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A Study on Prevalence, Speciation and Antifungal Susceptibility test of Candidemia in a Tertiary Care Hospital

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A B S T R A C T

Background: *Candida* species are the fourth most common cause of bloodstream infections in USA whereas rank eighth in India. There is a progressive shift from a predominance of *Candida albicans* toward *non-albicans candida* (NAC) spp., especially *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. The present study is undertaken to know the various risk factors, recovery of *candida* species and their susceptibility pattern in patients admitted in a tertiary care hospital. Material and methods: The paired blood culture samples were processed in The BacT/ALERT 3D Blood Culture System. The automated system used was Vitek-2 with YST-ID cards. The antifungal susceptibility testing of *Candida* isolates were done for fluconazole, voriconazole, amphotericin B, caspofungin and micafungin by using AST-YS07/08 card. Result: A total of 71 (5.70%) patients were included in the study. The various *Candida* spp isolated were 31 (43.66%) *C. tropicalis* followed by 19 (26.76%) *C. auris*, 9 (12.67%) *C. albicans*, 7 (9.85%) *C. parapsilosis*, 4 (5.63%) *C. glabrata*, 1 (1.40%) *C. guilliermondii*. The present study showed *non-albicans Candida* species isolates had higher azoles MIC values than *C. albicans* isolates. Conclusion: The changing epidemiology of candidemia highlights the need for close monitoring of *Candida* species distribution and susceptibility to optimize treatment and outcome. Resistance to antifungals is gradually increasing, and thus, there is an urgent need for antibiotic stewardship, maintaining aseptic techniques and better hand hygiene practices.

Introduction

Candidemia is one of the most common healthcare-associated bloodstream infections and generally occurs days to weeks after hospitalization (Magill *et al.*, 2014; Chen *et al.*, 2006). These infections result in increased length of stay, high medical costs, and crude mortality of up to 30% (Morgan *et al.*, 2005). The true burden of *Candida* bloodstream infection (candidemia) is larger as only ~33% occur in ICU. Therefore the population incidence in all hospital admissions in India is estimated to be 2,70,284 per year – equivalent to a population incidence of 21.1 per 100,000 people (Chakrabarti *et al.*, 2015).

Candida accounts for nearly 96% of all opportunistic mycoses (Wisplinghoff *et al.*, 2004). *Candida* species are the fourth most common cause

of bloodstream infections in the USA whereas rank eighth in India (Giri and Kindo, 2012).

Patients who are at high risk for developing invasive candidiasis include those who have been in long term healthcare facility, have a central venous catheter, weakened immune system (for example, patients of organ transplant, and low white blood cell counts i.e. neutropenic patients), recently received multiple antibiotics in the hospital, diabetes, kidney failure or who are on hemodialysis (Zhang *et al.*, 2020).

A large multicentric study from India suggests the overall incidence of 6.51 cases per 1000 ICU admissions causing ICU acquired candidemia (Chakrabarti *et al.*, 2015). The invasive *Candida* infections are mainly attributed to five species naming *albicans, glabrata, tropicalis, krusei* and *parapsilosis* accounting two-third cases of candidiasis (Pfaller and Diekema, 2007).

During recent decades, there is a progressive shift from a predominance of *Candida albicans* toward a predominance of *non-albicans candida* (NAC) spp., especially *C. tropicalis, C. parapsilosis* and *C. glabrata*, in both neutropenic and non-neutropenic critically ill patients. There is growing evidence suggesting a role of the increasing use of azole agents in this epidemiological shift. In some North American and European centers reduced susceptibility to commonly used antifungal agents has also been observed (Pfaller and Diekema, 2007; Sobel, 2006).

The aim of the study was undertaken to know the various risk factors, recovery of *candida* species and their susceptibility pattern in patients admitted in a tertiary care hospital.

Materials and Methods

The study was conducted in the Department of Microbiology at the Krishna Institute of Medical Sciences, Secunderabad from 1, January 2021 to 31, December 2021 after taking the approval from the ethics committee. The hospital provides multidisciplinary integrated healthcare services with ICUs, CCU and transplant units (total 13 ICU) having a total bed capacity of 750 functional beds.

