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RESEARCH ARTICLE OPEN ACCESS

Monitoring the Spectrum of Candidemia and its Anti-fungal Resistance in A Tertiary Care Centre – An Emerging Global Alarm

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Abstract

Candidemia is caused by healthcare-associated bloodstream infections ranking as a fifth cause especially in the United States as well as in European countries among intensive care units (ICUs). Despite ongoing advances in diagnostics and medical interventions, it remains associated with high mortality rates, along with the prolonged duration of hospitalization and elevated health care costs. The aim of the study is to characterize *Candida* species and to investigate the antifungal resistance pattern from blood samples in a Tertiary Care centre. 53 known *Candida* isolates from blood samples of various wards and ICUs were collected. All isolates are processed and speciated by the conventional identification method demonstrating its various virulence factors phenotypically and AFST patterns were studied. In the present study, among 53 *Candida* isolates, 25 (47.16%) *C. tropicalis* is a predominant pathogen followed by 11 (20.75%) *C. parapsilosis*, 9 (16.98%) *C. albicans*, 4 (7.54%) *C. glabrata* and 4 (7.54%) *C. krusei*. Phospholipase activity was observed in 30 (56.60%) isolates, 36 (67.92%) showed hemolysin production. AFST showed 15 (28.30%) isolates being resistant to Fluconazole and 2 (3.77%) showed resistance to Amphotericin B. The prevalence of candidemia was high, the fatality rate was alarming and non-*albicans Candida* species were predominant and fluconazole was the least effective drug owing to the high level of resistance.

Keywords: Candidemia, Intensive Care Units, Candida Species, Anti-Fungal Susceptibility Test

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Abbreviations: BSI – Blood stream infection, CNA – Candida Non-albicans, CLSI - Clinical and Laboratory Standards Institute, CMA – Corn meal agar, EPIC- Extended Prevalence in Intensive Care, IC – Invasive candidiasis, ICU – Intensive care unit, MIC – Minimal inhibitory concentration, SDA - Sabouraud Dextrose Agar

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INTRODUCTION

Invasive candidiasis (IC) refers to candidemia and deep-seated infection which involves peritonitis, osteo¬myelitis, or intra-abdominal abscess that has a high morbidity and mortality rates ranging from 29% to 76%.[1] IC ranks in top positions on the list of invasive fungal infections globally and as of now, candidemia ranks up in 4th place and ranks as the 7th most prevalent infection among healthcare-associated infections.[2] Several studies from India reported that nearly 6%-8% of candidemia cases have resulted in the increased isolation rate of *Candida* Non-*albicans* (CNA) species with high mortality rates and antifungal resistance.[3] Approximately 92% of candidemia cases are encountered by the five most prevalent *Candida* pathogens in humans involve *Candida albicans, Candida krusei, Candida glabrata, Candida tropicalis,* and *Candida parapsilosis*.[4] However, an exceptional position was held by *Candida albicans* which constitute around 40% and almost 60% was contributed by CNA species.[5] But several individual studies from India reported that nearly 6%-8% of candidemia cases have resulted in the increased isolation rate of CNA species, of which the most predominant species isolated was *C. tropicalis*. Incidence rates have been increasingly observed despite the periodic use of central venous catheters, antibiotics, and especially in ICUs among immunocompromised patients, abdominal surgery, and those requiring invasive procedures and devices.[6] In line with the prevalence study of Extended Prevalence in Intensive Care (EPIC) II-point, the incidence of candidemia accounts for about 17% and ranks as the 3rd most common cause of infection in ICUs all over the world.[7]

Recently, the rise of multidrug-resistant *Candida auris* has also occurred as a healthcare-associated infection across the globe as they are very difficult to identify by conventional methods and it shows a much high rate of resistance than other *Candida* species.[8] Notably, azole resistance mechanisms in *Candida* species are mainly moderated by the presence of certain amino acid substitutions in *ERG11* that would result in the reduction of affinity among azoles in order to target the drug, besides overexpression of efflux pumps.[9] Several studies had proven that the predominant azole resistance mechanisms are due to the presence of efflux pumps in all *Candida* species. However, the increase of fluconazole-resistant strains in healthcare centres constitutes a serious risk of cross-infection among inpatients.[10] Therefore, understanding the intrinsic mechanisms of fluconazole resistance is an essential portion of managing antifungal agents for treatment. Therefore, we have focused on characterising the isolated *Candida* species and identifying fluconazole resistance patterns among candidemia patients.

