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ORIGINAL ARTICLE

Time to positivity of blood cultures causing candidemia and its relation to mortality

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ABSTRACT

Background and Objectives: Early diagnosis of candidemia is of vital importance in reducing mortality and morbidity. The main objective of the study was to determine the TTP (Time to Positivity) of different species of *Candida* causing blood-stream infection and to see whether TTP can help differentiate *Candida glabrata* which is frequently fluconazole resistant from Fluconazole sensitive *Candida*.

Materials and Methods: TTP (Time to positivity) and AAT (Appropriate Antifungal therapy) were noted for Blood cultures becoming positive for *Candida*. Presence of Risk factors for candidemia like prolonged ICU stay, neutropenia, Total Parenteral Nutrition (TPN), use of steroids , broad spectrum antibiotics, use of Central Venous Catheter, Foleys catheter were also analyzed.

Results: The most frequent isolates were *Candida parapsilosis*, *Candida tropicalis* and *Candida albicans.* The median TTP for all *Candida* isolates in our study was 34 hours. The diagnostic sensitivity of TTP for detecting *C. glabrata* and *C. tropicalis* in patients with candidemia was 88% and 85% respectively. TTP showed that there was no difference in survival between TTP <24 hrs. and > 24hrs. Initiation of antifungal therapy <24 hours and > 24hrs after onset of candidemia had no association with survival.

Conclusion: Longer TTP maybe predictive of *C. glabrata* while shorter TTP may be predictive of *C. tropicalis.* In our study we found that fluconazole resistant *Candida* causing blood stream infection is quite unlikely if the TTP of the isolate is <48hrs.

Keywords: *Candida*; Fluconazole; Susceptibility; Resistance; Sensitivity

INTRODUCTION

Invasive candidiasis has become an important nosocomial infection and is mainly seen among patients with Central Venous Catheters (CVC), on broad spectrum antibiotics, on mechanical ventilation, prolonged ICU stay and on parenteral nutrition (1). Incidence of candidemia among ICU patients varies from 0.24 to 34.3 patients/1,000 ICU admissions and a high mortality rate of 35-75% (2-4). *Candida albicans* used to be the primary cause of candidemia but increase in infections caused by non-albicans species has therapeutic implications because some organisms are intrinsically resistant to at least one or more antifungal agents (5). Early diagnosis of candidemia is of vital importance in reducing mortality

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and morbidity. Non albicans species commonly causing blood stream infection (BSI) include *C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, Candida lusitaniae, Candida guilliermondii, Candida rugosa* and *Candida auris.*

Delay in the diagnosis of fungal infections is a common occurrence, and delayed initiation of appropriate antifungal therapy can lead to increased mortality. Availability of Continuous Monitoring Automated Blood Culture Systems (CMABS) in most laboratories has made early diagnosis of candidemia possible to a certain extent. Apart from self-monitoring of microbial growth, one of the main advantages with this equipment is the fact that it gives Time to positivity (TTP). TTP is defined as the time interval between start of blood culture bottle incubation to the blood culture positive alert signal (as documented by the monitoring system) (6). A study conducted in Uttar Pradesh by Verma et al. ranked *Candida* spp. eighth among all isolates from BSI (7).

According to other studies, *C. glabrata* which is usually resistant to fluconazole has a longer TTP when compared to other *Candida* (8). Studies in India showing TTP of different *Candida* species and comparing TTP of fluconazole resistant *Candida* to Pan sensitive *Candida* are very scanty. TTP has been used diagnostically and prognostically for bacteremia due to *Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumoniae* respectively in other studies (9-11). The main objective of the study was to determine the TTP of different species of *Candida* causing bloodstream infection and to determine whether TTP can differentiate *C. glabrata* which is frequently fluconazole resistant from the fluconazole sensitive *Candida*. This can help in earlier administration of the appropriate antifungal agent. The other objectives of the study was to assess the impact of TTP on clinical outcome, to assess the risk factors associated with these yeast isolates and to study the antifungal susceptibility of these yeast isolates.

