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Invasive yeast infections in the Intensive Care Unit of a tertiary care centre in South India

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Abstract:

INTRODUCTION: The incidence of invasive mycoses has increased enormously due to the rising population of critically ill patients. Emergence of rare yeasts with variable sensitivity patterns has underlined the need to monitor laboratory data for the emergence of resistance.

AIMS: To determine the prevalence and susceptibility patterns of invasive mycoses and analyse the causative risk factors in the Intensive Care Unit (ICU) of a tertiary care centre in South India.

MATERIALS AND METHODS: This is a retrospective, observational study performed on invasive Candida species isolated from patients admitted to the ICU. All the blood and cerebrospinal fluid (CSF) samples from ICU patients, over a period of one year, were included in the study. Preliminary identification of the isolates was done by VITEK. Genotypic identification of each isolate was done using colony polymerase chain reaction-restriction fragment length polymorphism and gene sequencing. Susceptibility pattern was ascertained by broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines in document M27-A3. Relevant clinical details of the patients were collected from the records for the analysis of risk factors and outcome.

RESULTS: The present study was done on 4629 blood and 341 CSF specimens, which were sent to the central clinical microbiology laboratory for diagnosis, between November 2013 and October 2014. A total of 49 Candida strains were isolated during the study period, with a prevalence of candidaemia in our ICU to be 1.05%. *Candida tropicalis* 19 (35.8%) was found to be the predominant species, followed by *Candida albicans*, *Candida parapsilosis*, *Candida auris*, *Candida glabrata*, *Candida duobushaemulonii* and *Candida krusei*. All the Candida species were found to be sensitive to Anidulafungin and Micafungin. Candida isolates were sensitive to Fluconazole - 39 (80.4%), Voriconazole - 49 (100%), Amphotericin B - 27 (54.7%) and Caspofungin - 48 (96.3%). Risk factor analysis revealed intravenous catheterisation as the major predisposing factor, followed by prior therapy with broad-spectrum antibiotics, mechanical ventilation, urinary catheterisation, diabetes mellitus and steroid therapy. Four isolates of *Cryptococcus neoformans* were also isolated from the blood samples. Three (0.8%) cases of fungal meningitis were diagnosed from 341 CSF samples, with *C. tropicalis*, *C. neoformans* and *Cryptococcus gattii* as causatives.

CONCLUSION: It is of paramount importance to promptly identify and precisely determine the susceptibility of these agents, to combat this medical battle.

Keywords:

Antifungal susceptibility, broth microdilution, candidaemia, colony polymerase chain reaction

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Introduction

Fungaemia is a medical emergency urging prompt diagnosis and treatment. The incidence and prevalence of invasive yeast infections have increased enormously due to widespread use of broad-spectrum antibiotics, prolonged treatment with corticosteroids, aggressive treatment modalities for cancers, catheter-borne

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infections, immunosuppression due to diabetes mellitus, AIDS, malignancies, transplantation and dialysis.[1]

Candidaemia is the fourth leading cause of hospital-acquired bloodstream infections in the USA, with a mortality rate ranging from 10% to 49% worldwide. Although *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis* and *Candida krusei* contribute to 90% of infections, reports of new Candida species are on the rise.

The upsurge of non-albicans Candida species and a shift in the antifungal susceptibility profile have emphasised the need for characterising these rare species. The current study was aimed at profiling the invasive yeast infections in the susceptible population.

Objectives

- Identification of the yeasts from blood and cerebrospinal fluid (CSF) samples of patients admitted to the Intensive Care Units (ICUs) by colony polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)
- Determination of the antifungal susceptibility pattern of the isolates
- Analysis of the risk factors predisposing to invasive yeast infections and the outcome of the infections.

Materials and Methods

The present study is a retrospective observational study, conducted at Sri Ramachandra Medical College and Research Institute, Chennai, over a period of one year, from November 2013 to October 2014. All the blood and CSF samples received from the ICU patients for routine culture were taken for the study. The samples which grew yeast were considered positive. The Institutional Ethics Committee approval was obtained for the study from the Ethical Committee, Sri Ramachandra University.

Aseptically collected blood and CSF samples were inoculated in blood culture bottles. The samples which were positive for growth were sub-cultured on Sabouraud dextrose agar (SDA) and these isolates were subjected to phenotypic and genotypic identification. Relevant clinical details of the patients were collected from the records for analysis of the risk factors and outcome. The details collected were age, clinical diagnosis, previous administration of long-term antibiotics, predisposing factors such as invasive catheterisation, mechanical ventilation, dialysis, total parenteral nutrition (TPN) and immunocompromised states such as diabetes, steroid therapy, neutropenia and chemotherapy.