The patients diagnosed as having candidemia were included in the study. Candidemia was defined as the isolation of any *Candida* spp. from paired blood culture samples of a patient. If repeated paired blood culture were flagged positive, they were not included in the study. A detailed proforma was filled for all patients included in the study. The proforma included patient's preliminary data (name, age, sex, ID number), type of sample, time of sample taken and relevant clinical history of the patient.

The blood culture samples were processed inBacT/ALERT 3D Blood Culture System (bioMérieux). Two sets of blood culture bottles approximately 15 minutes apart, with each set from two different venipuncture sites were collected. In adults FA plus and FN plus bottles were used and up to 10 ml sample was added. In pediatric patients PF plus bottles were used and up to 4 ml sample was added.

The blood culture bottles which flagged positive were unloaded and Gram stained. Subculture on blood agar and MacConkey agar was done if bacteria were observed on gram stain. An additional sabouraud dextrose agar plate was inoculated if Gram positive budding yeast-like cells were observed. Subcultures were incubated for 24-48 hours at 37°C. Bacterial isolates were excluded from the study.

Speciation of genus *Candida* was done by a combination of conventional and automated systems. The conventional tests employed included Gram-stain and germ tube test. The automated system used was Vitek-2 with YST-ID cards.

The antifungal susceptibility testing of *Candida* isolates were done for voriconazole, fluconazole, amphotericin B, caspofungin and micafungin by using AST-YS07/08 card (Chakrabarti *et al.*, 2009;

Xess *et al.*, 2007; Singh *et al.*, 2011). Reporting of voriconazole, fluconazole, caspofungin and micafungin were done based on MIC values according to CLSI guidelines document M60 document (1st edition, November 2017) (Chakrabarti *et al.*, 2009). For amphotericin B European Society of Clinical Microbiology and Infectious Diseases (EUCAST) clinical breakpoints were considered from EUCAST Antifungal Clinical Breakpoint Table v. 10.0, page no.6.

Currently no established *C. auris* specific susceptibility breakpoints are available. However breakpoints are considered those established for closely related *Candida* species. In the present study, breakpoints for *C. auris* are considered as per CDC guidelines for Antifungal susceptibility testing and interpretation for *C. auris* (Table.4).

Results and Discussion

A total of 6823 paired blood samples were received during the study period. 1245 samples flagged positive, 5578 were negative for bacterial and fungal growth. Out of 1245, bacterial pathogens were 1174 (94.29%) and fungal were 71 (5.70%). All of which were *Candida* species.

A total of 71 patients were included in the study. Males were 46 (64.78%). Females were 25 (35.21%).

The age group 0-9 had 7 (9.85%) patients, 10-19 had 6 (8.45%) patients, 20-29 had 6 (8.45%) patients, 30-39 had 10 (14.08%) patients, 40-49 had 12 (16.90%) patients, 50-59 had 15 (21.12%) patients, 60-69 had 8 (11.26%) patients and 70-79 had 7 (9.85%) patients. The various risk factors included in the study are shown in Table 1.

The various *Candida* spp isolated were 31 (43.66%) *Candida tropicalis* followed by 19 (26.76%) *C.auris*, 9 (12.67%) *C.albicans*, 7 (9.85%) *C.parapsilosis*, 4 (5.63%) *C. glabrata*, 1 (1.40%) *C.guilliermondii* as shown in Table 2.

The various species of *Candida* were divided into 3 groups for better understanding as

Group I: *Non albicans candida* (*C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. guilliermondii*)

Group II: *C. auris*

GroupIII: *C. albicans.*

The *in vitro* antifungal susceptibility profile of *Candida* species to the five antifungal drugs voriconazole, fluconazole, amphotericin B, caspofungin and micafungin were expressed as MIC in µg/ml.

The antifungal susceptibility pattern of group I, II and III are given in Table 3, 4 and 5 respectively.

Infection represents a frequent complication among the patients admitted to tertiary care hospitals. *Candida* spp. infections have increased in the last few decades, particularly those caused by the NAC, indicating the importance of laboratory diagnosis for the correct identification of species involved and initiation of timely and adequate treatment (Colombo and Guimarães, 2003).