MATERIALS AND METHODS

A cross-sectional study was carried out in a tertiary care centre and ethical approval (2896/IEC/2021) was obtained from the Institute Ethical Committee (Human Studies). A total of 957 blood samples were received from suspected septicemia patients in various wards and intensive care units (ICUs). Bottles flagged positive signals were gram stained directly. The routine culture was performed onto blood agar and MacConkey agar to isolate bacteria and on Sabouraud Dextrose Agar (SDA) for fungal isolation and incubated at 25˚C and 37˚C for 24 to 48 hours. The plates were examined macroscopically and microscopically. The bacteria were identified by using standard microbiological investigations. Further, the yeasts were recognized by standard mycological investigations and as follows Gram stain, Germ tube test, Chlamydospore formation on Corn meal agar (CMA) (Hi-Media Laboratories), colony characteristics on *Candida* CHROMagar (Hi-Media Laboratories), sugar fermentation test, sugar assimilation test (sugars disc - Hi-Media Laboratories), urease test (Hi-Media Laboratories), and methods to study virulence factors - Phospholipase activity, Biofilm formation, Hemolysis production were done. According to Clinical and Laboratory Standards Institute (CLSI) guidelines, an antifungal susceptibility test was performed for all the isolates on Cation-adjusted Muller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue (Hi-Media Laboratories) by Kirby Bauer disk diffusion methods. Antifungal disks were tested such as Amphotericin B (100 U), itraconazole (10 µg) and Fluconazole (25 µg) (Hi-Media Laboratories). Additionally, broth microdilution M27-A3 was

performed on resistant isolates to identify Minimal inhibitory concentration (MIC) values, and results were interpreted (CLSI M27-S4 supplement for yeasts).

RESULTS

A total of 957 blood samples of suspected septicemia patients were obtained from various wards and ICUs, 367 were flagged positive for various organisms (Table 1). Out of them, 212 (57.77%) were positive for Gram-positive cocci, 102 (27.79%) were Gram-negative *bacilli*; 53 (14.44%) shows gram positive budding yeast cells (Figure 1). Notably, most of the candidemia cases were obtained from ICUs than wards, and the occurrence of several predisposing factors among those who are presenting candidemia and without candidemia during this study period was analyzed and compared. One of the predominant risk factors related to candidemia was the use of central line catheters and the utilization of a wide array of antimicrobial agents. Also, the prolonged ICU stay beyond 15 days was also closely associated with the frequency of candidemia among the patients.

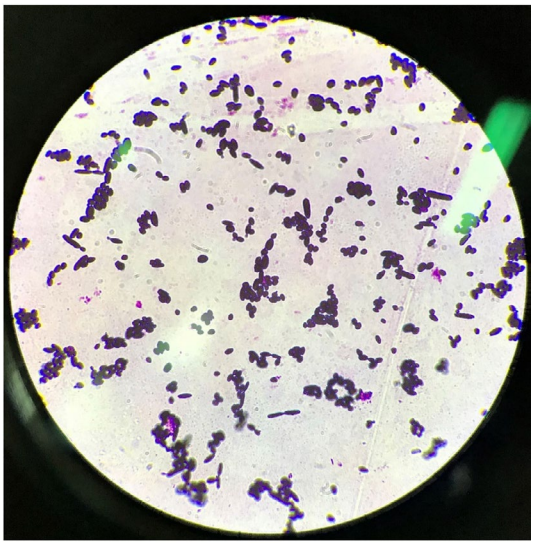

Figure 1. Gram Staining Showing Budding Yeast Cells

Table 1. Culture positive distribution (n=367)

Organisms	Total positive	Percentage (%)
Gram positive cocci	212	57.77%
Gram negative bacilli	102	27.79%
Candida species	53	14.44%
Total	367	100%

Out of 53 *Candida* isolates, 9 (16.98%) isolates were positive for the germ tube test whereas 44 (83.02%) isolates did not show positivity. Among them, 25 (47.16%) *C. tropicalis* is a predominant pathogen isolated followed by 11 (20.75%) *C. parapsilosis*, 9 (16.98%) *C. albicans*, 4 (7.54%), *C. glabrata*, and 4 (7.54%) *C. krusei*. A urease test was performed and all the isolates gave negative results (Table 2 and Figure 2).