Preliminary identification of the isolates was done by the automated identification system, VITEK2 Compact, BioMerieux Systems, USA.

The genotypic identification of each isolate was done using colony PCR-RFLP method.

Colony PCR is designed to screen the target DNA directly from the colony. In a 200 µl PCR tube, 15 µl of PCR Master Mix (Genei), 1 µl of forward primer (ITS-1:5′-CCGTAGGTGAACCTGCG-3′), 1 µl of reverse primer (ITS-4:5′-TCCTCCGCTTATTGATATGC-3′) and 13 µl of nuclease free water were added to make a reaction volume of 30 µl. Using a sterile pipette tip, an isolated colony of the test organism was picked up and added to this reaction mix and mixed well. The PCR tubes were then placed in a thermocycler and programmed to run in the following conditions. Initial denaturation was done at 95°C for 10 min, followed by denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for five minutes. This was programmed to repeat for thirty cycles after which the final PCR product was obtained.[2]

The PCR products, thus amplified, were electrophoresed in 1.5% agarose gel in 1X Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer, for approximately 30 min at 100V.

The isolates which showed positive bands in the PCR gel were subjected to RFLP, using the enzyme *Msp1*.

The products of RFLP were separated in 2% gel electrophoresis in TAE buffer and visualised under ultraviolet light. Table 1 shows the product size of the various Candida species based on which our test isolates identified.

Those isolates which could not identified by RFLP were identified by sequencing the internal transcribed spacer

Table 1: Size of ITS-1–ITS-4 products for Candida species before and after digestion with *Msp1*

Candida species	Size of the PCR product (bp)	Sizes of the RFLP product (bp)
C. albicans	535	297,238
C. tropicalis	524	340,184
C. krusei	510	261,249
C. glabrata	871	557,314
C. parapsilosis	520	520
C. auris	400	400
C. haemulonii	400	400

C. tropicalis: *Candida tropicalis*; *C. albicans*: *Candida albicans*; *C. auris*: *Candida auris*; *C. haemulonii*: *Candida haemulonii*; *C. glabrata*: *Candida glabrata*; *C. parapsilosis*: *Candida parapsilosis*; *C. krusei*: *Candida krusei*; bp: Basepair; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism

(ITS) regions (SciGenom Labs, Cochin, Kerala, India). The sequence was then used for a nucleotide-nucleotide search using the Basic Local Alignment Search Tool (BLAST) algorithm at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/). BLAST which hits >99% was considered for species identification.

The antifungal susceptibility was performed by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI document M27-A3) 'Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Third Edition' (CLSI 2008).[3]

Antifungal agents Fluconazole and Amphotericin B were procured from HiMedia, India, and Posaconazole, Itraconazole, Voriconazole, Echinocandins (Caspofungin, Anidulafungin and Micafungin) from Sigma-Aldrich, India. Stock solutions were prepared in dimethyl sulfoxide (DMSO) at 100 times concentration more than the highest concentration tested and stored at −80°C until use. The drug concentration tested ranged from 0.125 to 64 μg/ml for Fluconazole, 0.03–16 μg/ml for Itraconazole, Ketoconazole, Posaconazole, Voriconazole, Amphotericin B, 0.015–8 μg/ml for Caspofungin, Anidulafungin and Micafungin. Intermediate stocks of all the antifungal agents were prepared at fifty times the concentration higher than the range tested. The working concentration was prepared by diluting the intermediate concentration in RPMI 1640, which gave 2X concentration of the drugs. One hundred microlitres from the working concentration was dispensed into the corresponding wells in a microtiter plate. Colonies from 24 to 72 h old culture grown on SDA were suspended in sterile distilled water and the density was adjusted to optical density 0.45–0.55 at 530 nm in a spectrophotometer. 1:500 dilution of the inoculum was made in RPMI 1640 and 100 μl of this inoculum was added to the wells. RPMI as a growth control and inoculum with only DMSO as drug control was also included. It was incubated at 35°C for 24 h and the breakpoints were interpreted from the CLSI M27-S4 supplement document for yeasts.[4] *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control strains.

CLSI breakpoints are available for the antifungals, Fluconazole, Voriconazole, Amphotericin B, Caspofungin, Anidulafungin and Micafungin, only against the common Candida species, namely, *C. albicans*. *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. glabrata* and *Candida guilliermondii*. The antifungal susceptibility pattern of the rare Candida isolates and the Cryptococcal isolates could not be ascertained as susceptible/intermediate/resistant since the breakpoints have not been defined in the CLSI document.