The present study details the prevalence of *Candida* infection, their risk factors and antifungal susceptibility pattern in a tertiary care hospital. The prevalence of *Candida* infection in the present study is 5.7% with gender predominance of Candidemia seen in males 64.78%. The predominant *Candida* species isolated was *Candida tropicalis* 43.66% followed by *Candida auris* 26.76%. In the present study 57.77 % were susceptible to voriconazole, 50.70 % were susceptible to fluconazole, 57.74 % were susceptible to amphotericin B and 98 % were susceptible to echinocandins.

Decreased susceptibility to azoles could be due to indiscriminate use of the agent for therapy and prevention. Recent studies identified several risk factors for candidemia, including extreme age, prior antibiotics receiving, prior hemodialysis, parenteral

nutrition, blood transfusion and abdominal surgeries (Yapar *et al.,* 2011; Barberino *et al.,* 2006). In the study of Ding li *et al.,* (2017), the ratio of patients with length of hospital stay more than 30 days (31.3 %), mechanical ventilation of more than 2 days (10.0 %) and ICU admitted for more than 3 days (22.5 %) was observed**.** The present study also identifies similar risk factors including patients in long term care facility, have central venous catheters, received multiple antibiotics, diabetes mellitus and renal failure patients on hemodialysis as mentioned in Table 1.

In the study conducted by Nur Yapar *et al.,* (2011), 39.7% candidemia patients were hospitalized in intensive care units, 31.3% in surgical wards and 29% were in medical wards. In the present study various species of candida reported from different clinical departments are discussed in Table 6.

In the last 10 years, infections due to *non albicans* species account for a majority of invasive candidal infections. Worldwide *C. tropicalis* is now the most common (Adhikary and Joshi, 2011). More than 90% of invasive infections are attributed to five species *C. tropicalis, C. albicans, C. glabrata, C. parapsilosis, and C. krusei* (Giri and Kindo, 2012).

Significant geographic variation is observed among cases of candidemia in different parts of the world, which appears to follow a specific pattern. Blood stream infections caused by NAC were more common than those caused by *C. albicans*, more specifically in Asia, South Europe, South America, and the Indian subcontinent (Falagas *et al.,* 2010; Chakrabarti *et al.,* 2002).

Table.1 Risk factors in candidemia patients.

S. No.	Risk factor	Number of patients	Percentage (%)
1	Stay in long term care facility	71	100
2	Central venous catheters	71	100
3	Weakened immune system patients	71	100
4	Received multiple antibiotics	71	100
5	Diabetes mellitus	40	56.33
6	Hemodialysis	34	47.88

Table.2 Identification and speciation of various *candida* species isolated from patients.

S. No.	*Candida* species	Number of isolate	Percentage (%)
1	*C. tropicalis*	31	43.66
2	*C. auris*	19	26.76
3	*C. albicans*	9	12.67
4	*C. parapsilosis*	7	9.85
5	*C. glabrata*	4	5.63
6	*C. guilliermondii*	1	1.40
Total		71	100