MATERIALS AND METHODS

This is a five year retrospective study conducted from 2017-2021 in a 500 bedded tertiary care hospital after getting clearance from Ethical Committee (IEC/09/83/2019). Blood cultures were ordered for patients with clinical signs of sepsis as decided by the physician. Blood samples sent for culture were inoculated into one aerobic and one anaerobic bottle and immediately loaded into a BacT/ALERT 3D Microbial Detection System (bio-Me´rieux, Inc., Durham, NC, USA). When the blood culture system gave a positive signal, the bottles were taken out and Time to positivity was noted. TTP was defined as the time interval between start of blood culture bottle incubation to the blood culture positive alert signal (as documented by the monitoring system). Subcultures were made into Blood agar, MacConkey agar and Sabouraud's Dextrose agar plus a Gram smear was taken. Samples which showed presence of yeast like cells on Gram smear were included in the study. A total of 100 blood cultures which were positive for *Candida* were included in the study. Only one isolate per patient was included. Identification and antifungal susceptibility testing of the yeast was done by VITEK-2 Yeast Biochemical Card (bioMerieux, Inc.). Details of patients were collected from medical records which included age, gender, presence of comorbidities like diabetes, hypertension. Presence or absence of Risk factors for candidemia like prolonged ICU stay, neutropenia, Total Parenteral Nutrition (TPN), use of steroids, broad spectrum antibiotics, use of CVC, Foleys catheter were also analyzed.

Definitions. The timing of AAT (Appropriate Antifungal Therapy) was defined as the interval between the onset of candidemia and the administration of AAT. Infectious Diseases Society of America guidelines were used to determine appropriate doses of antifungals (5). The source of infection was identified based on clinical evidence of infection, regardless of whether causative organisms were recovered from the origin. CDC (Centre for Disease Control and Prevention) definition was used for identifying a case of catheter related blood stream infection. Gastrointestinal tract was considered as the source of candidemia only if patients had signs or symptoms related to the gastrointestinal tract prior to the onset of candidemia and did not have any other source as decided by physician. Neutropenia was defined as an absolute neutrophil count of <500/µl. These definitions were used in another study conducted by Kim et al. (12). *Candida* causing urinary tract infection is quite rare, hence urinary tract was identified as the source of *Candida* if the patient had signs and symptoms of UTI and the same *Candida* was isolated in two separate urine cultures and there was no other source of infection as decided by the physician.

Statistical analysis. Descriptive statistics included frequency and percentages of all categorical variables. Proportions were tested using Chi-square test. Time to Positivity (TTP) and Time to appropriate antibiotic therapy (TT-AAT) was summarized as median and Inter quartile range (IQR) and depicted using box-plots. Probability of survival was compared using Log-Rank test based on both TTP and TT-AAT. Level of significance used was 5%. The same was portrayed using Kaplan Meier Curves.

Evaluation of TTP to discriminate those resistant to fluconazole from those sensitive was done using the area under the curve (AUC) of a receiver-operator characteristic curve (ROC). The TTP was converted to binary using cut-offs based on Youden's J -Index. Validity of this cut points were assessed using Sensitivity, Specificity, Positive and Negative Predictive Values and by positive Likelihood Ratio. The above methods were used also to evaluate TTP as a diagnostic marker for each species and each source of infection. All analysis was done using SAS software, Version 9.4.

RESULTS

Among the 100 isolates included in the study, 68 were isolated from male patients, 32 were isolated from female patients. The yeasts isolated in our study and their frequency is shown in Table 1. The most frequent isolates were *C. parapsilosis, C. tropicalis* and *C. albicans*. The distribution of yeasts in different age groups are shown in Table 2. The demographic and clinical characteristics of patients with candidemia are shown in Table 3. Diabetes mellitus (47%) and Hypertension (49%) were the most common comorbidities. The CVC (48%) and Urinary tract (22%) were the major sources of candidemia. Among the 22 patients in whom urinary tract was mentioned as source of candidemia, 21 (95.5%) were on Foleys Catheter. Antifungal susceptibility profiles were available for 83% of the isolates (83/100) including 27 *C. tropicalis*, 27 *C. parapsilosis*, 21 *C. albicans* and 8 *C. glabrata* (Table 4). Most of these isolates were pan-susceptible except 3 isolates of *C. parapsilosis* (2 isolates resistant to amphotericin B, 1 isolate resistant to flucytosine), 1 isolate each of *C. albicans* and *C. tropicalis* (resistant to caspofungin) and 5 isolates of *C. glabrata* (1 isolate resistant to voriconazole and 4 isolates resistant to caspofungin). First-line antifungal therapy included fluconazole (68/100, 68%), amphotericin B (7/100, 7%), voriconazole (5/100, 5%) and echinocandins (7/100, 7%). AAT was started in majority of the patients (84/100, 84%) within 24 hours of onset of candidemia.

