Molecular epidemiology, risk factors, species distribution, antifungal susceptibility and outcome of candidemia in the capital of Iran: a prospective study

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Abstract

Background

Candidemia is a major cause of morbidity and mortality among patients receiving immunosuppressive therapy and those hospitalized with serious underlying diseases despite the commencement of antifungal therapy. We investigated the molecular epidemiology, clinical characteristics, comorbidity risk factors, species distribution, antifungal susceptibility profile, and outcome of candidemia to provide appropriate perspectives in Tehran, Iran.

Methods

A prospective observational study of all patients diagnosed with candidemia was performed at two referral teaching hospitals in Tehran. Demographic characteristics, underlying diseases, risk factors, clinical symptoms, and laboratory analyses were mined.

Results

One-hundred and fifty-two Candida isolates from 89 patients with candidemia were recovered. The overall crude mortality was 47.2%. The most common underlying disease was sepsis (48.3%) followed by malignancy (46.1%), renal failure/dialysis (43.8%), and Hypertension (40.0%). C. albicans (43.8%) was the most frequent causative agent followed by C. glabrata (21.3%), C. parapsilosis complex (15.7%), C. tropicalis (11.2%), and C. lusitaniae (3.4%). Result of antifungal susceptibility test show that activity of all the four azole antifungal agents was low against non-albicans Candida species, especially C. tropicalis.

Conclusion

Increase in non-albicans Candida species with reduced susceptibility to antifungal drugs might be alarming in high-risk patients. Therefore, accurate knowledge of predisposing factors and epidemiological patterns in candidemia are effective steps for managing and decreasing the mortality rate in candidemia.

1. Introduction

Despite advancement in clinical patient care, Candida species remain the most commonly encountered pathogens isolated from bloodstream infections (BSIs) globally and are associated with significant morbidity and mortality particularly, among hospitalized patients receiving immunosuppressive therapy or diagnosed with a serious underlying health condition [1, 2]. Depending on the yeast species, the mortality rate may vary from 30 to 85% [3, 4]. Although in general, C. albicans is still the leading cause of candidemia, a shift towards non-albicans Candida (NAC) species has been reported in recent years [5]. The changing face of candidemia is alarming because NAC species might be associated with increased mortality and antifungal drug resistance [6, 7]. Although empirical therapy partly depends on the epidemiological data, risk assessment for candidemia is critical for clinicians to commence appropriate empirical antifungal therapy [8]. There are several risk factors associated with candidemia such as exposure to broad-spectrum antibiotics, surgical procedures, prolonged use of central venous catheters, dialysis, use of corticosteroids, and cytotoxic chemotherapy, among others [9]. Again, the diagnosis of candidemia remains a challenging task, despite the development attained in the diagnosis of fungal BSIs during recent years [10–12]. Disparities in the epidemiology of candidemia exist between countries and this seriously influences the need for continuous surveillance to monitor the trend of the disease, the species distribution, and the emergence of antifungal drug resistance [13]. Although some studies have reported determinants of mortality in candidemia, their results were based on retrospective data and from a restricted viewpoint [14–17]. Accordingly, the reasons for the current poor outcome of candidemia are based on inadequate data. With this idea in mind, we investigated the molecular epidemiology, clinical characteristics, species distribution, antifungal susceptibility profiles, and outcome of candidemia among hospitalized patients in Tehran, the capital of Iran, to provide appropriate perspectives on these patients.