Results

Blood samples from a total of 4629 patients were received in the central laboratory, from the patients of ICU of Sri Ramachandra Medical College, for culture, during the study period of one year (November 2013–October 2014). Fifty-three blood samples were positive for yeast. The isolates identified were *C. tropicalis* 19 (35.8%), *C. albicans* 10 (18.8%), *C. parapsilosis* 10 (18.8%), *C. glabrata* 2 (3.7%), *C. krusei* 1 (1.8%), *C. auris* 6 (11.3%), *Candida duobushaemulonii* 1 (1.8%), *Cryptococcus gattii* 1 (1.8%) and *Cryptococcus neoformans* 3 (5.6%). Other specimens were also found to yield yeast from the candidaemia patients. They were urine, peritoneal fluid, CSF and pus in six, two, one and one samples, respectively. A total of 341 CSF samples were received for culture during the same period. Three CSF samples yielded yeast, namely, *C. tropicalis*, *C. neoformans* and *C. gattii.*

Fungaemia was reported in 19 paediatric (<18 years) patients and 34 adult patients, with Candida species being responsible for 18 and 31 cases, in the paediatric and adult age group, respectively. Cryptococcaemia was reported in one paediatric patient and three adult patients.

Fungi-specific universal primers, ITS-1 and ITS-4, were able to successfully amplify the ITS region of all the 56 (53 from blood and three from CSF) isolates. All the isolates were positive in the pan-fungal PCR. In RFLP, the PCR products of digestion by the enzyme, *Msp1*, generated bands which corresponded to the predicted sizes of the common Candida species. PCR-RFLP detected *C. tropicalis* (19) *C. albicans* (10), *C. parapsilosis* (10), *C. glabrata* (2) and *C. krusei* (1). However, there was no difference between PCR and RFLP products of *C. auris*, *Candida haemulonii*, Cryptococcus species as there were no recognition sites for this enzyme within the ITS region of above-mentioned species. Hence, these rare isolates were identified by gene sequencing as *C. auris* (6), *C. duobushaemulonii* (1), *C. gattii* (1) and *C. neoformans* (3). The gel pictures of PCR and RFLP products are shown in Figures 1 and 2, respectively.

Fifty-three (1.2%) blood samples tested positive for the presence of yeast. Among the 53 yeast isolates, 49 (92.5%) were identified as Candida species and 4 (7.5%) isolates were identified as Cryptococcus species. It was thus inferred that the overall prevalence of candidaemia in the ICU setup of our institute, over the study period, was 1.05%. *C. tropicalis* 19 (35.8%) was found to be the predominant species, followed by *C. albicans* 10 (18.8%), *C. parapsilosis* 10 (18.8%), *C. auris* 6 (11.3%), *C. glabrata* 2 (3.7%), *C. duobushaemulonii* 1 (1.8%), *C. krusei* 1 (1.8%) and *C. neoformans* 4 (7.5%). Speciation of the candidaemia isolates is depicted in Figure 3.

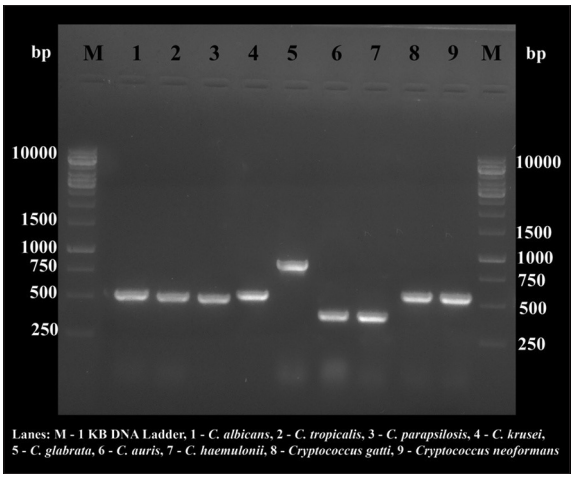

Figure 1: Banding pattern of the representative isolates of Candida species in pan-fungal polymerase chain reaction

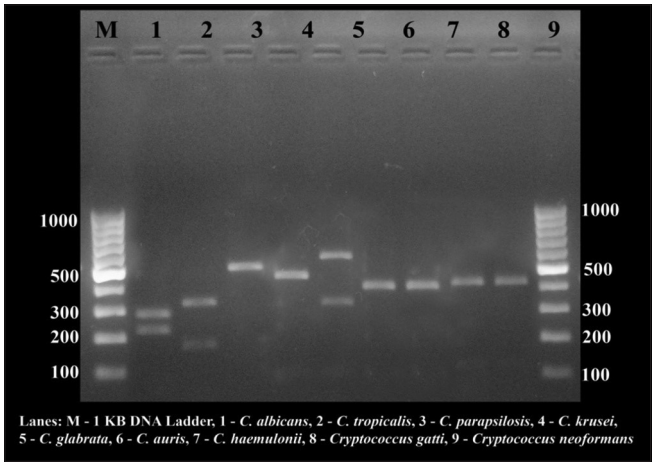

Figure 2: Banding pattern of the restriction fragment length polymorphism products from the representative isolates of Candida species