Table 1. Frequency of occurrence of yeast isolates in blood culture

Type of Yeast	No of isolates (%)
C. albicans	21 (21)
C. tropicalis	27 (27)
C. parapsilosis C.	27 (27)
glabrata	8 (8)
Trichosporon species	4 (4)
C. krusei	3 (3)
K. ohmeri	2 (2)
C. utilis	2 (2)
C. rugosa	1 (1)
C. auris	1 (1)
C. famata	1 (1)
C. kefyr	1 (1)
C. pelliculosa	1 (1)
C. norvegensis	1 (1)
Total Number of isolates	100 (100)

Time to positivity (TTP) for *Candida* isolates. The median TTP for all *Candida* isolates in our study was 34 hours (Interquartile range (IQR) 21.75-50 h). The median TTP varied for each species as shown by median (IQR). They were 42h (34-57h) for *C. albicans*, 20.5h (19-23.5h) for *C. tropicalis*, 45h (34-58h) for *C. parapsilosis*, 70.5h (60.5-73h) for *C. glabrata* and 25h (15-29h) for *C. krusei*. The TTP of different *Candida* species is shown by Box plot graph in Fig. 1.

ROC curves were plotted to evaluate the ability of TTP to detect fluconazole resistance in patients with candidemia (Fig. 2). Overall, the diagnostic sensitivity of TTP for detecting fluconazole resistance in patients with candidemia was 50% (95% CI: 23%-77%), the specificity was 78% (95% CI: 68%-86%), the positive predictive value was 27% (95% CI: 12%-48%) and the negative predictive value was 91% (95% CI: 81%-96%), with a TTP cut-off value of ≥48h.

ROC curves were plotted to evaluate the ability of TTP to identify *C. glabrata* isolates in patients with candidemia (Fig. 3). The diagnostic sensitivity of TTP for detecting *C. glabrata* in patients with candidemia was 88% (95% CI 0.47-1.00), the specificity was 82% (95% CI 0.72-0.89), the positive predictive value was

Table 2. Distribution of yeasts by age group

Age	N (%)	*C. tropicalis*	*C. albicans*	*C. parapsilosis*	*C. glabrata*	*Trichosporon*	*C. krusei*	Others*
0-1 month	5 (5)	Nil	1 (4.8)	1 (3.7)	Nil	Nil	2 (66.7)	1 (10)
>1- 12 month	4 (4)	Nil	Nil	3 (11.1)	Nil	Nil	1 (33.3)	Nil
>1-16 years	4 (4)	1 (3.7)	Nil	1 (3.7)	Nil	Nil	Nil	2 (20)
17-40 years	4 (4)	3 (11.1)	Nil	1 (3.7)	Nil	Nil	Nil	Nil
41-60 years	25 (25)	7 (25.9)	6 (28.6)	8 (29.6)	1 (12.5)	Nil	Nil	3 (30)
61-80 years	49 (49)	16 (59.3)	11 (52.4)	10 (37)	4 (50)	4 (100)	Nil	4 (40)
>80 years	9 (9)	Nil	3 (14.3)	3 (11.1)	3 (37.5)	Nil	Nil	Nil

Others*- *K. ohmeri, C. utilis, C. rugosa, C. auris, C. famata, C. kefyr, C. pelliculosa, C. norvegensis*
N- Total number

Table 3. Association of demographic and clinical characteristics of patients with candidemia with mortality at 6 weeks