2. Materials And Methods

2.1. Study design and patient selection

This study was conducted on hospitalized candidemia patients from February to December 2018 at two tertiary care training centers (Imam Khomeini hospital complex and Shariati hospital) affiliated with Tehran University of Medical Sciences, Tehran, Iran. Imam Khomeini hospital complex is the largest referral center in the country; admitted 25,410 patients in 2018 alone. Meanwhile, Shariati Hospital - with oncology and transplantation services, cares for patients in need of kidney, heart, and bone marrow transplantation - admitted 16,130 patients in 2018. All methods were performed in accordance with the relevant guidelines and regulations. The studied population included all culture-positive Candida BSI patients irrespective of their age or gender. In this study, we defined nosocomial candidaemia as the occurrence of one or more Candida species culture-positive blood drawn at least 48 h after admission. We excluded nosocomial candidemia episodes that represented relapses but included fresh episodes that occurred during separate admissions as new cases. The patient's baseline characteristics, clinical, laboratory, and microbiological data were collected upon confirmation of candidemia. Data were extracted from the patients' hospital records using a standardized case report form and included the baseline characteristics...
(age, sex); microbiological parameters (Candida species); comorbidities (diabetes mellitus, pulmonary disease, chronic renal failure/hemodialysis, malignancy, cardiovascular diseases, human immunodeficiency virus [HIV] infection, viral hepatitis, sepsis, and neutropenia [absolute neutrophil count < 500 cells/mm$^3$]); invasive procedures (including the insertion of a central venous catheter [CVC], nasogastric tube, urinary catheterization, immunosuppressive therapy, and intubation) and other risk factors such as total parenteral nutrition (TPN) within 72 h prior to the onset of candidemia, clinical manifestations, use of broad-spectrum antibiotics etc; antifungal therapy, and outcome parameters - hospital mortality (i.e., death within 30 days of the first documented candidemia episode). In cases where a patient had more than one episode of candidemia, the first episode was used in the risk factor analysis.

### 2.2. Clinical specimens and identification of Candida species

Blood samples were aseptically obtained from patients with suspected bloodstream infections. The samples were inoculated in aerobic blood culture medium bottles (BacT/ALERT® Culture Media/bioMerieux) and incubated within the automated Bactec system (BACT/ALERT® 3D). Through observation, we identified and then subcultured the initial positive blood cultures onto Sabouraud dextrose agar (SDA) supplemented with 0.5% chloramphenicol and incubated at 37°C for 24 to 48 hours. Yeast-like colonies were sub-cultured on CHROMagar Candida medium (CHROMagar Company, Paris, France) to ensure purity, and then identified using the automated Vitek 2 YST ID Card system (bioMérieux, Marcy-L’Etoile, France), according to the manufacturer's instructions [18]. Molecular identification was conducted for all recovered isolates. Briefly, we extracted the genomic DNA from cultures grown on SDA using the Genomic Extraction Kit (GeneAll, Korea), according to the manufacturer's instructions, and stored at −20 °C till next use. The internal transcribed spacer rDNA region (ITS1-5.8S-ITS2) was amplified and sequenced using ITS1 and ITS4 primers, as previously described by Leaw et al. [19]. Thereafter, we performed a bidirectional chain terminated Sanger sequencing with the same primers used for the amplification. We processed the sequence data using the Lasergene SeqMan software (version 9.0.4, DNASTAR) and aligned the results with the data in the GenBank database (https://blast.ncbi.nlm.nih.gov) and the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands) research database (http://www.westerdijkinstitute.nl/). Identification was defined by > 99.5% sequence similarity, with ≥ 95% query coverage.

### 2.3. Antifungal susceptibility testing

Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute broth microdilution guidelines (CLSI-M27-A3 and M60) [20, 21]. All tests were performed in duplicate, on two different days. C. krusei (ATCC 6258) and C. parapsilosis (ATCC 22019) were used as quality control strains, as recommended by the CLSI. Data interpretation was based on clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) [21, 22].

### 2.4. Statistical analyses

We used the SPSS statistical software version 22.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis in this study. Whereas the median, mean, standard deviation (SD), maximum, and minimum values were used to describe quantitative data frequencies were used to describe categorical data. The Chi-square test or Fisher’s exact test was used to evaluate categorical variables and the Student’s t-test to evaluate continuous variables. Logistic regression analyses were performed to identify independent variables associated with candidemia due to *C. albicans* and non-*C. albicans* spp. and the final outcome.

