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'Candidemia'- beyond albicans and azoles: clinical and epidemiological review in a tertiary centre

Raksha K[1], S Jayasree[2]

Abstract:

Candida being a major blood stream fungal pathogen, risk stratification in patients and correct identification of species is the need of the hour owing to emergence of non- *C. albicans* and anti-fungal resistance. We aimed to speciate Candida isolates from blood cultures, evaluate anti-fungal susceptibility pattern and assess risk factors in this retrospective cohort study between May 2013 to December 2014.Yeast isolates obtained by BacT/ALERT® 3D automated microbial detection system were subcultured and identified using Vitek 2 yeast identification and anti-fungal susceptibility testing system, germ tube test and HiCrome Candida differential agar medium. Predisposing risk factors were analyzed using Chi-square test and Fisher's exact test. Of the 12, 637 blood culture samples, 56 Candida species were isolated from 46 patients contributing to 5.15 % (56/1088) of total positives. All patients with candidemia were admitted in the intensive care units. Male: female ratio was 2.5. *C. tropicalis* -33.39% (19/56) was the most common isolate. 16.27 % (7/43) of non *C. albicans* isolates showed intermediate level susceptibility to fluconazole and 50 % (3/6) of *C. haemulonii* isolates were Amphotericin-B resistant. Among the risk factors- age, length of hospitalization, broad-spectrum antibiotics, diabetes mellitus, central venous catheters, mechanical ventilation, recent surgery and total parenteral nutrition were significantly related. Emergence of non- *C. albicans* is an important problem in tertiary set-ups. Active screening in high risk groups can shorten diagnostic delays and help achieve better clinical results.

Key words: anti-fungal susceptibility, bloodstream infections, critical care, neonatal candidemia, non- *Candida albicans*

Introduction:

Candidemia is a well recognized clinical challenge in critical care today, the reasons being multiple. Candida is the fourth most common etiological agent of nosocomial bloodstream infections (BSIs) and accounts for about 12% of hospital acquired BSIs. The expanding population of immunocompromised patients due to rapid advancements in medicine has led to a new group of susceptible hosts to this fungus and a new problem of anti-fungal resistance is now in the limelight, more so with the non- *Candida albicans* (NAC) which is well documented in many recent studies across the world. A systematic review based on geographic region and study design done by Falagas et al stressed on the need for local epidemiological data to know the significance of candidemia, the species variability and expression of anti-fungal resistance.[1] The Prospective Anti-fungal therapy alliance (PATH) study by Pfaller et al between 2004-2008 reported an increased incidence of 54.4% of non- *C. albicans*.[2] This emergence of NAC and less common species is also attributed to greater emphasis on species identification as a guide to initiate treatment choices. Thus, variability in species distribution across centres and expression of anti-fungal resistance necessitates strong local epidemiological study.[3] But few reports on unique country

specific epidemiology are available for candidemia in Indian critical care settings.[4-7] A recent study by Chakrabarti et al determined epidemiological and clinical parameters of candidemia cases among ICU patients across 25 hospitals in India.[8] The need for such comparative data for the real significance of candidemia as a nosocomial infection in our hospital and the lack of local epidemiological data to aid the diagnosis and treatment of this group of patients prompted us to conduct this study. The aim of our study was to speciate Candida isolates from blood cultures, to determine the risk factors associated with candidemia and to evaluate the anti-fungal susceptibility pattern in these isolates.

Materials & Methods:

This was a retrospective cohort study at a tertiary multi-specialty centre between the period of May 2013 to December 2014 conducted for a period of 20 months. The institutional ethics committee clearance was obtained. The data was obtained from laboratory information system and case records. All patients admitted in the ICUs during this study period who developed signs and symptoms of nosocomial bloodstream infection were screened for candidemia. Isolation of atleast one positive blood culture containing Candida species with supportive clinical correlation was considered as a case of candidemia. Patients already diagnosed of candidemia elsewhere before admission, patients on prophylactic anti-fungal therapy or patients with <48 hours of stay in hospital were excluded from the study. A detailed clinical history was obtained which included age, gender, length of hospitalization, treatment with broad-spectrum antibiotics, chemotherapy, serum procalcitonin levels, diabetes mellitus, central venous catheters, mechanical ventilation, recent surgery, total parenteral nutrition, anti-fungal medication, dialysis and other possible risk factors.