Variables	Total patients (n=100)	Survivors (n=63)	Died (n=37)	P value
Age (years), median (IQR)		63 (47-70)	66 (55-77)	
Male gender (%)	68 (68)	43 (68.3)	25 (67.6)	0.9
Comorbidities n (%)				
Diabetes Mellitus	47 (47)	26 (41.3)	21 (56.8)	0.1
Hypertension	49 (49)	30 (47.6)	19 (51.4)	0.7
Chronic Kidney Disease	26 (26)	15 (23.8)	11 (29.7)	0.5
Chronic Liver Disease	7 (7)	2 (3.2)	5 (13.5)	0.05
Malignancy	20 (20)	10 (15.9)	10 (27)	0.2
Risk Factors				
ICU stay	72 (72)	36 (57.1)	36 (97.3)	<0.0001
Neutropenia	29 (29)	17 (27)	12 (32.4)	0.6
Corticosteroid	63 (63)	33 (52.4)	30 (81.1)	0.0041
Total Parenteral Nutrition	57 (57)	22 (34.9)	35 (94.6)	<0.0001
Mechanical Ventilation	54 (54)	23 (36.5)	31 (83.8)	<0.0001
CVC	87 (87)	51 (81)	36 (97.3)	0.02
Foleys Catheter	76 (76)	41 (65.1)	35 (94.6)	0.0008
Recurrent Surgery	18 (18)	11 (17.5)	7 (18.9)	0.9
Broad Spectrum Antibiotics	96 (96)	60 (95.2)	36 (97.3)	0.6
Source of Candidemia, n (%)				
GIT	15 (15)	10 (15.9)	5 (13.5)	0.8
UTI	22 (22)	13 (20.6)	9 (24.3)	0.7
CVC	48 (48)	30 (47.6)	18 (48.6)	0.9
Unknown	15 (15)	10 (15.9)	5 (13.5)	0.8
Candida species, n (%)				
C. albicans	21 (21)	9 (14.3)	12 (32.4)	0.03
C. tropicalis	27 (27)	14 (22.2)	13 (35.1)	0.2
C. parapsilosis	27 (27)	25 (39.7)	2 (5.4)	0.0002
C. glabrata	8 (8)	5 (7.9)	3 (8.1)	0.98
C. krusei	3 (3)	3 (4.8)	0	0.2
C. auris	1 (1)	1 (1.6)	0	0.4
Other Species	13 (13)	6 (9.5)	7 (18.9)	0.2
TTP ≤ 24 hours, n (%)	34 (34)	19 (30.2)	15 (40.5)	0.3
First Line Drugs, n (%)				

Table 3. Continuing ...

Fluconazole	68 (68)	47 (74.6)	21 (56.8)	0.06
Amphotericin B	7 (7)	3 (4.8)	4 (10.8)	0.3
Echinocandin	7 (7)	3 (4.8)	4 (10.8)	0.3
Voriconazole	5 (5)	3 (4.8)	2 (5.4)	0.9
None	13 (13)	7 (11.1)	6 (16.2)	0.5
Timing of AAT, n (%)				
<24 hrs	84 (84)	55 (87.3)	29 (78.4)	0.2
24-48hrs	2 (2)	1 (1.6)	1 (2.7)	0.7
48-72hrs	0	0	0	
>72hrs	14 (14)	7 (11.1)	7 (18.9)	0.3

IQR- Interquartile range, ICU- Intensive Care Unit, CVC- Central Venous Catheter
GIT- Gastrointestinal Tract, UTI- Urinary Tract Infection,TTP- Time to Positivity, AAT- Appropriate Antifungal Therapy

Table 4. Antifungal susceptibility profile

Drugs	***C. parapsilosis*** **n (%)**	***C. albicans*** **n (%)**	***C. tropicalis*** **n (%)**	***C. glabrata*** **n (%)**
Amb				
≤0.25 (S)	8 (29.6)	5 (23.8)	20 (74.1)	1 (12.5)
0.5-1 (S)	17 (63)	16 (76.2)	7 (25.9)	7 (87.5)
>2 (R)	2 (7.4)			
Flucytosine				
<4-(S)	24 (88.9)	20 (95.2)	27 (100)	8 (100)
4-16 (I)	2 (7.4)	1 (4.8)		
>16 (R)	1 (3.7)			
Fluconazole				
≤2 (S)	27 (100)	21 (100)	27 (100)	
4 (SDD)				
≥8 (R)				
≤32 (SDD)				
≥64 (R)				8 (100)
Voriconazole				
≤0.12 (S)	27 (100)	21 (100)	27 (100)	5 (62.5)
0.25-0.5 (SDD)				2 (25)
≥1 (R)				1 (12.5)
Caspofungin				
≤0.12 (S)	2 (7.4)	20 (95.2)	25 (92.6)	3 (37.5)
0.25 (I)			1 (3.7)	1 (12.5)
≥0.5 (R)			1 (3.7)	4 (50)
0.25 (S)	11 (40.7)			
0.5 (I)				
≥1 (R)				
≤2 (S)	14 (51.9)			
4 (I)				
≥8 (R)		1 (4.8)		