### 3. Results:

#### 3.1. Patient characteristics and risk factors

Eighty-nine candidemia patients among 41,540 hospitalized patients were enrolled in this study. The demographic characteristics, clinical manifestation, types of the underlying disorder, comorbidities with increased risk of candidemia, medications, and outcome of the disease are summarized in Table 1. The incidence of candidemia in the two centers was 0.21%, overall. The patient’s age ranged from 21 days to 93 years, with a mean age of 49.6 years. The majority of patients (61/89; 68.5%) were over 40 years of age and only six patients were under 16 years. Forty-two patients (42/89; 47.2%) were male. The prevalence of candidemia in various hospitalized wards was as follows: 39.3% in the intensive care units (ICUs), 18.0% in hemato/oncology wards, 10.1% in the internal ward, 5.6% in the surgical ward, and 6.7% in the kidney/urology unit. The most common underlying disease was sepsis (43/89; 48.3%) followed by malignancy (41/89; 46.1%), renal failure/dialysis (39/89; 43.8%), hypertension (32/89; 40.0%) and lung disorders (28/89; 31.5%). The majority of patients had multiple risk factors. The most common risk factors for candidemia in the present study were central vein catheter (67/98; 75.3%), intubation (49/89; 52.8%), and urinary catheterization (46/89; 51.7%). Fever was the most frequent clinical manifestation of candidemia (59/89; 66.3%) followed by diarrhea (27/89; 30.3%), cough (22/89; 24.7%), chills (20/89; 22.5%) and pleural effusion (16/89; 18.0%).

