

Candidemia in patients with prolonged fever in Kashan, Iran

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Abstract

Background and Purpose: *Candida* species are considered a common cause of fungal blood stream infections, which are associated with considerable mortality and morbidity rates, especially in the admitted and immunocompromised patients. Despite the increase in new and available antifungal agents, the emergence of resistant strains is growing. Regarding this, the aim of the present study was to assess the fungal epide-miology of candidemia and the antifungal susceptibility patterns against five current antifungal agents among the patients with prolonged fever, who were admitted to Beheshti Educational Hospital, Kashan, Iran.

Materials and Methods: This cross-sectional study was conducted on 253 hospitalized patients with prolonged fever despite receiving broad-spectrum antibiotic therapy. Blood samples were collected aseptically, and then cultured using an automated blood culture system and conventional broth culture bottle. *Candida* isolates were identified at species level using morphological and physiological properties and produced color on the CHROMagar *Candida*. Furthermore, the antifungal susceptibility testing was performed using CLSI M27-A3 and CLSI M27-S4 (broth microdilution methods).

Results: The most positive cultures were detected by the automated blood culture system. *C. albicans* (%50) was the most prevalent species, followed by *C. glabrata* (%40), and *C. parapsilosis*, (%10) respectively. The mortality rate was high (%60) and most patients with candidemia were admitted to the Intensive Care Unit and Neonatal Intensive Care Unit. All isolates were susceptible to amphotericin B, while the highest resistance belonged to caspofungin.

Conclusion: In this study, high resistance was reported, especially for caspofungin, which can be regarded as the emergence of caspofungin-resistant strains. Regarding this, the establishment of a surveillance and prevention program for the reduction of the emergence of resistant species is necessary.

Keywords: Antifungal susceptibility, Candidemia, Iran, Kashan, Prolonged fever

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Introduction

Despite the therapeutic effectiveness of antifungal agents, the incidence of fungal infections has increased during the past few decades due to the higher use of chemotherapeutic and immunosuppressive agents and organ transplantation [1]. Out of more than 100 yeast species recognized as pathogens, only less than ten species are considered as common causes of fungal infections in humans [2]. *Candida* species are the most prevalent fungal pathogens. These species, especially *C. albicans*, are considered as the most important cause of fungal bloodstream infection (BSI) described as candidemia.

However, the incidence of candidemia due to non-*albicans* species (e.g., *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*) have increased during

the recent years [1, 3, 4]. In addition, *Candida* species are known as the fifth common cause of nosocomial infection and the fourth common cause of nosocomial BSI (8-10%) [5-7]. Despite the effectiveness of the antifungal therapy, candidemia remains an important cause of human mortality (30-60%). This increase mostly occurs among the susceptible patients with intense disruption in the immune system. This condition is mostly observed among those admitted to the Intensive Care Units (ICUs) [8].

There are numerous predisposing factors for candidemia some of which are more familiar such as using central venous catheter, total parenteral nutrition, prolonged and broad-spectrum antibiotic therapy, previous steroid therapy, undergoing repeated

abdominal sur-geries, and low birthweight. Most of these predisposing factors usually occur when the patients are admitted to the internal medicine units and ICUs [9, 10]. Therefore, this can explain why more than 50% of all candidemia episodes occur in the patients admitted to these units [11].

Resistance to antifungal agents is becoming more common among the *Candida* spp., especially in non-*albicans* species such as *C. glabrata*, which is inherently or secondarily resistant to fluconazole. Amphotericin B is considered as a selected treatment for the systemic fungal infections; nevertheless, resistance to amphotericin B has been reported for some species such as *C. lusitaniae*, *C. guilliermondii*, and *C. kefyr* [12, 13]. Resistance to antifungal agents has a high impact on the fungal infection prognoses and is significantly correlated with increased treatment failure, mortality, and prolonged hospital stay.

The isolation of the *Candida* spp. from blood culture using traditional aerobic and anaerobic bacteriological media can detect the occurrence of candidemia. However, the chance of fungal isolation from blood stream by these media is limited and estimated to be about 50% in the patients with invasive candidiasis. Furthermore, this method requires additional blood samples, an increasing amount of incubator space, and additional processing, which are time-consuming and need more experience [14]. On the other hand, the BACTEC blood culture system (Becton Dickinson Diagnostic Systems), which is specifically formulated for this purpose, can provide a faster and more efficient method for fungal isolation from blood stream [15].