Virulence factors interpretation among 53 *Candida* isolates, 30 (56.60%) were demonstrated phospholipase activity (Table 3), out of which 18 (60%) were *C. tropicalis* followed by 7 (23.33%) were *C. albicans*, 3 (10%) were *C. glabrata*, 1 (3.33%) isolate of *C. krusei* and 1 (3.33%) isolate of *C. parapsilosis*. Among 53 *Candida* isolates, 36 (67.92%) showed hemolysin production (Table 4 and Figure 3), out of 36 isolates, 22 (61.11%) of *C. tropicalis* followed by 8 (22.22%) *C. albicans*, 3 (8.33%), *C. glabrata*, 2 (5.56%) *C. parapsilosis* and 1 (2.77%) *C. krusei*. Biofilm production was performed on all the 53 isolated *Candida* species by spectrophotometric method and Congo red agar method (Table 5), 11 (20.75%) *Candida* isolates produced biofilm by spectrophotometric method, and 7 (13.20%) *Candida* isolates produced biofilm in Congo red agar plate method.

Antibiogram shows that 17 *Candida* isolates exhibited a resistance pattern. Of them, a high level of resistance was observed to Fluconazole 15 (28.30%) in contrast to 2 (3.77%) isolates that were recognized to be resistant to

Table 2. Species-wise distribution of *Candida* isolates (n=53)

Candida species	Total No. of isolates	Percentage (%)
Candida albicans	9	16.98%
C. tropicalis	25	47.16%
C. glabrata	4	7.54%
C. krusei	4	7.54%
C. parapsilosis	11	20.75%
Total	53	100%

Amphotericin B. Notably, all strains of *C. glabrata* were sensitive to Amphotericin B and 2 (50%) were found to be resistant to Fluconazole, whereas all *C. albicans* strains were susceptible to Amphotericin B and 1 (11.11%) showed resistant to Fluconazole. Among *C. tropicalis*, 19 (76%) were resistant to Fluconazole and 24 (96%) were sensitive to Amphotericin B and all the isolates of *C. krusei* were sensitive to Amphotericin B and 3 (75%) were resistant to Fluconazole. In the present study, 43(81.13%) isolates were sensitive to Itraconazole, and 10 (18.86%) isolates have resulted as resistant to Itraconazole mostly by *C. albicans* which was in agreement with the previous study findings (Table 6 and Figure 4).

MIC for fluconazole-resistant *Candida* isolates showed that 5 (33.33%) shows MIC of ≤ 2 µg/mL, 7 (53.33%) were in the intermediate category with MIC of 4-32 µg/mL and 2 (13.33%) showed a higher MIC of ≥ 64 µg/mL. A higher MIC of ≥ 64 µg/mL was observed in 1 isolate of *C. glabrata*. 1 isolate of *C. krusei* showed a high MIC

Figure 2. *Candida* species on *Candida* CHROMAGAR

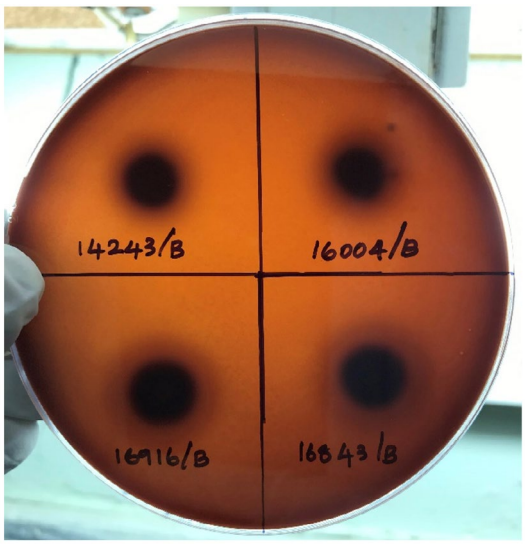

Figure 3. Hemolysin Production of Various *Candida* Isolates

Figure 4. Antifungal Susceptibility Testing Showing Fluconazole resistant

Table 3. Phospholipase activity among *Candida* species (n=53)

Candida species	Total No. of isolates	Phospholipase activity among *Candida* species (%)
C. albicans	9	7 (23.33%)
C. tropicalis	25	18 (60%)
C. glabrata	4	3 (10%)
C. krusei	4	1 (3.33%)
C. parapsilosis	11	1 (3.33%)
Total	53	30 (56.60%)

Table 4. Haemolysin production among *Candida* species (n=53)

Candida species	Total No. of isolates	Haemolysin production among *Candida* species (%)
C. albicans	9	8 (22.22%)
C. tropicalis	25	22 (61.11%)
C. glabrata	4	3 (8.33%)
C. krusei	4	1 (2.77%)
C. parapsilosis	11	2 (5.56%)
Total	53	36 (67.92%)

of ≥ 64 µg/mL, which was excluded from this study as they are intrinsically resistant to fluconazole (Table 7).