Blood samples were collected aseptically in Bact Alert 3D culture bottles and incubated at 37°C for 7 days in BacT/ALERTX® 3D automated microbial detection system. Subsequent samples were also collected during the course of treatment from some patients. When BacT Alert indicated positive, the bottles were unloaded, preliminary Gram stain was made to look for yeast-like cells and subcultures were made on 5% sheep blood agar and Sabouraud's dextrose agar and incubated at 25°C and 37°C. The colonies appeared within 24 hours of incubation in all the cases. Speciation of these isolates was further done using (a) germ tube production, (b) pigmentation on chromogenic medium (HiCHROM Candida agar) (c) chlamydospore formation and (d) carbohydrate assimilation tests using the VITEK 2 compact system (BioMérieux). Quality control measures were undertaken with reference strains *C. krusei* ATCC 6258 and ATCC 22019. Anti-fungal susceptibility was also done against fluconazole, flucytosine, voriconazole and amphotericin-B, caspofungin and micafungin in VITEK 2 compact system. Following interpretive susceptible criteria for anti-fungals were used. For fluconazole, breakpoints were as follows: susceptible(S), MIC ≤8 µg/ml; susceptible-dose dependent (SDD), MIC = 16 to 32 µg/ml; resistant(R), MIC ≥64 µg/ml. For flucytosine, isolates showing MIC's ≤4 µg/ml were considered as susceptible, 8-16 µg/ml as intermediate and ≥32 µg/ml as resistant. For voriconazole, breakpoints followed were: susceptible ≤1.0 µg/ml, 2.0 µg/ml as dose-dependent and ≥4 µg/ml as resistant. For amphotericin-B, isolates showing a MIC of ≤1.0 µg/ml were taken as susceptible and those with MIC >1 µg/ml were considered as resistant (**Table I**).

Categorical data of demographic and clinical parameters were analyzed using Chi-square and Fisher's exact tests, as

appropriate. A p-value ≤ 0.05 was considered statistically significant. Candidemic patients treated with anti-fungals were followed up for outcome and the effect of nosocomial candidemia on mortality was assessed.

Results:

There were 56 episodes of candidemia in 46 patients during the study period. Of the 12,637 blood culture samples processed, 1088 had indicated positive (8.60 %). Among the total blood culture positives, 5.15% (1088/12,637) were cases of candidemia. All 46 patients were admitted in the intensive care units (100%). Seven patients had more than one episode of candidemia. Out of the 56 blood isolates of various Candida species, the most common was *C. tropicalis* (33.92%). It was followed by *C. albicans*, *C. glabrata*, *C. haemulonii*, *C. parapsilosis*, *C. famata*, *C. guilliermondii and C. lusitaniae* (**Figure I**). Majority of the infected patients were males (72%). Patients having candidemia belonged to the age group ranging from 17 days to 89 years with a mean of 54.82 years(**Figure II**). The length of hospitalization ranged from 1 to 67 days with an average duration of stay in the hospital of ~19.86 days.

A number of predisposing factors were found among the candidemia cases. The majority of patients had multiple predisposing factors that have been associated with candidemia. For 33 patients (71.73%), ≥3 risk factors were identified (**Figure III**). The risk factors which were found to be associated with candidemia with p-value <0.05 considered significant were- antibiotic therapy (95.65%), invasive mechanical ventilation (60.86%), diabetes mellitus(58.69%), recent surgery (58.69%), use of central venous catheters (28.26%), chemotherapy (19.56%), dialysis (15.21%) and total parenteral nutrition (10.86%)(**Table II**).