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Page 3/13
| Characteristics                      | All cases (n = 89) | C. albicans (n = 39) | C. glabrata (n = 19) | C. parapsilosis (n = 14) | C. tropicalis (n = 10) | C. lusitaniae (n = 3) | C. kefyr (n = 1) | C. guilliermondii (n = 1) | C. krusei (n = 1) | C. dubliniensis (n = 1) |
|-------------------------------------|-------------------|---------------------|---------------------|-------------------------|-----------------------|----------------------|-----------------|---------------------------|-----------------|------------------------|
| **Demographic**                     |                   |                     |                     |                         |                       |                      |                 |                           |                 |                        |
| < 1 year                            | 3 (3.4)           | 2 (5.1)             | 1 (5.3)             | 0                       | 0                     | 0                    | 0               | 0                          | 0               | 0                      |
| 1–15 years                          | 3 (3.4)           | 1 (2.5)             | 1 (5.3)             | 0                       | 0                     | 0                    | 0               | 0                          | 0               | 0                      |
| 16–40 years                         | 22 (24.7)         | 10 (25.6)           | 0                   | 6 (42.9)                | 4 (40.0)              | 0                    | 1 (100)         | 1 (100)                   | 0               | 0                      |
| 41–60 years                         | 36 (40.4)         | 17 (43.6)           | 8 (42.1)            | 4 (28.6)                | 4 (40.0)              | 2 (66.7)             | 0               | 0                          | 1 (100)         | 0                      |
| > 60 years                          | 25 (28.1)         | 9 (23.1)            | 9 (47.4)            | 3 (21.4)                | 2 (20.0)              | 1 (33.3)             | 0               | 0                          | 0               | 1 (100)                |
| Mean age, years (range)             | 49.6 (1–93)       | 47.8 (1–86)         | 58.8 (1–147)        | 40.5 (14–79)            | 50.3 (27–89)          | 65.3 (50–93)         | 29              | 16                         | 53              | 65                     |
| Male sex (%)                        | 42 (47.2)         | 22 (56.4)           | 7 (36.8)            | 6 (42.9)                | 5 (50)                | 0                    | 1 (100)         | 0                          | 1 (100)         | 0                      |
| Median days in hospital (range)     | 42 (1–347)        | 41 (1–347)          | 42.6 (5–126)        | 89.1 (7192)             | 50.9 (1–130)          | 46 (31–54)           | 197             | 101                       | 30              | 4                      |
| **Hospital Ward**                   |                   |                     |                     |                         |                       |                      |                 |                           |                 |                        |
| ICUs                                | 35 (39.3)         | 16 (41.0)           | 7 (36.8)            | 6 (42.9)                | 3 (30.0)              | 2 (66.7)             | 1 (100)         | 0                          | 0               | 0                      |
| Hemato-oncology                     | 16 (18)           | 4 (10.3)            | 4 (21.1)            | 3 (21.4)                | 2 (20.0)              | 1 (33.3)             | 0               | 0                          | 1 (100)         | 1 (100)                |
| Internal                            | 9 (10.1)          | 2 (5.1)             | 2 (10.5)            | 2 (14.3)                | 2 (20.0)              | 0                    | 0               | 1 (100)                   | 0               | 0                      |
| Surgical                            | 5 (5.6)           | 3 (7.7)             | 0                  | 1 (7.1)                 | 1 (10.0)              | 0                    | 0               | 0                          | 0               | 0                      |
| Kidney/Urology                      | 6 (6.7)           | 3 (7.7)             | 3 (15.8)            | 0                       | 0                    | 0                    | 0               | 0                          | 0               | 0                      |
| Infectious                          | 5 (5.6)           | 2 (5.1)             | 1 (5.3)             | 1 (7.1)                 | 1 (10.0)              | 0                    | 0               | 0                          | 0               | 0                      |
| Other                               | 13 (14.6)         | 9 (23.1)            | 2 (10.5)            | 1 (7.1)                 | 1 (10.0)              | 0                    | 0               | 0                          | 0               | 0                      |
| **Underlying disease**              |                   |                     |                     |                         |                       |                      |                 |                           |                 |                        |
| DM (%)                              | 27 (30.3)         | 11 (28.2)           | 8 (42.1)            | 3 (21.4)                | 2 (20.0)              | 2 (66.7)             | 0               | 0                          | 1 (100)         | 0                      |
| Malignancy (%)                      | 41 (46.1)         | 11 (28.2)           | 11 (57.9)           | 8 (57.1)                | 7 (70.0)              | 1 (33.3)             | 0               | 1 (100)                   | 1 (100)         | 1 (100)                |
| HTN                                 | 32 (36.0)         | 15 (38.5)           | 10 (52.6)           | 3 (21.4)                | 2 (20.0)              | 2 (66.7)             | 0               | 0                          | 0               | 0                      |
| HIV infection (%)                   | 3 (3.4)           | 2 (5.1)             | 0                  | 1 (7.1)                 | 0                    | 0                    | 0               | 0                          | 0               | 0                      |
| Viral Hepatitis (%)                 | 6 (6.7)           | 4 (10.3)            | 1 (5.3)             | 1 (7.1)                 | 0                    | 0                    | 0               | 0                          | 0               | 0                      |
| Transplant (%)                      | 6 (6.7)           | 3 (7.7)             | 1 (5.3)             | 2 (14.2)                | 0                    | 0                    | 0               | 0                          | 0               | 0                      |
| Vascular and heart events (%)       | 26 (29.1)         | 10 (25.6)           | 9 (47.4)            | 4 (28.4)                | 2 (20.0)              | 1 (33.3)             | 0               | 0                          | 0               | 0                      |
| Renal failure/dialysis (%)          | 39 (43.8)         | 20 (51.3)           | 9 (47.4)            | 4 (28.6)                | 6 (60.0)              | 0                    | 0               | 0                          | 0               | 0                      |
| Sepsis (%)                          | 43 (48.3)         | 17 (43.6)           | 9 (47.4)            | 10 (71.4)               | 3 (30.0)              | 2 (66.7)             | 1 (100)         | 0                          | 1 (100)         | 0                      |
| Pneumonia/Lung diseases (%)         | 28 (31.5)         | 16 (41.0)           | 3 (15.8)            | 4 (28.4)                | 2 (20.0)              | 2 (66.7)             | 0               | 0                          | 0               | 1 (100)                |
| **Risk factors**                    |                   |                     |                     |                         |                       |                      |                 |                           |                 |                        |
| Characteristics          | All cases (n = 89) | C. albicans (n = 39) | C. glabrata (n = 19) | C. parapsilosis (n = 14) | C. tropicalis (n = 10) | C. lusitaniae (n = 3) | C. kefyr (n = 1) | C. guilliermondii (n = 1) | C. krusei (n = 1) | C. dubliniensis (n = 1) |
|--------------------------|-------------------|---------------------|---------------------|--------------------------|------------------------|-----------------------|----------------|--------------------------|----------------|-------------------------|
| CVC (%)                  | 67 (75.3)         | 28 (71.8)           | 15 (78.9)           | 11 (78.6)                | 6 (60.0)               | 3 (100)               | 1 (100) | 1 (100)                  | 1 (100) | 1 (100)                 |
| Urinary catheterization (%) | 46 (51.7)       | 18 (43.9)           | 11 (57.9)           | 9 (64.3)                 | 4 (40.0)               | 2 (66.7)               | 1 (100) | 0                        | 1 (100) | 0                      |
| Total Parenteral nutrition (%) | 10(11.2)        | 4 (9.8)             | 3 (15.8)            | 3 (21.4)                 | 0                      | 0                     | 0                   | 0                        | 0                   | 0                       |
| Immunosuppressive (%) therapy | 22 (24.7)      | 9 (22)              | 7 (31.6)            | 3 (35.7)                 | 2 (40.0)               | 0                     | 0                   | 1 (100)                  | 0                   | 0                      |
| Neutropenia (%)          | 15 (16.9)         | 4 (9.8)             | 3 (15.8)            | 5 (35.7)                 | 2 (40.0)               | 0                     | 0                   | 1 (100)                  | 0                   | 0                      |
| Intubation (%)           | 47 (52.8)         | 19 (48.7)           | 10 (52.6)           | 8 (57.1)                 | 6 (60.0)               | 2 (66.7)               | 1 (100) | 0                        | 0                   | 1 (100)                 |