Amb- Amphotericin B, S- Susceptible, I- Intermediate, R-Resistant
SDD- Susceptible Dose Dependent

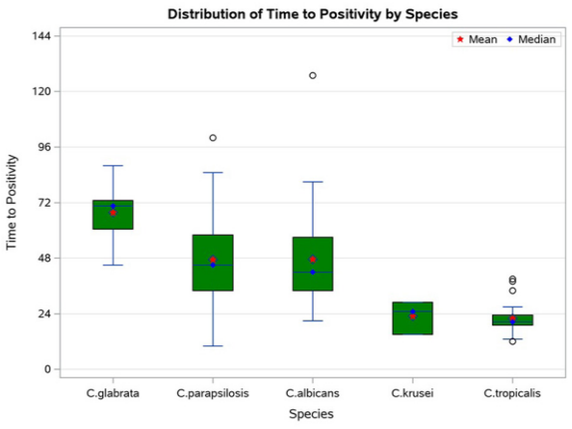

Fig. 1. Time to positivity of different *Candida* species

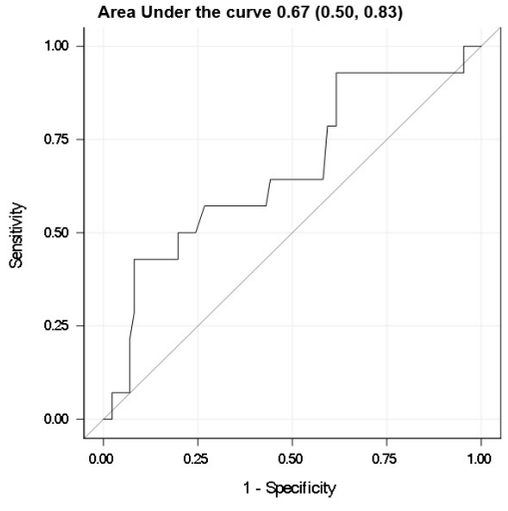

Fig. 2. ROC Curve to discriminate resistant vs sensitive fluconazole using time to positivity

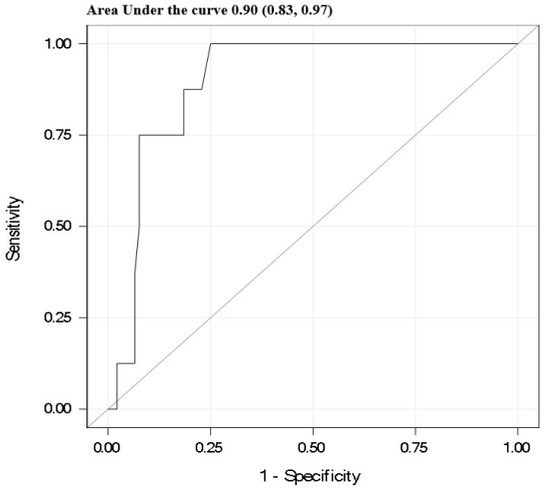

Fig. 3. ROC curve to discriminate *Candida glabrata* vs the others using time to positivity

29% (95% CI 0.13-0.51) and the negative predictive value was 99% (95% CI 0.93-1.00), with a TTP cut-off value of ≥51h.