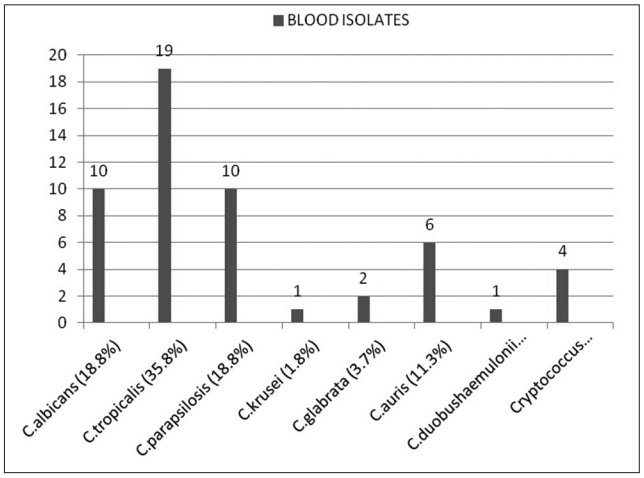

Figure 3: Bar diagram representing the distribution of the Candida species from blood samples of Intensive Care Unit patients

Among the three cases of fungal meningitis, we reported in our study, *C. tropicalis*, *C. neoformans* and *C. gattii* were the causative agents.

Antifungal susceptibility testing was performed by broth microdilution method using the CLSI reference guidelines M27-A3. Minimum inhibitory concentration (MIC) values of the test isolates are tabulated in Table 2.

All the 42 common Candida isolates, except one, were susceptible to Echinocandins, which was resistant to Caspofungin. Among the azoles, all isolates were susceptible to Voriconazole. Thirty-four (81%) of the isolates were susceptible to Fluconazole, while 4 (9.5%) isolates were resistant and 4 (9.5%) had intermediate susceptibility. Intermediate susceptibility and complete resistance to Amphotericin B were seen in 18 (42.85%) and 2 (4.7%) of the isolates, respectively. The seven rare Candida species had very high MIC for Fluconazole and Amphotericin B and low MIC for Echinocandins.

C. neoformans isolates had low MICs for the Azoles and Amphotericin B. *C. gattii* isolate had very high MICs for the above drugs.

Colony PCR-RFLP was done to rapidly speciate the isolates, and the results were compared with the identification by the automated VITEK2 system. Speciation results of the automated identification system, VITEK2 system, correlated with the molecular diagnosis in only 45 (80.3%) of cases. The comparison of the results is tabulated in Table 3.

On analysing the risk factors for developing invasive yeast infections, the most common predisposing factor was found to be the presence of central venous catheter 48 (85.7%), followed by prior therapy with broad-spectrum antibiotics 42 (75%). Risk factors predisposing to invasive candidiasis (candidaemia and meningitis) have been depicted in Figure 4.

Leucopenia and retropositivity were found to be the major risk factors, leading to invasive cryptococcosis.

Overall mortality of 37 (66%) was found among our study population. Fatality of 32 (64%) patients was attributed to invasive candidiasis and 5 (83.8%) patients to invasive cryptococcosis.

Discussion

Invasive fungal infections are reported in increasing incidence in the patients admitted to the ICU. The estimated annual incidence of invasive mycoses due to Candida species is 72–228 infections per million population and 30–66 infections per million population for *C. neoformans*.[5]

According to the national surveillance efforts by the Centers for Disease Control and Prevention (CDC), there