Dose-dependent susceptibility to fluconazole was seen in 16.27 % (7/43) of NAC isolates. Fifty percent (3/6) of *C. haemulonii* isolates were resistant to Amphotericin-B. All isolates showed ~ 100 % susceptibility to echinocandins (**Table III**).

Table I: Interpretative criteria for anti-fungal susceptibility testing using Vitek 2 Compact system (BioMérieux)

ANTI-FUNGAL	Breakpoints of Minimum inhibitory concentration(MIC) in µg/ml		
	Susceptible(S)	Susceptible dose dependent(SDD)	Resistant(R)/non-Susceptible(NS)
FLUCYTOSINE	≤ 4	8-16	≥ 32
FLUCONAZOLE	≤ 8	16-32	≥ 64
VORICONAZOLE	≤ 1	2	≥ 4
AMPHOTERICIN-B	≤ 1	-	≥ 1
CASPOFUNGIN*	≤ 2	-	≥ 2
MICAFUNGIN	≤ 2	-	≥ 2

**C. glabrata*- variable

Figure I: Spectrum of Candida species (n=56)

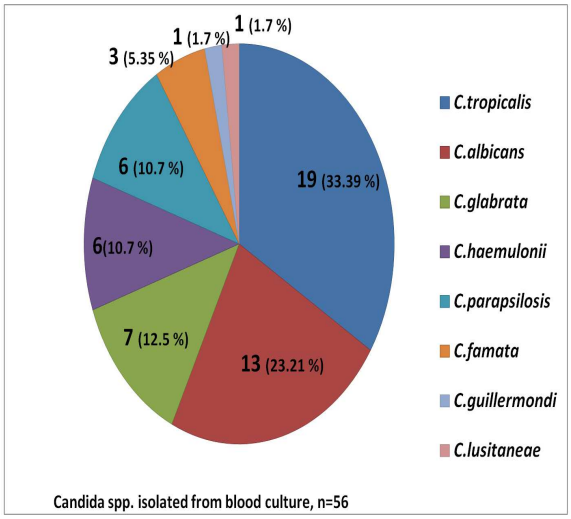
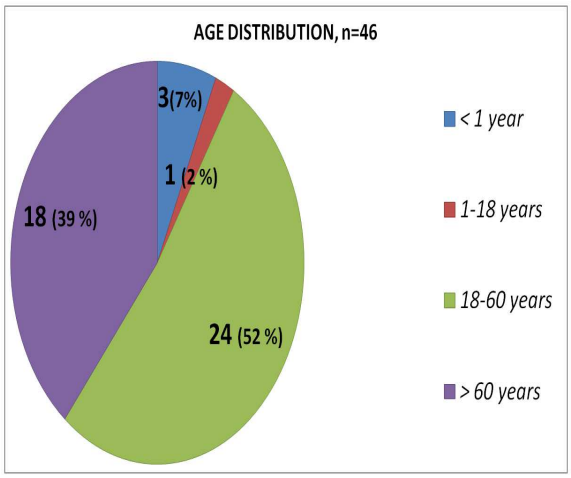


Figure II: Age distribution of patients with candidemia (n=46)



Anti-fungal therapy was instituted in 24 patients after blood culture results. Fluconazole was the commonly administered anti-fungal for 54.16% (13/24) of the treated patients. Fluconazole was escalated to caspofungin in five patients based on the anti-fungal susceptibility results (**Table IV**). The clinical outcome of patients with candidemia, both *C. albicans* & NAC are summarized (**Table V**). The mortality rate in our study was 47.8% (22/46). Mortality was higher in patients with non-*albicans* candidemia (59%) when compared to those with candidemia due to *C. albicans* (41%). We also looked at the mean serum procalcitonin levels in these patients during candidemia episodes which was 3.99 ng/ mL (**Figure IV**).