DISCUSSION

Globally, candidemia ranks top position among invasive fungal infections. The incidence of candidemia has been evolving due to the rise of CNA spp. and the emergence of antifungal drug resistance with the aid of a growing population. This cross-sectional study highlights the occurrence of candidemia which accounts for about 53 of the 367 confirmed septicemia cases with a predominance of 14.4%. Similar results were reported by Gupta

Table 5. Comparison of various methods on biofilm formation in *Candida* spp (n=53)

Candida species	Total No. of isolates	ELISA Method	Congo red agar plate method
C. albicans	9	3 (27.27%)	2 (28.57%)
C. non-*albicans*	44	8 (72.73%)	5 (71.43%)
Total	53	11 (20.75%)	7 (13.20%)

Table 6. Antifungal susceptibility pattern of *Candida* isolates by Kirby Bauer disc diffusion method (n=53)

Candida species	FLC		ITR		AMP B	
	S	R	S	R	S	R
C. albicans (n=9)	7	1	3	6	9	0
C. tropicalis (n=25)	19	5	24	1	24	1
C. glabrata (n=4)	2	2	3	1	3	0
C. krusei (n=4)	1	3	3	1	4	0
C. parapsilosis (n=11)	7	4	10	1	9	1
Total (n=53)	38	15	43	10	51	2
Percentage (%)	71.70%	28.30%	81.14%	18.86%	92.23%	3.77%

Table 7. MIC of Fluconazole-resistance *Candida* spp (n=15)

Candida species	MIC of Fluconazole (0.25- ≥ 64 µg/mL)								
	≥ 64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	≤ 0.25 µg/mL
C. albicans (n=1)	-	-	-	-	-	-	1	-	-
C. tropicalis (n=5)	-	1	2	1	-	1	-	-	-
C. glabrata (n=2)	1	1	-	-	-	-	-	-	-
C. krusei (n=3)	1	1	-	1	-	-	-	-	-
C. parapsilosis (n=4)	-	-	-	-	1	1	2	-	-

et al.,[11] which show the prevalence of candidemia of about 16% among suspected septicemia cases. Several other studies reported a candidemia prevalence of 19% and 17.3% among a population of pediatric patients in ICUs with BSIs. Notably, the occurrence of CNA spp over *C. albicans* has become significant because over 70% of BSIs were obtained by CNA spp. However, the occurrence of candidemia in ICUs is estimated at around 33%-50%. In this cross-sectional study, the prevalence of candidemia in ICUs was reported to be 39.63%. Our study results were in concordance with the study findings of Diekema DJ et al.[12] (40%), Medeiros et al.[13] (37.5%), and Mazzanti S et al.[14] (36%).

In this study out of 53 *Candida* spp., the incidence of CNA spp. accounts for about 83.01% whereas *Candida albicans* showed 16.99% and these results were consistent with the study findings of Mazzanti S et al.[14] showed CNA spp. of 82.35% and 17.65% of *C. albicans*. In contrast, several studies were conducted by Oliveira et al.[15] and Al-Rawahi GN et al.[16] reported a high prevalence of *C. albicans* over CNA spp. Out of 44 NAC spp, *C. tropicalis* 47.16% was the predominant species isolated that were correlated with the study findings of Thomas M et al.,[17] Chakrabarti A et al.[18] and Chander et al.[19] which showed 50.5%, 47%, and 40.8% respectively. Hence, it is observed that *C. tropicalis* is the most prevalent species in India which is consistent with our study reports. Our study also reports 11 (20.75%) *C. parapsilosis*, 4 (7.54%) *C. glabrata*, and 4 (7.54%) *C. krusei*. These results were correlated with the study findings of Tak V et al.[20]

The pathogenesis of *Candida* spp. is based on distinct virulence factors such as phenotypic switching, host cell adherence, extracellular hydrolytic enzyme production, germination, and biofilm formation. The present study was directed to demonstrate hemolysin, biofilm formation, and phospholipase activities from isolated *Candida* spp. Out of 53 *Candida* spp., 30 (56.60%) isolates demonstrated positive results for phospholipase activity. In which, 7 (23.33%) were *C. albicans* and 23 (76.67%) were CNA spp. Similar results were reported by Mayer FL et al.[21] Conversely, Fule et al.[22] and Khater et al.[23] resulted that the phospholipase activity was more in *C. albicans* than in CNA spp.