Table 2: Susceptibility patterns of the fungal species isolated

Isolate	Antifungal drug MIC (μg/ml)							
	Fluconazole	Voriconazole	Posaconazole	Itraconazole	Amphotericin B	Caspofungin	Anidulafungin	Micafungin
C. gattii	8	0.06	0.5	1	4	-	-	-
C. tropicalis	1 (S)	0.03 (S)	0.25	0.25	1 (S)	0.125 (S)	0.125 (S)	0.06\ (S)
C. tropicalis	0.125 (S)	0.03 (S)	0.25	0.25	2 (I)	0.007 (S)	0.007 (S)	0.007 (S)
C. tropicalis	0.125 (S)	0.03 (S)	0.25	0.25	1 (S)	0.015 (S)	0.015 (S)	0.007 (S)
C. tropicalis	0.25 (S)	0.03 (S)	0.25	0.25	1 (S)	0.015 (S)	0.015 (S)	0.007 (S)
C. albicans	0.25 (S)	0.03 (S)	0.25	0.25	1 (S)	0.125 (S)	0.03 (S)	0.015 (S)
C. albicans	0.25 (S)	0.03 (S)	0.25	0.25	1 (S)	0.125 (S)	0.03 (S)	0.015 (S)
C. parapsilosis	8 (R)	0.03 (S)	0.5	0.25	2 (I)	0.25 (S)	0.25 (S)	0.25 (S)
C. parapsilosis	8 (R)	0.03 (S)	0.5	0.25	2 (I)	0.25 (S)	0.25 (S)	0.25 (S)
C. glabrata	0.5	0.06	0.125	0.5	2 (I)	0.25 (I)	0.125 (S)	0.03 (S)
C. albicans	0.25 (S)	0.03 (S)	0.06	0.125	2 (I)	0.06 (S)	0.06 (S)	0.03 (S)
C. auris	≥64	0.5	0.25	0.5	8	0.25	0.125	0.125
C. tropicalis	0.5 (S)	0.06 (S)	0.125	0.25	1 (S)	0.125 (S)	0.125 (S)	0.06 (S)
C. auris	4	0.125	0.125	0.06	4	0.015	0.015	0.007
C. parapsilosis	0.5 (S)	0.03 (S)	0.125	0.125	2 (I)	0.25 (S)	0.25 (S)	0.5 (S)
C. glabrata	0.5	0.03	0.125	0.25	2 (I)	0.06 (S)	0.06 (S)	0.015 (S)
C. tropicalis	0.125 (S)	0.03 (S)	0.125	0.06	2 (I)	0.007 (S)	0.007 (S)	0.007 (S)
C. tropicalis	≥64 (R)	0.5 (I)	0.25	1	8 (R)	0.125 (S)	0.125 (S)	0.125 (S)
C. parapsilosis	0.25 (S)	0.03 (S)	0.125	0.125	2 (I)	0.03 (S)	0.125 (S)	0.25 (S)
C. parapsilosis	0.25 (S)	0.03 (S)	0.125	0.125	2 (I)	0.25 (S)	0.25 (S)	0.25 (S)
C. neoformans	1	0.03	0.25	0.5	2	-	-	-
C. neoformans	1	0.03	0.25	0.5	2	-	-	-
C. neoformans	1	0.03	0.25	0.5	2	-	-	-
C. albicans	0.125 (S)	0.03 (S)	0.25	0.25	1 (S)	0.125 (S)	0.03 (S)	0.015 (S)
C. albicans	0.125 (S)	0.03 (S)	0.25	0.25	1 (S)	0.125 (S)	0.03 (S)	0.015 (S)
C. tropicalis	4 (I)	0.125 (S)	0.25	0.25	1 (S)	0.25 (S)	0.125 (S)	0.06 (S)
C. tropicalis	4 (I)	0.125 (S)	0.25	0.25	1 (S)	0.25 (S)	0.125 (S)	0.06 (S)
C. parapsilosis	4 (I)	0.06 (S)	0.25	0.5	2 (I)	0.25 (S)	0.125 (S)	0.125 (S)
C. tropicalis	4 (I)	0.06 (S)	0.06	0.06	2 (I)	1 (R)	0.015 (S)	0.015 (S)
C. parapsilosis	0.25 (S)	0.03 (S)	0.06	0.06	1 (S)	0.06 (S)	0.06 (S)	0.015 (S)
C. albicans	2 (S)	0.06 (S)	0.25	0.25	2 (I)	0.25 (S)	0.25 (S)	0.06 (S)
C. tropicalis	2 (S)	0.125 (S)	0.25	0.5	2 (I)	0.25 (S)	0.125 (S)	0.125 (S)
C. parapsilosis	1 (S)	0.06 (S)	0.125	0.125	0.5 (S)	0.25 (S)	0.25 (S)	0.125 (S)
C. duobushaemulonii	≥64	0.06	0.125	0.125	4	0.125	0.06	0.03
C. parapsilosis	0.5 (S)	0.03 (S)	0.125	0.125	1 (S)	0.125 (S)	0.125 (S)	0.125 (S)
C. tropicalis	4 (I)	0.125 (S)	0.5	0.5	2 (I)	0.25 (S)	0.25 (S)	0.125 (S)
C. tropicalis	1 (S)	0.06 (S)	0.25	0.25	2 (I)	0.125 (S)	0.125 (S)	0.125 (S)
C. albicans	0.25 (S)	0.03 (S)	0.125	0.125	0.5 (S)	0.06 (S)	0.06 (S)	0.015 (S)
C. tropicalis	0.25 (S)	0.03 (S)	0.125	0.125	0.5 (S)	0.06 (S)	0.06 (S)	0.03 (S)
C. auris	≥64	0.25	0.25	0.25	8	0.25	0.25	0.125
C. krusei	1 (int R)	0.06 (S)	0.125	0.25	2 (I)	0.125 (S)	0.125 (S)	0.015 (S)
C. parapsilosis	1 (S)	0.06 (S)	0.25	0.25	1 (S)	0.125 (S)	0.125 (S)	0.06 (S)
C. albicans	0.25 (S)	0.03 (S)	0.06	0.06	4 (R)	0.125 (S)	0.06 (S)	0.03 (S)
C. tropicalis	0.5 (S)	0.06 (S)	0.125	0.125	0.5 (S)	0.06 (S)	0.06 (S)	0.06 (S)
C. tropicalis	0.5 (S)	0.06 (S)	0.25	0.125	1 (S)	0.125 (S)	0.06 (S)	0.06 (S)
C. tropicalis	0.5 (S)	0.06 (S)	0.25	0.125	1 (S)	0.125 (S)	0.06 (S)	0.06 (S)
C. tropicalis	0.5 (S)	0.03 (S)	0.125	0.25	2 (I)	0.06 (S)	0.03 (S)	0.03 (S)
C. albicans	0.5 (S)	0.06 (S)	0.06	0.06	1 (S)	0.125 (S)	0.125 (S)	0.06 (S)
C. auris	≥64	0.5	0.25	0.5	8	0.125	0.125	0.125
C. neoformans	1	0.125	0.25	0.25	1	-	-	-
C. tropicalis	0.25 (S)	0.06 (S)	0.125	0.125	1 (S)	0.06 (S)	0.03 (S)	0.03 (S)
C. albicans	0.5 (S)	0.03 (S)	0.125	0.25	1 (S)	0.06 (S)	0.06 (S)	0.06 (S)
C. auris	32	0.125	0.125	0.5	8	0.25	0.125	0.125
C. auris	32	0.125	0.125	0.5	8	0.25	0.125	0.125