ROC curves were plotted to evaluate the ability of TTP to identify *C. tropicalis* isolates in patients with candidemia (Fig. 4). The diagnostic sensitivity of TTP in identifying *C. tropicalis* isolates in patients with candidemia was 85% (95% CI 0.66-0.96), the specificity was 85% (95% CI 0.75- 0.92), the positive predictive value was 68% (95% CI 0.49-0.83) and the negative predictive value was 94% (95% CI 0.85-0.98), with a TTP cut-off value of ≤ 24h. ROC curves were also plotted to evaluate the ability of TTP to predict CVC as the source of candidemia (Fig. 5). The diagnostic sensitivity of TTP in predicting CVC as the source of candidemia was 90% (95% CI 0.77-0.97), the specificity was 23% (95% CI 0.13- 0.37), the positive predictive value was 52% (95% CI 0.41-0.63) and the negative predictive value was 71% (95% CI 0.44-0.90), with a TTP cut-off value of <57h.

The mortality rate at 6 weeks after the onset of candidemia was 37% (37/100). Clinical features of patients who survived and died are compared in Table 3. The two groups did not differ in terms of comorbidities or source of candidemia. With regards to risk factors for a *Candida* infection, the patients who died were more likely to stay in the ICU at the onset of candidemia and to have received total parenteral nutrition, corticosteroid, mechanical ventilation, CVC and Foleys catheter (P<0.05). Among the *Candida* species, isolation of *C. albicans* was significantly associated with mortality, whereas the isolation of *C. parapsilosis* was significantly associated with survival (P<0.05).Among the antifungal agents, fluconazole was more frequently administered to survivors when compared to the patients who expired. Survival curves based on Time to positivity showed that there was no difference in survival between TTP <24 hrs and > 24hrs (Fig. 6). Survival curves based on appropriate antibiotic therapy showed that intiation of antifungal therapy <24 hours and > 24hrs after onset of candidemia had no association with survival (Fig. 7).

DISCUSSION

Among the *Candida* species, isolation of *Candida* non *albicans* was more frequent when compared to *C. albicans* (73% vs 21%). The most frequent isolate among the non-albicans species were *C. tropicalis*

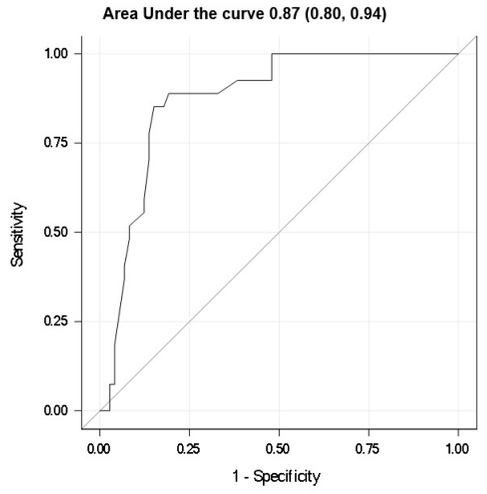

Fig. 4. ROC Curve to discriminate *Candida* tropicalis vs others using time to positivity

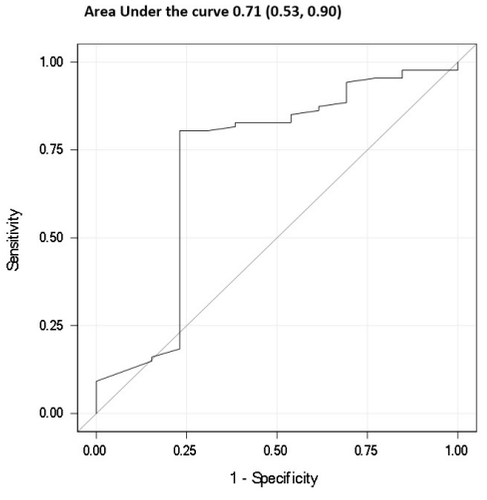

Fig. 5. ROC Curve to discriminate CVC (Central Venous Catheter) vs others using time to positivity

(27%) and *C. parapsilosis* (27%). The types of yeast isolates in a hospital differ according to geographical area, type of patient population and risk factors. Higher prevalence of *Candida* non-*albicans* in blood stream infections was also reported by Caggiano et al. (13). A study conducted in Korea by Lee et al. however found *C. albicans* as the most common isolate causing infection (14). Studies conducted in India by Chander et al. and Chakrabarti et al. showed that *Candida* non-*albicans* was more common with *C. tropicalis* being the predominant isolate, which was concordant with the findings in our study (15, 16). Our study reported higher occurrence of yeast in 41-60 years (n=25) and 61-80 years (n=49) age group.