Table II: Statistical analysis of risk factors for candidemia

Risk factor	p- value
Age	< 0.001
Antibiotic therapy	< 0.001
Invasive mechanical ventilation	0.003
Diabetes mellitus	0.002
Surgery	0.018
Central venous catheterization	0.045
Chemotherapy	0.013
Dialysis	0.025
Total parenteral nutrition	0.03

Discussion:

All patients in our study were admitted in the ICUs and the length of hospitalization was a significant risk factor similar to other reports in India. Age, antibiotic therapy, mechanical ventilation, diabetes mellitus, surgery, central venous catheterization, chemotherapy, dialysis, total parenteral nutrition were other statistically significant risk factors for acquiring candidemia. *C. tropicalis* (33.92%) was the most common isolate in conformity with the other single centre

Table III: Anti-fungal susceptibility of Candida isolates (n=46)

S-susceptible, I-intermediate, R-resistant, SSD-Susceptible dose dependent, NS-Not susceptible

ANTIFUNGAL	SUSCEPTIBILITY	*C. lusitaniae* (1)	*C. guilliermondi* (1)	*C. famata* (3)	*C. parapsilosis* (6)	*C. haemulonii* (6)	*C. glabrata* (7)	*C. tropicalis* (19)	*C.albicans* (13)
FLUCYTOSINE	S	100	100	-	83.4	100	100	94.7	100
	I	0	0	-	16.6	0	0	0	0
	R	0	0	-	0	0	0	5.3	0
FLUCONAZOLE	S	100	0	-	83.4	16.6	100	89.4	92.3
	SSD	0	100	-	16.6	83.4	0	0	0
	R	0	0	-	0	0	0	10.6	7.7
VORICONAZOLE	S	100	100	-	83.4	83.4	100	89.4	100
	SSD	0	0	-	16.6	16.6	0	5.3	0
	R	0	0	-	0	0	0	5.3	0
AMPHOTERICIN-B	S	100	100	-	100	50	100	100	100
	R	0	0	-	0	50	0	0	0
CASPOFUNGIN	S	100	100	-	100	83.4	100	100	100
	NS	0	0	-	0	16.6	0	0	0
MICAFUNGIN	S	100	100	-	100	100	100	100	100
	NS	0	0	-	0	0	0	0	0

Figure III: Risk factors in patients with candidemia (n=46)