Contd...

Table 2: Contd...

Isolate	Antifungal drug MIC (μg/ml)							
	Fluconazole	Voriconazole	Posaconazole	Itraconazole	Amphotericin B	Caspofungin	Anidulafungin	Micafungin
C. albicans	0.5 (S)	0.06 (S)	0.125	0.125	1 (S)	0.125 (S)	0.125 (S)	0.06 (S)
C. neoformans	0.5	0.06	0.125	0.25	0.5	-	-	-

-: Not tested, since intrinsically resistant; *C. neoformans*: *Cryptococcus neoformans*; *C. gattii*: *Cryptococcus gattii*; *C. albicans*: *Candida albicans*; *C. auris*: *Candida auris*; *C. parapsilosis*: *Candida parapsilosis*; *C. krusei*: *Candida krusei*; *C. duobushaemulonii*: *Candida duobushaemulonii*; *C. glabrata*: *Candida glabrata*; *C. tropicalis*: *Candida tropicalis*; MIC: Minimum inhibitory concentration; S: Sensitive; I: Intermediate; R: Resistant

Table 3: Comparison of the identification by the VITEK2 automated system and molecular techniques

Sample	Automated system	PCR-RFLP/gene sequencing result
CSF	*C. laurentii*	*C. gattii*
Blood	*C. albicans*	*C. glabrata*
Blood	*C. haemulonii*	*C. auris*
Blood	*C. haemulonii*	*C. auris*
Blood	*C. haemulonii*	*C. duobushaemulonii*
Blood	*C. famata*	*C. auris*
Blood	*C. famata*	*C. auris*
Blood	*C. famata*	*C. albicans*
Blood	*C. tropicalis*	*C. auris*
Blood	*C. haemulonii*	*C. auris*

PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; *C. laurentii*: *Cryptococcus laurentii*; *C. gattii*: *Cryptococcus gattii*; *C. albicans*: *Candida albicans*; *C. auris*: *Candida auris*; *C. duobushaemulonii*: *Candida duobushaemulonii*; *C. haemulonii*: *Candida haemulonii*; *C. famata*: *Candida famata*; *C. tropicalis*: *Candida tropicalis*; *C. glabrata*: *Candida glabrata*

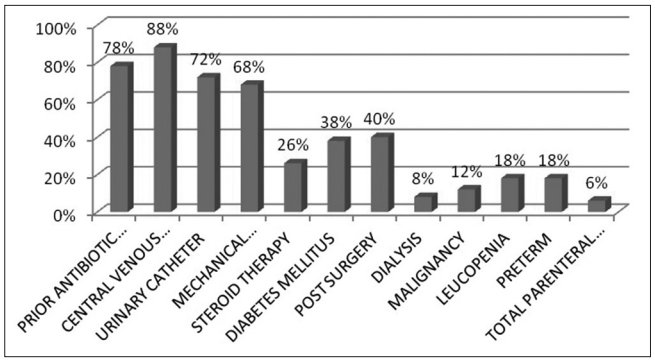

Figure 4: Bar diagram depicting the percentage of risk factors predisposing to invasive candidiasis

has been >10-fold increase in invasive candidiasis among critically ill patients since the 1980s.