Since few hospital centers in Iran are accustomed to using the automated blood culture system as their main method, the traditional aerobic and anaerobic blood cultures remain as a routine media for fungal blood culture in the majority of the hospitals. However, this method provides limited information about the *Candida* spp. detection and identification at the species level and their antifungal susceptibility patterns, which are necessary for the selection of appropriate antifungal medications, especially in the patients with candidemia.

Regarding this, the aim of the current study was to assess the fungal epide-miology of candidemia and the antifungal susceptibility patterns against five current antifungal agents including fluconazole, itraconazole, voriconazole, amphotericin B, and caspofungin among the patients with prolonged fever.

Materials and Methods

Study population

This cross-sectional study was conducted on

253 patients with persistent fever, who were suspected of candidemia based on the clinical symptoms, admitted to the Beheshti Educational Hospital, Kashan, Iran, during May 2011–November 2013. These patients had prolonged fever in spite of receiving broad-spec-trum antibiotic therapy for more than seven days. The study procedure was approved by the Medical Research Ethics Committee of Kashan University of Medical Sciences. All the patients were enrolled in the study after obtaining their informed consents.

Clinical data

The demographic and clinical data collected in this study were age, gender, duration of stay in the ICU, current history of broad-spectrum antibiotic therapy for more than seven days, antifungal therapy, presence of central venous catheter (CVC), urinary catheter (UC), mechanical ventilation (MV), and history of surgery or other predisposing factors, and clinical outcomes.

Fungal cultures

During this study, 361 blood samples (i.e., approximately 20 ml for each patient) were collected aseptically from 232 patients via venipuncture using a sterile syringe after skin disinfection through a standard technique [16]. The equal volumes of blood samples were inoculated into the following two media: the biphasic fungal media containing the brain heart in-fusion (BHI) broth and BHI Agar (Tebo sadegh, Kashan, Iran). Subsequently, the samples were incubated at 37°C for at least two weeks and the remaining processes were implemented following the procedures previously described by Lotfi et al. [17]. The BACTEC Myco/F Lytic culture vials (Becton Dickinson, Le Pont de Claix, France) were placed in the BACTEC 9120 System (Becton Dickinson Microbiology Systems, Maryland, DE, USA), and automatically controlled every 10 min. The conventional broth bottles and BACTEC bottles with sign of fungal growth were sub-cultured on the plates containing the BHI agar (Merck, Germany) and Sabouraud dextrose agar (Biolife, Italy) supplemented with chlor-amphenicol (0.5µg/ml) following the previously described study [18].

The isolated yeasts were identified at species level using morpho-logical and physiological properties and produced color in CHROM agar *Candida* (bioMérieux, Marcy l’Etoile, France). Since one strain could not be identified based on the color, it was identified by polymerase chain

reaction (PCR) sequencing using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') for amplification of the ITS1-5.8 S rRNA-ITS2 regions according to the method described previously by Ghahri et al. [1].

The PCR product was subjected to DNA sequencing with the same primers. The obtained sequences were analyzed in the GenBank database. The identified species were inoculated for further investigation into the tubes containing BHI broth with 20% glycerol and kept at -70°C.

***In vitro* antifungal susceptibility testing**

The antifungal susceptibility testing was performed using microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI, document M27-A3) [19]. Interpretative breakpoints were assessed according to the MICs ($\mu\text{g/mL}$) from the 24-h M27-S4 [20] and M27-A3 CLSI broth microdilution methods [19].

The antifungal drugs were obtained from the following companies: amphotericin B (Sigma-Aldrich, USA.), fluconazole (Sigma-Aldrich, USA.), itraconazole (Sigma-Aldrich, USA.), voriconazole (Pfizer Central Research, UK), and caspofungin (Merck, USA.). The selected antifungals were diluted in the standard Roswell Park Memorial Institute 1640 Medium with L-glutamine without bicarbonate (Sigma-Aldrich, USA.), buffered to pH 7.0 with 0.1653-(N-morpholino) propane sulfonic acid (MOPS) (Sigma-Aldrich, USA). Subsequently, they were dispensed in a 96-well microplate to supply twice stronger concentrations.

