Candidemia isolates, identification and antifungal susceptibility testing: a study from a tertiary care centre

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Abstract

Aim: This study is aimed to characterize the epidemiology and antifungal susceptibility profile of *Candida* isolates from candidemia cases in a tertiary care centre in both ICU and non- ICU patients.

Materials and Method: This study was conducted in Department of Microbiology, St. John's Medical College and Hospital, Bangalore for a period of 6 months. Ethical approval was obtained from the Institutional Ethics Committee. Blood culture bottles from the adult and paediatric wards were incubated for a period of 7 days at 37^0C.Once the blood cultures showed growth, further processing was done. The *Candida* isolates were speciated using conventional morphological and biochemical tests.

Results: *C.tropicalis* was the predominant isolate accounting for 53.3% out of the 30 *Candida* isolates obtained during this study period. The incidence of candidemia was higher in ICU patients(77%). An emerging drug resistance to Fluconazole was observed among *C.albicans*.

Conclusion: The frequency of candidemia among hospitalized patients has increased greatly during the recent years.

Keywords: Candidemia, *Candida*, Antifungal susceptibility testing, *C.tropicalis*

Introduction

Candidiasis is the commonest fungal disease affecting the mucosa, skin, nails and internal organs of humans. Invasive fungal infections are an important cause of morbidity and mortality among critically ill patients. Candidemia is a serious problem among adults and neonates, particularly among those in ICUs and account for approximately 10% of ICU blood stream infections.[1,2,3] *Candida* is the seventh most common nosocomial pathogen and fourth most common blood stream pathogen.[1,2] In the recent past non-*Albicans* species resistant to Fluconazole have emerged as important pathogens[4] and candidemia due to these are associated with high mortality.[2] Hence early diagnosis of candidemia due to non-*Albicans Candida*(NAC) species is critical in the management of patients.[1] Most of the studies on candidemia have been carried out in western countries and there is paucity of such data from India especially South India. It is therefore important to speciate all *Candida* isolated from blood in the laboratory and perform antifungal susceptibility testing especially to Fluconazole which is a commonly used first line azole drug for which resistance is being reported among non-*Albicans* species.

Materials and Method

All the *Candida* isolates obtained as a single pathogen from blood cultures during the study period from June 2011 to November 2011 were included in the study. This study was done at Department of Microbiology, St. John's Medical College and Hospital, Bangalore, India. The blood culture bottles (routine conventional and automated) from adult and paediatric wards were incubated for a period of 7 days at 37^0C. Once the blood culture showed growth, Gram staining was carried out. If Gram positive budding yeast cells were observed, subculture was made onto 2 SDA tubes. One was incubated at room temperature and the other at 37^0C to get a pure growth and was incubated for four weeks. Identification and speciation was carried out using the following tests-

Growth at 37^0C, Gram staining, Chlamydospore formation, germ tube formation, carbohydrate assimilation test, carbohydrate fermentation test and urea hydrolysis. Germ tube test was carried out by emulsifying colony directly in human serum and also by inoculating 10-20 µl broth into human serum and incubating at 37^0C for 2-3 hours. Chlamydospore formation was studied using the Dalmau method. Antifungal susceptibility testing (AFST) was done by disc diffusion test and broth micro dilution method according to Clinical and Laboratory Standards Institute (CLSI) document M27-A2 for Fluconazole. For Amphotericin B, AFST was carried using broth micro dilution test according to the same M27-A2 CLSI document.

Results

A total of 30 *Candida* isolates were obtained as a single pathogen from blood cultures that were received in the diagnostic division of the Department of Microbiology. All the 30 isolates were speciated by morphological and biochemical tests. *C. tropicalis* was the predominant species (53.3%) followed by *C.parapsilosis* (20%), *C.krusei* (16.7%) and *C. albicans* (10%). The 3 *C. albicans* isolates were positive by direct and conventional germ tube test