Various studies globally have determined the incidence of candidaemia to be 0.24–34.3 patients/1000 ICU admissions.[6] An Indian study involving 27 ICUs reported an incidence of 6.51 cases/1000 ICU admissions.[6] Our study gives a incidence of 10.5 candidaemia cases/1000 ICU admissions. The prevalence of candidaemia was found to be 1.08%. It is nearly twice as high in a similar study, conducted five years earlier, which stated the prevalence of candidaemia among ICU patients as 0.65%.[7] In a five-year study from New Delhi, Xess *et al*. found a prevalence of 6% for candidaemia.[8]

An incidence of cryptococcosis of 0.4–1.3 cases/100,000 population was reported in a US-based study conducted in 2000, with a mortality rate of 12%.[9] A one-year study in India reported a prevalence of 2.79% among retropositive patients.[10] The current study gave a prevalence of 0.12%, with a mortality of 83.8%.

The mean age of the present study was 34.9 years. Twenty (35.7%) of paediatric population and 36 (64.2%) of adult population in the ICU were affected by invasive mycoses. In our study, 18 (36.7%) candidaemia patients were in the paediatric age group, the leading cause being *C. tropicalis*. Many studies in paediatric age group identified *C. albicans* as the most prevalent species contributing to candidaemia, followed by *C. parapsilosis*.[11] Neonatal population with candidaemia in our study was 7 (13.2%). A North Indian study reported an isolation rate of 8.1% for Candida species from cases of neonatal septicaemia.[12]

One out of the six cryptococcal cases was seen in paediatric age group. In a population-based surveillance program in South Africa, cryptococcosis was reported in 2% of the paediatric population.[13]

The epidemiology of fungal infections changes over time. Therefore, it is important to verify the incidence of each key pathogenic fungal species every decade, in order to provide an update on the epidemiology of invasive fungal infections and susceptibility of strains to antifungal medications.

In our study, out of the 49 candidaemia cases, *C. tropicalis* was found as the predominant species, accounting to 19 (35.8%) of the cases. It was followed by *C. albicans* and *C. parapsilosis*, both being responsible for 10 (18.8%) of candidaemia cases each. *C. auris*, *C. glabrata*, *C. krusei* and *C. duobushaemulonii* were found to be the causatives of 6 (11.3%), 2 (3.7%), 1 (1.8%) and 1 (1.8%) of candidaemia, respectively. A study similar to this current study, conducted five years earlier, reported the prevalence of *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. krusei* and *C. glabrata* to be 74.35%, 10.25%, 7.69%, 5.13% and 2.56%, among candidaemia patients, respectively.[7] A major surveillance study, involving 11 ICUs all over

India, also had results comparable with ours. They reported *C. tropicalis* (41.6%) as the most prevalent cause of candidaemia, followed by *C. albicans* (20.9%) and *C. parapsilosis* (10.9%).[6]

This study also portrays the increasing incidence of emerging rare fungal species, implicated in opportunistic mycoses. We have reported *C. auris* in 6 (11.3%) of candidaemia cases and *C. duobushaemulonii* in 1 (1.8%) of them. A New Delhi-based study identified 12 isolates of *C. auris* over a period of two years, from bloodstream infections.[14]

In vitro antifungal susceptibility testing plays a major role in prompt management of the invasive fungal infections and AIDS to track the development of antifungal resistance among the common pathogenic species and to detect the intrinsic resistance of the rare emerging yeasts, which are now being increasingly reported. Approximately 7% and 1% of all the candidaemia isolates tested at CDC are found to be resistant to Fluconazole and Echinocandins, respectively.[15] Among our study isolates, all the Candida species were found to be sensitive to Anidulafungin and Micafungin. Thirty-four (81%), 42 (99.5%), 23 (53.7%) and 40 (95.3%) of the common Candida isolates were sensitive to Fluconazole, Voriconazole, Amphotericin B and Caspofungin, respectively. Twenty-one (52.38%) of our Candida isolates were found to be pan-susceptible. One isolate of *C. tropicalis* was found to be multidrug resistant, being resistant to Fluconazole and Caspofungin. Although Fluconazole is considered a safe and effective choice of treatment for candidaemia, an increasing trend a Fluconazole resistance is being reported among the Candida species causing bloodstream infections. One *C. albicans* and one *C. tropicalis* isolates were resistant to Amphotericin B, and intermediate susceptibility pattern was expressed by seven isolates of *C. tropicalis*, six isolates of *C. parapsilosis*, two isolates of *C. albicans* and *C. glabrata* each and one isolate of *C. krusei*. There are very few reports of Amphotericin B resistance in Candida isolates from cases of candidaemia in India.[16] Although the susceptibility pattern of the Echinocandins are better, they are highly expensive and cannot be afforded by a majority of the Indian population. Among the seven rare Candida species isolated in our study, comprising six *C. auris* and one *C. duobushaemulonii*, all of them had a low MIC for Echinocandins, ranging from 0.06 to 0.25 μg/ml, with lowest MICs for Micafungin. All of them had a very high MIC for Amphotericin B and Fluconazole, ranging from 4 to 8 μg/ml and 4–>64 μg/ml, respectively.