The final concentrations were 0.0313-16 $\mu\text{g/ml}$ for amphotericin B, itraconazole, and voriconazole; 0.125-64 $\mu\text{g/ml}$ for fluconazole; and 0.015-8 $\mu\text{g/ml}$ for caspofungin. The *Candida* isolates were sub-cultured on the Sabouraud dextrose agar and incubated at 35°C overnight. Subsequently, they were suspended in 5 mL of sterile water and vigorously vortexed. The turbidity was adjusted spectrophotometrically at 625 nm to achieve the 0.5 McFarland standard (approximately 1×10^6 to 5×10^6 CFU/mL). The suspension was diluted to 0.5×10^3 - 0.25×10^3 CFU/mL on the RPMI-1640 medium. Then, the microplates were inoculated with 100 μL of suspension and incubated at 35°C in a humid environment for 24 h.

The MICs were visually determined and described as the lowest drug concentration, which could create a prominent decrease in turbidity, compared to the drug-free growth control well. The MICs for amphotericin B were determined as

the lowest concentration at which no visible growths were detected. The clinical breakpoints (CBPs) values are not determined for all drugs in the CLSI M27-S4; therefore, the CBPs were identified based on both CLSI M27-S4 and M27-A3 broth microdilution methods.

Caspofungin MIC values of ≤ 0.25 and ≥ 1 $\mu\text{g/ml}$ were determined as susceptible and resistant to *C. albicans*; however, these MIC values for *C. glabrata* were ≤ 0.12 and ≥ 0.5 $\mu\text{g/ml}$, respectively. Regarding fluconazole, these values were ≤ 2 and ≤ 8 $\mu\text{g/ml}$ as susceptible and ≤ 8 and ≥ 64 $\mu\text{g/ml}$ as resistant to *C. albicans* and *C. glabrata*, respectively. In addition, for voriconazole and itraconazole, MIC value of ≤ 0.12 $\mu\text{g/ml}$ was defined as susceptible, whereas MIC values of ≥ 64 and ≥ 4 $\mu\text{g/ml}$ were determined as resistant to *C. albicans* and *C. glabrata*, respectively. Furthermore, the *C. albicans* isolates with MIC value of ≥ 2 $\mu\text{g/ml}$ were considered as resistant to amphotericin B. Each test was carried out in duplicates, and *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used as quality controls.

Results

During 30 months, a total of 10 episodes of candidemia were identified by BACTEC blood culture system, and six specimens were detected using the conventional culture methods. The mean age of the patients with positive cultures was 48.2 ± 30.9 years. As the results demonstrated, the highest positive cultures were observed in the females (60%). Moreover, the *C. albicans* (5, 50%) was the most prevalent species followed by *C. glabrata* (4, 40%) and *C. parapsilosis* (1, 10%). The mean duration of hospital stay was 83.2 ± 23 days.

Additionally, all patients had at least one underlying disease such as cancer (20%), diabetes (20%), premature birth (20%), as well as multiple trauma and vast surgery (40%). Moreover, 20% of the patients had multiple underlying diseases simultaneously such as major surgery and dialysis or diabetes and renal failure.

Among different predisposing factors, the use of central venous catheter was reported for all the patients, and the majority of the patients (80%) had the experience of antibiotic therapy during the hospital stay just before getting infected with candidemia. In addition, three patients (30%) had a medical device such as mechanical ventilation and urine catheter. As the results indicated, the mortality rate was high (60%) among these patients. Furthermore, many patients (70%) with candidemia were admitted to the ICU and Neonatal Intensive Care Unit (NICU). All the demographic and clinical

Table 1. Characteristics of patients with positive blood Culture admitted in Beheshti hospital