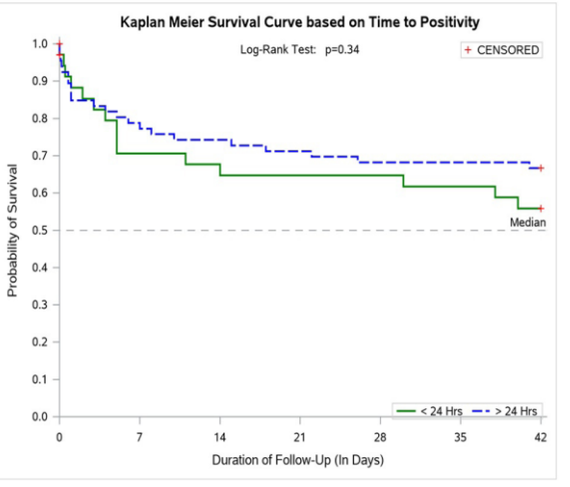

Fig. 6. Survival curves based on time to positivity

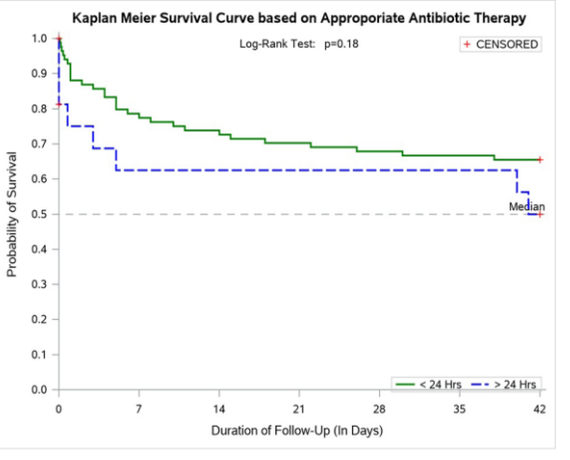

Fig. 7. Survival curve based on appropriate antibiotic therapy

This finding was similar to the study conducted by Diekema et al. who reported higher incidence of candidemia in >64 years (46%) age group (17). Chang et al. also reported that majority of patients (55.2%) who had candidemia were adults (18). In our study the majority (87.5%) of the *C. glabrata* isolates were from patients in the >60 years age group.

In this study, among the different *Candida* species causing candidemia, *C. tropicalis* had the shortest TTP and *C. glabrata* had the longest TTP. Study conducted by Lai et al. and Ben-Ami et al. also reported similar findings with respect to TTP of *C. glabrata* and *C. tropicalis* (19, 20). Study conducted by Park et al. reported mean TTP of *C. glabrata*, *C. albicans*, *C. parapsilosis* and *C. tropicalis* to be 50.8 hrs, 37.5 hrs, 35.3 hrs and 22.4 hrs respectively (21). Study conducted in India by Butta et al. also reported that

TTP of *C. tropicalis* and *C. glabrata* was found to be significantly shorter and longer respectively when compared to other isolates (22). The findings in our study as well as those in other studies suggest that while TTP differs among different species of *Candida*, shorter TTP may be predictive of candidemia caused by *C. tropicalis* and longer TTP maybe predictive of candidemia caused by *C. glabrata*.

Drug resistance patterns differ among each species of *Candida* and the study conducted in India by Kaur et al. showed that fluconazole resistance among isolates causing candidemia over a twenty year period was ranging from 7.4-8% (23). It is of extreme importance for the clinicians to know the species of *Candida* so that appropriate antifungal agents can be administered. In our study we analyzed whether TTP could differentiate between fluconazole resistant and sensitive *Candida*. We found out that the negative predictive value (NPV) of TTP for identifying fluconazole resistance in patients with candidemia was 91% with a TTP cut-off value of >48h, indicating that fluconazole resistant *Candida* is quite unlikely if the TTP of the isolates is <48h. The high negative predictive value implies that it is a useful supplementary test for excluding fluconazole resistant candidemia. This is particularly useful because it normally takes 48-72 hrs from the time blood culture becomes positive for *Candida* to the final species identification. *C. glabrata* is one of the more common fluconazole resistant *Candida* and we analyzed whether TTP could accurately predict whether the candidemia is caused by *C. glabrata*. The NPV of TTP to predict *C. glabrata* blood stream infection was 99% if a TTP cut off of >48 hours is used. This indicates that the probably of *C. glabrata* blood stream infection is extremely minimal if the TTP is <48h. Lai et al. found that *C. glabrata* fungaemia can be excluded if the TTP of the isolates is <27.7 hrs (19). The NPV of TTP for diagnosing *C. tropicalis* candidemia in our study was 94% if a TTP cut off of ≤24hrs was used. This indicates that the probability of *C. tropicalis* fungaemia is minimal if the TTP is > 24 hrs. In our study it was shown that TTP could predict Central venous Catheter related candidemia with a sensitivity of 90% and NPV of 71% if the TTP cut off used was ≤57 hrs.