### Clinical manifestations

| Fever (%)               | 59 (66.3)         | 29 (70.7)           | 12 (63.2)            | 8 (57.1)                | 5 (50.0)               | 3 (100)               | 0                   | 1 (100)                  | 1 (100) | 0                      |
| Diarrhea (%)            | 27 (30.3)         | 12 (29.3)           | 6 (31.6)            | 5 (35.7)                | 3 (30.0)               | 1 (33.3)               | 0                   | 0                        | 0                   | 0                      |
| Cough (%)               | 22 (24.7)         | 13 (31.7)           | 4 (21.1)            | 3 (21.4)                | 2 (40.0)               | 0                     | 0                   | 0                        | 0                   | 0                      |
| Chills (%)              | 20 (22.5)         | 10 (24.4)           | 4 (21.1)            | 5 (35.7)                | 1 (10.0)               | 0                     | 0                   | 0                        | 0                   | 0                      |
| Pleural effusion (%)    | 16 (18.0)         | 6 (14.6)            | 2 (10.5)            | 4 (28.6)                | 2 (20.0)               | 1 (33.3)               | 1 (100)             | 0                        | 0                   | 0                      |
| Sputum (%)              | 15 (16.9)         | 7 (17.1)            | 3 (15.8)            | 3 (21.4)                | 2 (20.0)               | 0                     | 0                   | 0                        | 0                   | 0                      |
| Peritonitis (%)         | 11 (12.4)         | 6 (14.6)            | 3 (15.8)            | 1 (7.1)                 | 1 (10.0)               | 0                     | 0                   | 0                        | 0                   | 0                      |
| Dysuria (%)             | 11 (12.4)         | 6 (14.6)            | 2 (10.5)            | 3 (21.4)                | 0                      | 0                     | 0                   | 0                        | 0                   | 0                      |
| Ascites (%)             | 11 (12.4)         | 6 (14.6)            | 3 (15.8)            | 1 (7.1)                 | 0                      | 1 (33.3)               | 0                   | 0                        | 0                   | 0                      |
| Seizures (%)            | 7 (7.9)           | 2 (4.9)             | 2 (10.5)            | 2 (14.3)                | 1 (10.0)               | 0                     | 0                   | 0                        | 0                   | 0                      |
| Candiduria (%)          | 29 (32.6)         | 11 (28.2)           | 8 (42.1)            | 4 (28.6)                | 3 (30.0)               | 2 (66.7)               | 0                   | 0                        | 0                   | 1 (100)                 |