Speciation results of the automated identification system, VITEK2 system, correlated with the molecular diagnosis in only 46 (80.3%) of cases.

Our study reports the presence of intravenous catheters as the predominant risk factor, for candidaemia in ICU patients. We found central venous catheterisation in 88% of our study population with candidaemia. The isolate was isolated from the venous catheters in 23 (47%) of the cases. Many studies state that indwelling catheter is the most important risk factor for *C. parapsilosis* candidaemia.[17]

The second most common predisposing factor among the candidaemia cases in our study was the prior therapy with broad-spectrum antibiotics, accounting as a risk factor in 38 (78%) of our study population. A nationwide study stated 93% of their candidaemia patients to be treated with broad-spectrum antibiotics.[6] A five-year long study found that prolonged use of antibiotics was the second most common risk factor for the development of candidaemia (34.5%).[18]

Thirty-five (72%) of the candidaemia patients in our study had urinary catheter. The same Candida species was isolated from the urinary catheter in 8 (22.8%) of the candidaemia patients. A review study suggested that as many as 10% of candiduria cases may be associated with candidaemia.[19]

Nineteen (40%) of our study population developed candidaemia post-surgery. Out of them, majority of the patients 10 (53%) underwent gastrointestinal surgery, 5 (23.5%) underwent neurological procedures, and 2 (11.7%) underwent cardiothoracic and orthopaedic surgeries each. It has been established that gastrointestinal surgeries may lead to direct invasion of the bloodstream by the commensal flora of the gut.

In India, which has a very high prevalence of diabetes, invasive candidiasis becomes a matter of great concern and has to be approached with great caution and these patients must be treated with a high level of suspicion for these lethal opportunistic infections. In our study, 19 (38%) of the patients were diabetic. Several studies associate diabetes mellitus with fungal sepsis, accounting for a mortality rate of 39%. High blood glucose concentration is a significant marker of increased in-hospital mortality in patients with diabetes and candidaemia.

TPN, a lifesaving mode of delivering nutrition in chronically debilitated individuals, is said to cause opportunistic invasive candidiasis if administered injudiciously. In our study, we found TPN in 3 (6%) of candidaemia patients. TPN was associated with 25 (23.6%) of candidaemia patients in the ICU in a study involving 105 patients.[20]

Immunosuppression, due to steroid therapy, neutropenia, malignancies or chemotherapy, is an important risk factor

for invasive mycoses. Steroid therapy, leucopenia and malignancies were seen associated with 13 (26%), 9 (18%) and 6 (12%) of our candidaemia patients, respectively. An Indian study attributes 10% of bloodstream infections among haemato-oncology patients in the ICU, to Candida species.[21]

Immunosuppression is considered the major risk factor for cryptococcal infections. *C. neoformans* is associated with bloodstream infections and meningitis in immunocompromised patients, while reports of cryptococcosis even in immunocompetent patients are increasing at a striking rate. We reported one *C. gattii* meningitis in an adult with no overt immunodeficiency.[22] The four patients with cryptococcaemia, caused by *C. neoformans* were retropositive.

In our study, mortality rate due to candidaemia was found to be 32 (66%). Mortality associated with fungal infections in patients hospitalised in ICUs reaches 67%.[23]

Conclusion

The discovery of new pathogenic yeasts and the rise in the population at risk warrant a very high degree of suspicion among the clinicians and the medical microbiologists, to identify the etiological agents and to initiate prompt therapy, in order to decrease the overall morbidity and mortality rates. Molecular techniques, yielding prompt identification, are very useful therapeutic and epidemiological tools in the field of invasive fungal infections. Correct etiological diagnosis and availability of culture with speciation and susceptibility help in planning effective treatment strategies.

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Conflicts of interest
There are no conflicts of interest.

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