In our study it was shown that the TTP and early or late initiation of antifungal therapy had no association with mortality. This was discordant with the study conducted by Kim et al. who showed that mortality rate at 6 weeks was significantly higher in the group with a TTP ≤ 24 hrs when compared to those with TTP ≥ 24 hrs. Kim et al. also showed that mortality at 6 weeks was higher in those patients where AAT was initiated > 72 hrs when compared to those in whom AAT was initiated< 72 hrs after onset of candidemia. Although the reason for discrepancy in our findings is not clear, it may have been influenced by various factors including study design and study population. Another study conducted by Nunes et al. showed longer TTP was associated with higher mortality for *C. albicans* blood stream infections (24). In our study, stay in the ICU at the onset of candidemia, total parenteral nutrition, corticosteroid, mechanical ventilation, CVC and Foleys catheter were significantly associated with mortality. Similar findings were reported by Kim et al. (12). In our study isolation of *C. albicans* from the blood was significantly associated with mortality. Study conducted by Hirano et al. also showed higher 30 day mortality rate (42.9%) with *C. albicans* (25).

In this study *C. albicans* and *C. tropicalis* showed no resistance for azoles and amphotericin B. This was similar to the findings in the study conducted by Butta et al. (22). Our study showed that *C. parapsilosis* showed 7.4% resistance for amphotericin B where as Butta et al. reported resistance of 6.25% (22). Study conducted by Lotfali et al. showed that 1.7% isolates of *C. parapsilosis* were amphotericin resistant (26). In our study there was no fluconazole or echinocandin resistance in *C. parapsilosis*, while Chakrabarti et al. reported resistance rates of 2%, 4% and 0% for amphotericin B, fluconazole and echinocandins (15). In this study echinocandin resistance in *C. glabrata* was 50% which is a grave concern and the detection of echinocandin resistance in *C. tropicalis* (3.7%) and *C. albicans* (4.8%) is concerning. However the mechanism of echinocandin resistance in various species of *Candida* could not be ascertained. Increasing echinocandin resistance was reported by Alexander et al. who found that during that course of a ten year study, the echinocandin resistance in *C. glabrata* increased from 4.9 to 12.3% (27). Emerging echinocandin resistance in *C. albicans* was also reported by Coste et al. (28). In 2018 a study conducted by Khan et al. showed emerging echinocandin resistance in *C. tropicalis* (29).

Finally, this study had a few limitations. TTP can be influenced by several factors like volume of blood drawn, incubation conditions and time from spec-

imen inoculation to receipt in the laboratory, prior use of antifungal agents. The variation in the findings caused by these factors would have been minimal due to the fact that volume of blood drawn was similar and incubation conditions were stable due to the usage of automated system. Another potential drawback is that number of isolates of *C. glabrata* and other fluconazole resistant *Candida* were very few when compared to others.

To the best of our knowledge this is the second study from India, analyzing the TTP of different *Candida* species. We found that *C. glabrata* causing blood stream infection is quite unlikely if the TTP of the isolate is <48 hrs. Eventhough the study showed that fluconazole resistant candidemia is quite unlikely if the TTP of the isolates is <48h, a much larger number of fluconazole resistant *Candida* needs to be analysed before coming to a conclusion. TTP and early or late initiation of antifungal therapy had no association with mortality. Echinocandin resistance in *C. glabrata* (50%) and emerging echinocandin resistance in *C. tropicalis* (3.7%) and *C. albicans* (4.8%) is a grave concern and needs immediate attention.

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