Patient ID	Sex	Age (years)	Hospitalization (Month)	Hospital unit	Underline disease	Antibiotic treatment	Isolated <i>Candida</i>	Medical device	MIC, µg/ml					Outcome
									FLU	ITZ	VZV	AmB	CASP	
1	M	52	2	ICU	Diabetes & Renal failure	+	<i>C. albicans</i>	CVC MV	2	0.25	0.031	0.5	1	died
2	M	2.7	2	NICU	Premature	+	<i>C. albicans</i>	CVC MV	2	0.125	0.25	0.25	0.125	died
3	M	61	0.4	IU	Cancer	-	<i>C. parapsilosis</i>	CVC	1	0.0625	0.125	0.125	0.125	survived
4	F	38	2	ICU	Multiple Trauma & Surgery	+	<i>C. albicans</i>	CVC	0.5	0.125	0.125	0.5	0.125	Coma
5	M	24	3	ICU	Multiple Trauma & Vast Surgery & Dialysis	+	<i>C. albicans</i>	CVC UC	0.25	0.125	0.25	0.5	0.125	died
6	F	60	5	ICU	Multiple Trauma & Vast Surgery	+	<i>C. albicans</i>	CVC	8	8	4	0.5	2	died
7	F	61	3	IU	Surgery (tracheotomy)	+	<i>C. glabrata</i>	CVC MV	4	16	1	1	0.5	died
8	F	75	5	ICU	Diabetes	+	<i>C. glabrata</i>	CVC UC MV	4	4	2	1	0.5	survived
9	F	41	4	ICU	Multiple Trauma & Vast Surgery	-	<i>C. glabrata</i>	CVC	4	4	2	1	0.5	Coma
10	F	70	1.1	IU	Cancer	+	<i>C. glabrata</i>	CVC MV	4	4	1	0.5	0.25	died

Abbreviations: ICU, intensive care unit; NICU, Neonatal intensive care unit; IU, internal unit; CVC, central venous catheter; MV, mechanical ventilation ; UC, urine catheter; MIC, minimal inhibitory concentration.

data are summarized in Table 1.

For amphotericin B, the breakpoint was not determined; however, several studies have reported ≤ 1 µg/ml as susceptible. All isolates (100%) were found to be susceptible to amphotericin B. nevertheless, for fluconazole and voriconazole, five, four, and one isolates were susceptible (50%), susceptible-dose dependent (40%), and resistant (10%), respectively.

Furthermore, regarding the itraconazole, four, five, and one isolates were susceptible (40%) susceptible-dose dependent (50%), and resistant (10%), respectively. More resistance (50%) belonged to caspofungin; in addition, four isolates

(40%) were susceptible, and one isolate (10%) was susceptible-dose dependent.

MIC ranges and geometric mean for all drugs are shown in Table 2.

Discussion

Candida spp. are considered as predominant causes of candidemia, morbidity, and mortality in the patients admitted to the ICU [21]. The high mortality rate (60%) found in the present study is in line with the findings of other studies, reporting between 38% and 76% mortality among the adults in the developing and developed countries [17, 22, 23].

The results also showed that 80% of the

Table 2. *In vitro* susceptibility testing of 10 *Candida* species isolated from blood of patients with candidemia to five antifungal agents

<i>Candida</i> species (n)	Antifungal agent MIC ^a , µg/ml ^b				
	Fluconazole	Itraconazole	Voriconazole	Amphotericin B	Caspofungin
<i>C. albicans</i> (5)	0.25-8 (1.31)	0.125-8 (0.24)	0.031- 4 (0.24)	0.125-0.5 (0.42)	0.125-2 (0.42)
<i>C. glabrata</i> (4)	4 (4)	4-16 (5.65)	1-2 (1.41)	0.5-1 (0.84)	0.25-0.5 (0.42)
<i>C. parapsilosis</i> (1)	1	0.0625	0.125	0.125	0.125

a Abbreviation: MIC, minimal inhibitory concentration.

b Range (Geometric Mean).

candidemia episodes occur in the patients admitted to the ICU, which was higher than the findings of the previous Latin American and European studies reporting 56.6% and 44.4%, respectively [24, 25]. Only in one study across five sites in Italy and Spain, a low rate (19.6%) was reported for the episodes of nosocomial candidemia in the ICU patients [26]. This discrepancy could be due to the variations in healthcare services and patients' immune status. Moreover, this predominant number of patients with candidemia in the ICU may be related to the intensity of immunosuppressive therapy and other common risk factors for candidemia in the ICU admitted patients.

