilosis	6	-	-	-	-	-	-	-	-	6
C. guilliermondii	3	-	-	-	-	-	-	-	-	3
C. glabrata	2	-	-	-	-	-	-	-	-	2
C. krusei	1	-	-	-	-	-	-	-	-	1
C. dubliniensis	-	-	1(100%)	-	-	-	-	-	-	1
Total	**46**	**-**	**3(6.1%)**	**-**	**-**	**-**	**-**	**-**	**-**	**49**

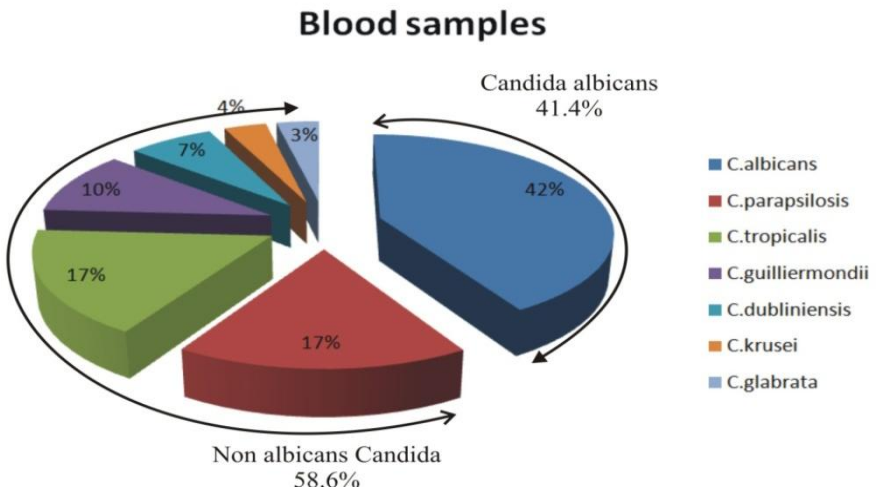
Figures in parenthesis indicate percentage
(MIC of >0.125 µg /ml by M27- A are less likely to respond to ketoconazole)

Table.9 MIC of amphotericin B. (Broth microdilution method)

Candida spp.	MIC of amphotericin B (µg/ ml)									Total
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	
C. albicans	29	-	-	-	-	-	-	-	-	29
C. tropicalis	7	-	-	-	-	-	-	-	-	7
C. parapsilosis	5	-	-	-	1 (16.7%)	-	-	-	-	6
C.guilliermondii	3	-	-	-	-	-	-	-	-	3
C. glabrata	2	-	-	-	-	-	-	-	-	2
C. krusei	1	-	-	-	-	-	-	-	-	1
C. dubliniensis	1	-	-	-	-	-	-	-	-	1
Total	**48**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**49**

Figures in parenthesis indicate percentage
(MICs of >1 µg/ml are probably resistant;M27-A)

Fig.2



Strains of *C. parasilosis, C. glabrata* were sensitive to fluconazole 1 (16.6%) *C. parasilosis* isolates was amphoterecin B resistant. In China, Ying Liang Yang *et al.*, found that all *C. parapsilosis* isolates were sensitive to fluconazole and the *C. krusei* isolates had the highest resistance rate to fluconazole (42).

We found that C. glabrata was sensitive to all the drugs tested. High degree of resistance to azoles was seen among *C. krusei* (78.57%) and *C. glabrata* (63.16%) by Deepak J., *et al.*, (24). Steinbach W.J. (43) found only 63% of *C. glabrata* isolates in children < 1 year of age to be susceptible to fluconazole. Although resistance to AMB was quite low (3.79%), but is a matter of concern as emergence of such isolates may pose serious therapeutic challenges and also increases risk of nosocomial infection. AMB constitutes the most preferred agent for the treatment of neonatal candidiasis (44).

In our study the results of susceptibity by disc diffusion method and with broth microdilution method were found to be almost same. 3.4% isolates of *C. albicans* had MIC value of 32 µg/ ml. 6.9% of *C. albicans* and 16.7% of *C. tropicalis* had a MIC value of >32 µg/ ml and 100% of *C. dubliniensis* had a MIC value of 64 µg/ ml. This showed that 6.9% isolates of *Candida albicans* were resistant to fluconazole and 1(3.4%) isolate was dose dependent sensitive, while 1(16.7%) isolate of *C. tropicalis* and 1(100%) isolate of *C. dubliniensis* were resistant to fluconazole. Pfaller *et al.*, (45) have also reported similar MICs for fluconazole (32 to 128µg/ ml). Colombo *et al.*, (46) also reported MIC value of >32 µg/ ml for fluconazole. However, they reported a MIC range of 0.125 to>32 µg/ ml.

Overall 10.2% Candida spp. were resistant to fluconazole by broth micro dilution method which was approximately same as by disc diffusion method (12.2%), as isolate of *C.albicans* was dose dependent sensitive.

The results of susceptibility pattern of ketoconazole and amphotericin B were found to correlate with the findings of disc diffusion method. We did not find any resistance to itraconazole and nystatin. These findings were in accordance with Pfaller *et al.*, (47).

The predominant Candida spp. causing candidaemia were the non albicans *Candida* (NAC) although *Candida albicans* were the overall predominant group in Candidiasis patient. Among the NAC Blood stream infection (BSI), *C.tropicalis* and *C. parapsilosis* were the common species isolated. Other NAC species causing BSI among neonates and infants were *C.guilliermondii, C.glabrata, C.dubliniensis* and *C.kursei*.

On studying the susceptibility pattern of various Candida isolates, we observed that all the isolates were sensitive to itraconazole and nystatin. Among individual species, the incidence of fluconazole resistance among *C.albicans* isolates was 10.3%, while ketoconazole was 6.9% resistant, clotrimazole was 6.8% resistant. Among *C.tropicalis*, 1(14.2%) and 2(28.6%) isolates were fluconazole and clotrimazole resistant respectively. 1 (16.6%) *C.parasilosis* isolate was amphoterician B resistant. *C.glabrata* was sensitive to all antifungal agents tested. 1(100%) isolate of *C.dubliniensis* was fluconazole resistant. 1(100%) isolate of *C.kursei* was resistant to clotrimazole and fluconazole.

Candida is significantly rising. Non albicans candidiasis should be considered when initiating antifungal prophylaxis as they possess a different antifungal susceptibility spectrum from *C. albicans.*

Therefore clinicians should strongly suspect Candida infection other than a bacterial infection in neonates and infants belonging to high risk group. Pediatricians should request for fungal culture and antifungal susceptibility testing before initiating antifungal prophylaxis.

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