Table.3 Antimicrobial susceptibility pattern in Group I *non albicans candida*

***Candida* species**	**Guidelines Used**	**Voriconazole n (%)**	**Fluconazole n (%)**	**Amphotericin B n (%)**	**Caspofungin n (%)**	**Micafungin n (%)**
I.Nonalbicans n=43		CLSI M60 1st edition	CLSI M60 1st edition	EUCAST Antifungal Clinical Breakpoint v. 10.0	CLSI M60 1st edition	CLSI M60 1st edition
1 *C.tropicalis* n=31	Minimum Inhibitory Concentration (MIC)	S: ≤0.12 µg/ml I: 0.25-0.5 µg/ml R: ≥ 1 µg/ml	S: ≤ 2 µg/ml SDD: 4 µg/ml R: ≥ 8 µg/ml	S: ≤ 1 mg/L R: >1 mg/L	S: ≤ 0.25 µg/ml I: 0.5 µg/ml R: ≥1 µg/ml	S: ≤ 0.25 µg/ml M: 0.5 µg/ml R: ≥1 µg/ml
	Sensitive	17 (54.83)	17 (54.83)	22(70.96)	29(93.54)	29(93.54)
	Mod Sen	2 (6.45)	0	0	2(6.45)	0
	Resistant	12 (38.70)	14(45.16)	9(29.03)	0	2(6.45)
2 *C.parapsilosis* n=7	Minimum Inhibitory Concentration (MIC)	S: ≤0.12 µg/ml I: 0.25-0.5 µg/ml R: ≥ 1 µg/ml	S: ≤ 2 µg/ml SDD: 4 µg/ml R: ≥ 8 µg/ml	S: ≤ 1 mg/L R: >1 mg/L	S: ≤2 µg/ml I: 4 µg/ml R: ≥8 µg/ml	S: ≤2 µg/ml I: 4 µg/ml R: ≥8 µg/ml
	Sensitive	6(85.71)	5(71.42)	7(100)	7(100)	7(100)
	Mod Sen	0	0	0	0	0
	Resistant	1(14.28)	2(28.57)	0	0	0
3 *C.glabrata* n=4	Minimum Inhibitory Concentration (MIC)	NA	SDD: ≤ 32 µg/ml R: ≥64 µg/ml	S: ≤ 1 mg/L R: >1 mg/L	S: ≤0.12 µg/ml I: 0.25 µg/ml R: ≥0.5 µg/ml	S: ≤0.06 µg/ml I: 0.12 µg/ml R: ≥0.25 µg/ml
	Sensitive	-	4(100)	4(100)	4(100)	4(100)
	Mod Sen	-	0	0	0	0
	Resistant	-	0	0	0	0
4 *C.guilliermondii* n=1	Minimum Inhibitory Concentration (MIC)	NA	NA	NA	S: ≤2 µg/ml I: 4 µg/ml R: ≥8 µg/ml	S: ≤2 µg/ml I: 4 µg/ml R: ≥8 µg/ml
	Sensitive	-	-	-	1 (100)	1 (100)
	Mod Sen	-	-	-	0	0
	Resistant	-	-	-	0	0

Table.4 Antimicrobial susceptibility pattern in Group II *Candida auris.*

***Candida* species**	**Guidelines Used**	**Voriconazole n (%)**	**Fluconazole n (%)**	**Amphotericin B n (%)**	**Caspofungin n (%)**	**Micafungin n (%)**
		CDC	CDC	CDC	CDC	CDC
II C.auris n=19	Minimum Inhibitory Concentration (MIC)	Not Applicable	R: ≥ 32 µg/ml	S: ≤ 1 mg/L R: ≥ 2 mg/L	S: ≤ 1 µg/ml R: ≥1 µg/ml	S: ≤ 8 µg/ml R: >8 µg/ml
	Sensitive	-	0	0	18(94.73)	19(100)
	Mod Sen	-	1(5.26)	0	0	0
	Resistant	-	18(94.73)	19(100)	1(5.26)	0

Table.5 Antimicrobial susceptibility pattern in Group III *Candida albicans.*

Candida species	Guidelines Used	Voriconazole n (%)	Fluconazole n (%)	Amphotericin B n (%)	Caspofungin n (%)	Micafungin n (%)
		CLSI M60 1st edition	CLSI M60 1st edition	EUCAST Antifungal Clinical Breakpoint v. 10.0	CLSI M60 1st edition	CLSI M60 1st edition
III-C.albicans n=9	Minimum Inhibitory Concentration (MIC)	S: ≤0.12 µg/ml I: 0.25-0.5 µg/ml R: ≥ 1 µg/ml	S: ≤ 2 µg/ml SDD: 4 µg/ml R: ≥ 8 µg/ml	S: ≤ 1 mg/L R: >1 mg/L	S: ≤0.25 µg/ml I: 0.5 µg/ml R: ≥1 µg/ml	S: ≤0.25 µg/ml I: 0.5 µg/ml R: ≥1 µg/ml
	Sensitive	9 (100)	9 (100)	8(88.89)	9 (100)	9 (100)
	Mod Sen	0	0	0	0	0
	Resistant	0	0	1 (11.11)	0	0

Table.6 Distribution of *Candida* species amongst various clinical departments.