Among 53 Candida isolates, 36 isolates showed hemolysin production, of which 8 (22.22%) were *C. albicans* and 28 (77.78%) were CNA spp. Our study results were similar to those obtained by Luo et al.[24] and M. A. Galan-Ladero et al.[25] On the contrary, Deorukhkar et al.[26] reported that 30.4% of *C. tropicalis* was found to be a hemolysin producer, this percentage was quite lesser when compared to our study findings [*C. tropicalis* (61.11%)]. Several studies have shown hemolysin production in *C. tropicalis*, but it is very much essential to understand if the hemolytic activity is really noticed or because of phospholipase production. Hence, the need for enhanced molecular studies to interpret the hemolysin production in the pathogenesis of *C. tropicalis* is justified.

In the present study, both *C. albicans* and CNA spp demonstrated biofilm production in vitro by spectrophotometric method and Congo red agar plate method. But the biofilm production was predominantly higher in CNA spp. (72.73%) than *C. albicans* (27.27%). Similar to our study analysis, Khater et al.[23] reported CNA spp. as the predominant biofilm producer. In contrast to the results of Mohandas et al.[27] and Mane et al.[28] reported that high biofilm production was shown by *C. albicans* (78%) over CNA spp. (22%).

The present study reports were in accordance with the other studies with respect to predisposing factors for candidemia; the major significant risk factors were early antifungal management for >14 days, and subsequent use of central lines.

In the present study, *Candida* species showed the highest sensitivity to Amphotericin B (92.45%) and Itraconazole (81.13%) than Fluconazole (67.92%). However, the highest resistance was noted to Fluconazole (28.30%) than Amphotericin B (3.77%) and Itraconazole (18.86%). Of which, CNA spp. (93.33%) showed significantly high resistance to fluconazole than *C. albicans* (6.66%). These results were compatible with the study findings of Yamin DH et al.[29] who reported 30.8%. The frequency of fluconazole resistance was highly noticed among *C. tropicalis* (33.33%) than in *C. albicans* (6.67%) and these results were closely associated with the study findings of Kothari et al.[30]

In our study, MIC for fluconazole-resistant *Candida* isolates showed that 5 (33.33%) shows ≤ 2 µg/mL, 8 (53.33%) were in the intermediate category with MIC of 4-32 µg/mL and 2 (13.33%) showed a higher MIC of ≥ 64 µg/mL. A higher MIC of ≥ 64 µg/mL was observed in 1 isolate of *C. glabrata*. However, 1 isolate of *C. krusei* showed a higher MIC of ≥ 64 µg/mL, and this resistant strain of *C. krusei* is excluded from this study as they are intrinsically resistant to fluconazole. These results were correlated with the study findings of Gandham et al.[31] showed a higher MIC of ≥ 2 µg/ml in 4 isolates of *C. tropicalis* and 1 of *C. parapsilosis*. The extensive use of a wide array of antifungal drugs urges the propagation of drug resistance among *Candida* spp. constitutes an added challenge in the appropriate management of candidemia. Thus, it is always very essential to analyze and identify the cause of candidemia by *Candida* spp. so as to commence an empirical regimen.

CONCLUSION

Candida species is an extremely significant and alarming pathogen responsible for Blood stream infections (BSIs) with a predominance of 14.4%. Even while *C. albicans* is still the most common species found among patients with suspected septicemia in different wards and ICUs, our analysis clearly showed a shift to *Candida* non-albicans candidemia, particularly by *C. tropicalis* 25 (47.16%). When compared to CNA species, *C. albicans* isolates have lower rates of antifungal agent resistance. The antifungal stewardship program and targeted management are given priority because of the elevated resistance among CNA species and the association between empirical antifungal usage. Thus, ongoing investigations can contribute to a better understanding of hindering drug resistance, detection of resistant isolates, and drug repurposing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study is approved by the Institutional ethical committee of SRM Medical College Hospital and Research Centre (2896/IEC/2021).

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