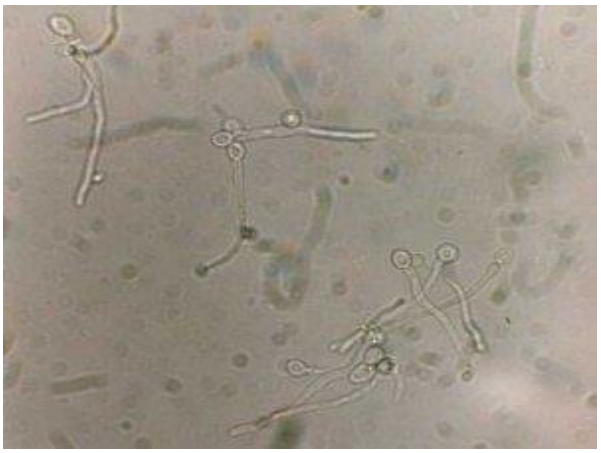

Fig. 1: photograph showing *Candida albicans* germ tube formation

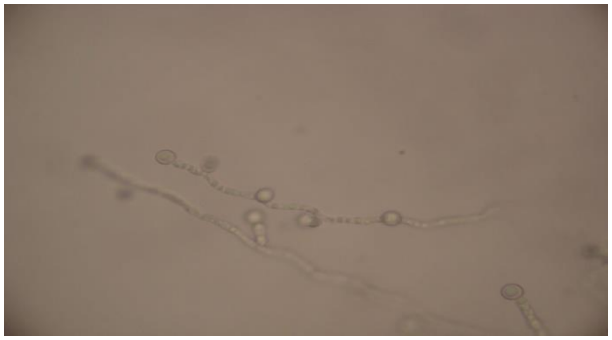

Fig. 2: Photograph showing chlamydospore formation using Dalmau technique

Table 1: Comparison of direct germ tube test and conventional germ tube test

Species	DGTT*	CGTT**
C.albicans	3	3
C.tropicalis	-	-
C.parapsilosis	-	-
C.krusei	-	-

*Direct Germ Tube Test ** Conventional Germ Tube Test

Out of the 30 isolates, 11 (37%) were neonates and adults accounted for 63% of which 10 were males (33%) and 9 (30%) were females. As per this study it is higher among ICU patients (23) compared with that of non-ICU patients (7), and also incidence rates were higher among patients who were either catheterised or undergoing therapy with more than one antibiotic. Graph 1 shows the distribution of ICU and non- ICU patients. Susceptibility testing by Disc Diffusion using 25µg Fluconazole disc gave the following results. Among the 30 isolates, 22 were susceptible, 3 were susceptible dose dependent and 5 were resistant Table 2 shows the results of disc diffusion test using Fluconazole. By MIC method, 21 were susceptible, 3 were susceptible dose dependent and 6 were resistant for Fluconazole.

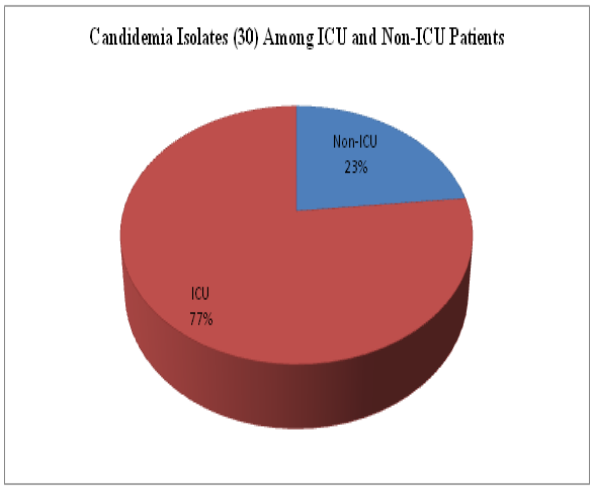

Table 2: Disc diffusion test using Fluconazole 25µg disc

Species	S*	SDD**	R***
C.albicans		2	1
C.tropicalis	16		
C.parapsilosis	6		
C.krusei		1	4

* Susceptible ** Susceptible Dose Dependent *** Resistant

However, all the 30 isolates were susceptible to Amphotericin B by MIC method. A good correlation between the disc diffusion and MIC methods for Fluconazole was evidenced among the same isolates, except for one *C.albicans* isolate which was susceptible dose dependent with disc diffusion and susceptible with MIC method. One isolate each of *C.tropicalis* and *C. parapsilosis* was susceptible with disc diffusion and susceptible dose dependent with MIC. One *C. krusei* isolate was susceptible dose dependent with disc diffusion and resistant with MIC. However one of the isolates of *C.albicans* exhibited resistance to Fluconazole by both the methods. Table: 3shows the antifungal susceptibility testing by MIC method.

Table 3: Antifungal Susceptibility Testing by MIC Method for both Fluconazole and Amphotericin B

Species (No. of isolates)	MIC				
	Fluconazole			Amphotericin B	
	S	SDD	R	S	R
C.albicans	1	1	1	3	
C.tropicalis	15	1		16	
C.parapsilosis	5	1		6	
C.krusei			5	5	