During the past decades, some studies have reported a shift to the emergence of non-*albicans* as the common etiology of candidemia [16, 27]. Lotfi et al. reported *C. parapsilosis* and *C. tropicalis* as the most common *Candida* species isolated from burn patients with candidemia [17]. Inconsistent with their finding, *C. albicans* was the most common cause of candidemia found in this study. Based on the data collected for this study, *C. albicans* and non-*albicans* had an equal frequency; however, *C. albicans* was the predominant species. This finding is in line with that of a similar study carried out by Bassetti et al. [11]. Furthermore, *C. glabrata* was isolated as a predominant species among the non-*albicans* species (40%). The emergence of non-*albicans* species could be related to some medical conditions, which varied for each species [26].

Previous exposure to azoles was considered as the most predisposing factor for candidemia due to *C. glabrata*, which was the second isolated species in our study [25]. Therefore, empiric antibiotic therapy and such antifungal drugs as azoles, which is the common practice implemented in Beheshti Hospital, could be the greatest cause for the high prevalence of *C. glabrata* candidemia and limitation in candidemia detection. In several studies, *C. glabrata* has been reported to be more common in the elderly patients. In the present study, the minimum age of the patients with *C. glabrata* candidemia was 41 years, which was consistent with the reported results of the previous studies [22, 28, 29].

In our study, all strains were susceptible to amphotericin B, which is in line with the findings of some previous studies [30, 31]. However, inconsistent with this finding, several studies reported a few amphotericin B-resistant strains isolated from the patients with candidemia [32, 33]. Amphotericin B is the first choice treatment drug for candidemia in Beheshti Hospital. It is also considered as an antifungal agent with rapid fungicidal potency against the majority of *Candida*

spp. strains, which is also inexpensive. These findings can have important and valuable implications for the healthcare systems.

According to the CLSI M27-S4, the CBPs for caspofungin susceptibility were defined at ≤ 0.25 , ≤ 0.12 , and ≤ 2 $\mu\text{g/ml}$ for *C. albicans*, *C. glabrata*, and *parapsilosis*, respectively. More resistance to caspofungin (50%) was reported in this study, which is higher, compared to the results reported in other studies [11, 34].

Based on the results of several studies, caspofungin had the best effect against *Candida* spp. with resistance to fluconazole, itraconazole, and amphotericin B at the same time [35, 36]. A study examined the *Candida* spp. isolates recovered from the cancer patients with oropharyngeal candidiasis in the north of Iran. In the mentioned study, caspofungin had the best antifungal activity against the oropharyngeal non-*albicans* *Candida* spp. isolates, particularly among the species that had reduced susceptibility to amphotericin B, fluconazole, and itraconazole [37]. In addition, caspofungin was recommended as a suitable treatment for the oropharyngeal candidiasis [37]. More resistance was shown among *C. glabrata* isolates. However, the emergence of resistance to caspofungin among *C. glabrata* isolates has also been demonstrated during the treatment of a critically ill patient [38]. Inconsistent with the findings of other studies, in the present study, an increased resistance to azole group was observed, which was higher than the reported rates in isolates from the Europe (12.6%, 6.3%) [11, 39] and North America (6.6%) [40].

Voriconazole, a recently-discovered drug, showed 10% resistance in the current study. However, in a study that was conducted in the largest teaching hospital in Brazil, 97% of the *Candida* spp. isolated from candidemia were susceptible to voriconazole [41]. In a study carried out in a tertiary care institute in India, 56% of the *Candida* isolates were resistant [32]. Several studies have shown that fluconazole-resistant strains have higher MICs for voriconazole than the fluconazole-susceptible isolates [42-44]. However, the rate of resistance reported in this study could be justified by over-the-counter or empirical therapy by azoles, especially fluconazole for vaginal candidiasis [45].

Conclusion

This study suffered from several limitations including the low number of patients and hospitals. A single center of study could be affected by local health policy; therefore, the comparison and interpretation of the findings, especially antifungal susceptibility patterns, should be carried out

carefully. However, based on the findings of the present study, candidemia should be considered as a significant source of morbidity in *Kashan*. Regarding this, the authorities need to establish a surveillance and control program for candidemia prevention and the minimization of drug resistance.

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Author's contribution

M.N. and R.R. designed and managed the study. R.R. and M.MH. performed the specimen collection. M.E. performed the tests, and M.N. prepared the manuscript.

Conflicts of interest

No conflicts of interest declared.

Financial disclosure

There was no financial interest related to the materials of the manuscript.

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