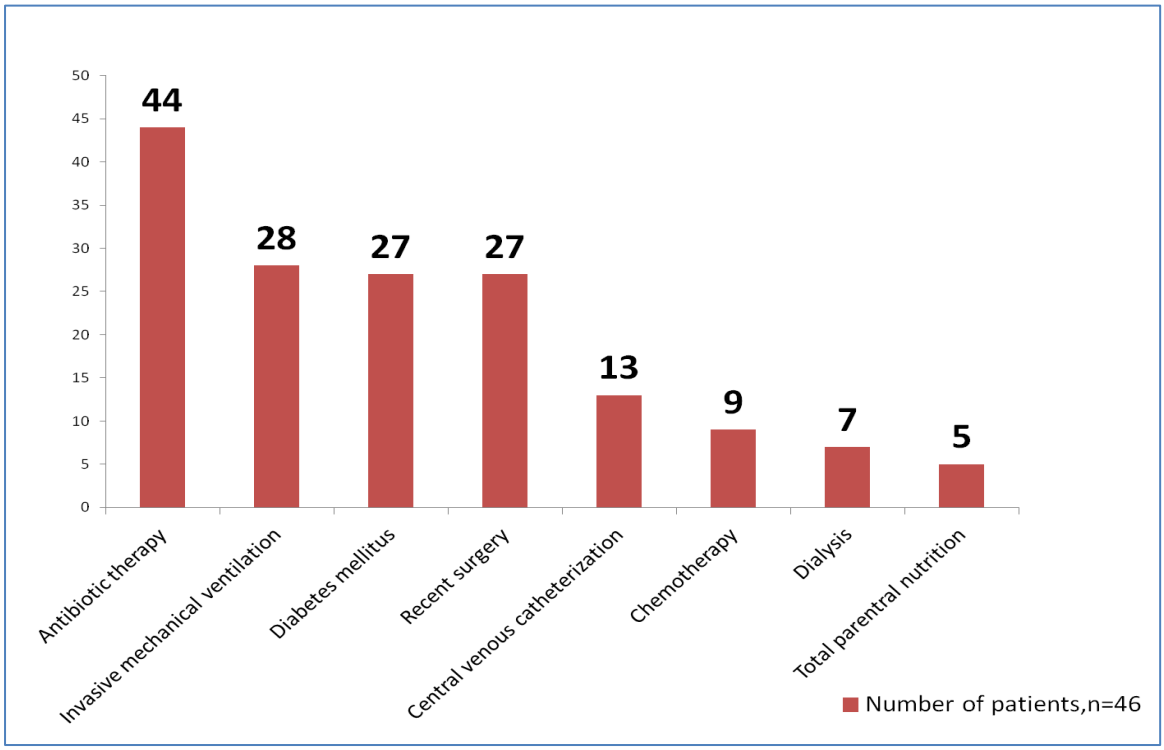


Figure IV: Mean serum procalcitonin levels in patients during candidemia episodes (n=56)

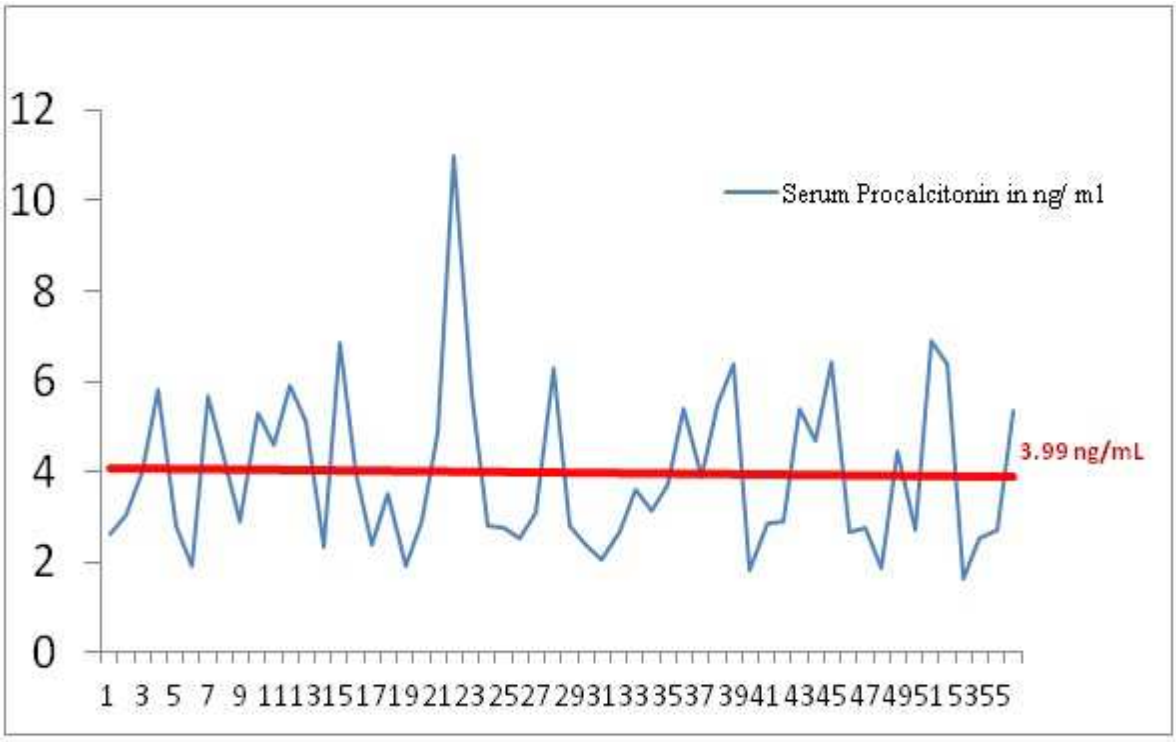


Table IV: Anti-fungal therapy instituted in patients after blood culture results

Anti-fungal	Number of patients, n=24
Fluconazole	13
Voriconazole	1
Caspofungin	2
Micafungin	1
Anidulafungin	2
Fluconazole escalated to Caspofungin	5