Group	***Candida spps.***	***HLTU***	***BMT***	***Pulmo***	***Neuro***	***Nephro***	***Emg***	***Paed***	***Rheu***	***Gen Sx***	***Int Med***
Group I. NAC n=43	*C.tropicalis* n=31	11	3	6	3	-	2	2	1	1	2
	C.parapsilosis n=7	1	-	-	-	1	1	3	-	-	1
	C. glabrata n=4	1	1	-	-	-	-	1	1	-	-
	C.gullerimondii n=1	-	-	-	-	-	1	-	-	-	-
Group I. NAC %		30.23	9.30	13.95	6.97	2.32	9.30	13.95	4.65	2.32	6.97
Group II n=19 C.auris	*C.auris* n=19	9	1	1	1	1	2	1	-	-	3
Group II. *C.auris* %		47.36	5.26	5.26	5.26	5.26	10.52	5.26	-	-	15.78
Group III n= 9 *C.albicans*	*C.albicans* n=9	1	1	2	1	2	1	-	-	1	-
Group III *C.albicans* %		11.11	11.11	22.22	11.11	22.22	11.11	-	-	11.11	-

Note: HLT= Heart Lung Transport Unit, BMT=Bone Marrow Transplant Unit, Pulmo= Pulmonology, Neuro= Neurology, Nephro= Nephrology, Emg= Emergency, Paed= Pediatrics, Rheu= Rheumatology, Gen Sx= General Surgery, Int Med= Internal Medicine

Other recent studies have shown an increase in the incidence of candidemia due to NAC, with the isolation rate ranging from 50 to 96% from tertiary care centers in India (Tak *et al.,* 2014; Chakrabarti *et al.,* 2009; Xess *et al.,* 2007; Singh *et al.,* 2011). NAC caused about 86% cases of candidemia in a study (Tak *et al.,* 2014). *C. tropicalis* and *C. parapsilosis* are an emerging cause of candidemia in India. Previous studies by Chakrabarti *et al.,* (2009); Xess *et al.,* (2007); Shukla *et al.,* (2020) and Singh *et al.,* (2011) are in agreement with our study, and it appears that *C. tropicalis* is the predominant species causing candidemia.

The present study showed NAC species isolates had higher azoles MIC values than *C. albicans* isolates but remained sensitive to low drug concentrations of amphotericin B. The present study susceptibility pattern correlates with the study of Godoy *et al.,* (2003). In the present study one isolate of *C. guilliermondii* was resistant to amphotericin B and fluconazole. Contrary results were found in the study of Godoy *et al.,* (2003) in which all isolates were susceptible.

Present study shows higher susceptibility to echinocandins for *C. albicans* than *C. tropicalis* and *C. parapsilosis*. Study by Jung *et al.,* (2020) are in agreement with the present study. The patients who reported *C. auris* were kept in isolation, the infection control team and infection control nurse were involved in educating and training the staff regarding adherence to hand hygiene, appropriate use of transmission-based precautions, cleaning and disinfecting the patient care and education with practical training given to all cadre of health care worker.

The changing epidemiology of candidemia highlights the need for close monitoring of *Candida* species distribution and susceptibility to optimize treatment and outcome. Differences in drug susceptibility profile and frequent isolation of NAC species initiates the use of accurate *in vitro* antifungal susceptibility testing methods.

There is an urgent need for antibiotic stewardship and maintenance of aseptic techniques, while inserting and maintaining invasive devices, frequent clinical checks for weaning these patients off these invasive monitoring devices, and better hand hygiene practices. In the present scenario, there is a need to develop guidelines for empiric therapy based on the disease epidemiology in India.

Even after treatment for invasive infections, patients generally remain colonized with *C. auris* for long periods, and perhaps indefinitely. Therefore, all recommended infection control measures need to be followed during and after treatment for *C. auris* infection including interfacility communication about the patient's *C. auris* status. Screening contacts of newly identified case patients and laboratory surveillance of clinical specimens to detect additional cases.

Conflicts of interest

Authors declare no conflicts of interest.

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