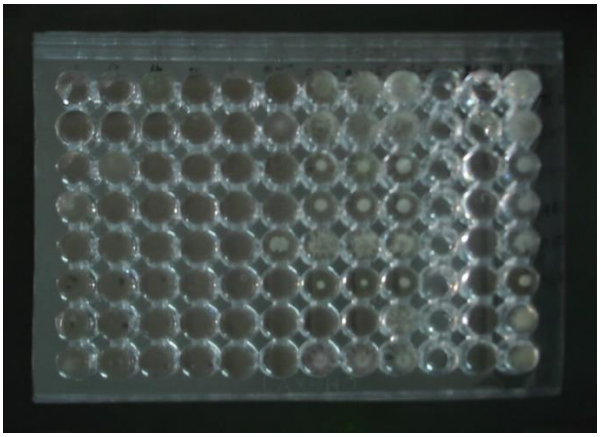

Fig. 3: Broth microdilution test for Amphotericin B

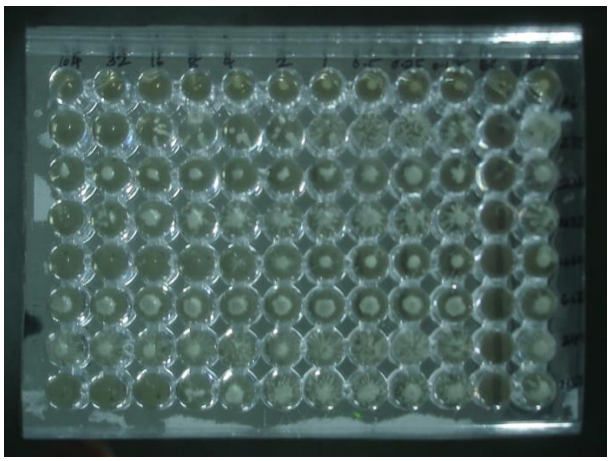

Fig. 4 Broth microdilution test for Fluconazole

Discussion

This study characterizes the epidemiology and antifungal susceptibility profile of *Candida* isolates from blood cultures in a tertiary care hospital. In this study period, a total of 30 *Candida* isolates were obtained from blood cultures received. Speciation of these isolates revealed *C.tropicalis* to be the predominant isolate accounting for 53.3% followed by *C.parapsilosis (*20%), *C.krusei (*16.7%) and *C.albicans (*10%). The studies by Xess. I et al.[5] discussed the predominance of NAC. NAC is the dominant one across the world evidenced from the study by Sandven. P,[6] Jain. N et al.[7] which is similar to the findings of our study. Chakrabarti A et al. in a recent multi centric study found that candidemia is high in ICU setting which is similar to the findings of this study.[8] However, in this study we did not isolate any species of *C.glabrata* which is a predominant isolate in the western literature as documented by Antoniadocs. A et al.[9] As evidenced from the results, this study provides similar findings as the data obtained by Donald et al.[10] in his study regarding the direct germ tube test (GTT). A 100% correlation between the direct GTT and the conventional GTT was observed in the present study. Appropriate antifungal therapy is a critical factor in improving outcomes during bloodstream infections with *Candida* species. Given that most bloodstream isolates of *C.albicans* remain susceptible to azoles such as Fluconazole, the rapid identification of *C.albicans* is a key step in the diagnostic and treatment algorithm for blood stream *Candida* infection to guide targeted and cost-effective antifungal strategy. One of our main objectives was to compare the results of Fluconazole disc diffusion testing of *Candida* species and the MIC results obtained.[11] The disc diffusion test using 25µg Fluconazole disc is an easy method that is performed in many of the laboratories. Fluconazole is the main drug for Candidemia, it being a cheaper drug.

A good correlation between the disc diffusion and MIC (micro broth dilution) methods for Fluconazole was evidenced among the same isolates. However, one of the *C.albicans* isolate exhibited resistance to Fluconazole by both methods. Overall, the level of categorical agreement between the disk diffusion test results and the reference MIC results were quite good.

All the isolates were susceptible by MIC method to Amphotericin B. However, no disc diffusion test could be done for Amphotericin B as it is water insoluble. Amphotericin B still remains the drug of choice for treating Candidemia. But it cannot be used for the treatment of patients with renal diseases as the drug is highly nephrotoxic.

Conclusion

The incidence of candidemia in India has increased considerably since the late 1990's. There is definitely a shift in the species distribution with non *Albicans* being reported as the common agents. Continued collaboration of antifungal susceptibility testing results such as that presented here will ensure the generation of useful antifungal surveillance data and result in continued improvement of antifungal susceptibility testing practices. The data from this study indicates that the microdilution test gives an accurate MIC determination by a simple visual reading. Determination of MIC will help the practitioner to determine the appropriate dosage, as this drug is severely toxic. With the advent of standardized antifungal susceptibility testing methods and microdilution adaptations, it is now possible to perform large-scale surveys of clinical isolates. Such surveys may be used to develop population distribution profiles of MICs for antifungal agents against various species of fungi and to begin to correlate invitro data with clinical response.

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