Table V: Clinical outcome in candidemic patients (n=46)

Anti-fungal	Patients treated, n=24	Patients not treated with anti-fungals n=22
Discharged	63%(15)	32%(7)
Death	37%(9)	59%(13)
Discharged against medical advice	-	9%(2)

studies from India.[4-7] *C. glabrata, C. haemulonii, C. parapsilosis, C. famata, C. guilliermondii & C. lusitaniae* were the other NAC isolates. Prophylactic anti-fungal therapy is not a routine practice in our institute. Restricted use of anti-fungals is practiced and anti-fungal therapy is initiated on evidence of positive fungal culture reports unless the patients fall in the high risk category of candidemia and are suffering from high-grade fever with negative bacterial blood cultures, anti-fungal therapy is initiated empirically in such cases. Fluconazole was the commonest targeted therapy, followed by caspofungin in this group of patients of our study. *C. haemulonii* (6) was a highly resistant species showing less activity against amphotericin-B and azoles which can be attributed to intrinsic resistance. The mortality rate of 47.8 % in our study on comparison to another recent study by Adhikar et al in our region in which it was 37 % has thus shown an increase in fatal cases of candidemia over the recent years, although this falls within the range of 10-49%.[5] Mortality in candidemic patients occurs due to multiple factors like age of the patient, underlying co-morbid conditions, timing of the treatment, concomitant infections and prompt administration of appropriate antibiotic or anti-fungal therapy. Therefore, only an attributable mortality can be determined if we compare these statistics with other suitable controls in a case-control study. As this was not a case-control study, only crude mortality could be determined. The role of serum procalcitonin in fungal sepsis is an interesting area of research, which may help to differentiate candidemia from bacterial sepsis.

The limitations of our study were that this was a single institution study with retrospective design. There is lack of uniformity in clinical practice with regards to empiric therapy based on local epidemiology; also there were small number of isolates of *C. haemulonii, C. parapsilosis, C. famata, C. guilliermondii* and *C. lusitaniae* with limited follow-up.

Assessing risk factors and more active screening can shorten the diagnostic delays in candidemia especially in the intensive care settings. A bedside scoring system- 'CANDIDA SCORE' as described by

Leon et al in patients admitted to the ICUs should be used by clinicians. Four independent risk factors: multi-focal Candida species colonization, surgery upon ICU admission, severe sepsis, and total parenteral nutrition (TPN) are considered in this scoring tool. The Candida score is obtained by adding the statistical weight of each risk factor: clinical sepsis (2 points), surgery (1 point), TPN (1 point), and multifocal colonization (1 point).[9] A Candida Score of >2.5 is associated with a >7-fold increase in the likelihood of documented Candida infection.[9]

Integrated effort on the part of all treating physicians and microbiologists to restrict risk factors especially the use of antibiotics, central venous lines, total parenteral nutrition, mechanical ventilation and long stay in the hospital especially in intensive care should be the primary goal in a preventive direction. Life threatening candidemia in patients admitted in tertiary set-ups for treatment of a primary disease can be prevented with close monitoring of species distribution of Candida and anti-fungal resistance like it is being done in few centres in India.[10] National guidelines for a rational empiric anti-fungal usage should be established for better treatment and outcome of patients with candidemia; more hospital-based epidemiological studies for comparison is also the need of the hour.

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Authors details:

1- **Corresponding author:** Registrar, Apollo Hospitals, Department of Microbiology, Bannerghatta Road, Bangalore-560076; **E-mail:** rakshakbhat@gmail.com

2- Consultant & Head, Department of Microbiology, Bannerghatta Road, Bangalore