### Medication

| Broad spectrum antibiotic (%) | 82 (92.1) | 35 (89.7) | 19 (100) | 14 (100) | 8 (80.0) | 3 (100) | 1 (100) | 0 | 1 (100) | 1 (100) |
| Corticosteroid (%)            | 22 (24.7) | 9 (23.1)  | 6 (31.6) | 4 (28.6) | 2 (20.0) | 0       | 0       | 0 | 1 (100) | 0       |
| Fluconazole (%)               | 33 (37.1) | 13 (33.3) | 7 (36.8) | 8 (57.1) | 3 (30.0) | 0       | 0       | 0 | 1 (100) | 1 (100) |
| Amphotericin B (%)            | 22 (24.7) | 7 (17.9)  | 4 (21.0) | 8 (57.1) | 2 (20.0) | 0       | 0       | 0 | 1 (100) | 0       |
| Caspofungin (%)               | 29 (32.6) | 9 (23.0)  | 10 (52.6) | 7 (50.0) | 2 (20.0) | 1 (33.3) | 0 | 0 | 0 | 0 |
| Nystatin (%)                 | 3 (3.4)  | 1 (2.6)   | 1 (5.2)  | 1 (7.1)  | 0       | 0       | 0       | 0 | 0 | 0 |
| Voriconazole (%)              | 7 (7.7)  | 3 (7.7)   | 1 (5.2)  | 3 (21.4) | 0       | 0       | 0       | 0 | 0 | 0 |
| Clofibratezole (%)            | 4 (4.5)  | 2 (5.1)   | 1 (5.2)  | 1 (7.1)  | 0       | 0       | 0       | 0 | 0 | 0 |

### Outcome
### 3.2. Distribution of *Candida* species

In the study, 152 *Candida* isolates were recovered as agents of candidemia in culture-positive blood from 89 patients. Forty-four patients (44/89; 49.4%) had a single positive blood culture, while 35 cases with two positive cultures, 6 cases with three positive cultures, 2 cases with four positive cultures, 1 case with five positive cultures, and 1 patient with seven positive cultures. The leading agents of nosocomial candidemia were *C. albicans* (39/89; 43.8%), followed by *C. glabrata* (19/89; 21.3%), *C. parapsilosis* complex (14/89; 15.7%), *C. tropicalis* (10/89; 11.2%), *C. lusitaniae* (3/89; 3.4%), and other species (*C. dubliniensis*, *C. kefyr*, *C. krusei* and *C. guilliermondii*) (4/89; 4.4%). Table 1 illustrates the distribution of *Candida* species per patients’ age categories. *Candida albicans*, *C. glabrata*, and *C. parapsilosis* were the most common in the age range of 41–60 years (17/39; 43.6%), > 60 years (9/19; 47.4%), and 16–40 years (6/14; 42.9%), respectively. The common *Candida* species – [*C. albicans* (16/39; 41.0%), *C. glabrata* (7/19; 36.8%), *C. parapsilosis* complex (6/14; 42.9%), and *C. tropicalis* (3/10; 30%)] – were more frequently isolated from ICU. Based on *Candida* species, fever (29/39; 74.4%) and cough (13/39; 33.3%) were more prevalent in *C. albicans* infections, while diarrhea was more prevalent in *C. glabrata* (6/19; 31.6%) and *C. parapsilosis* complex (5/14; 35.7%) and pleural effusion in *C. parapsilosis* infections (4/14; 28.6%). The most common underlying disease in *C. albicans*, *C. glabrata*, *C. parapsilosis* complex, and *C. tropicalis* infection were renal failure/dialysis (20/39; 51.3%), malignancy (11/19; 57.9%), sepsis (10/14; 71.4%), and malignancy (7/10; 70%), respectively. In all the cases, CVC was the most common risk factor of candidemia in *C. albicans* (28/39; 71.8%), *C. glabrata* (15/19; 78.9%), *C. parapsilosis* (11/14; 78.6), and *C. tropicalis* (6/10; 60%) infection. Multivariate analysis of risk factors for candidemia due to *C. albicans* and NAC species was summarized in Table 2. This analysis revealed that malignancy was an independent risk factor for candidemia (*P* = 0.013).

#### Table 2

**Multivariate analysis of risk factors for candidemia due to *Candida albicans* and non-albicans Candida**

| Characteristics                  | Population data (n = 89) | 
|----------------------------------|--------------------------|
|                                  | *C. albicans* (n = 39)   | *Non-albicans Candida* (n = 50) | OR (95% CI) | p-value |
| Gender, male                     |                          |                             | 0.62 (0.27, 1.43) | 0.26    |
| Mean age, years (range)          | 47.8 (1–86)              | 50.9 (1–93)                 | 1.01 (0.99, 1.02) | 0.59    |
| **Predisposing factors**         |                          |                             |               |         |
| DM                               | 11                       | 16                          | 1.36 (0.55, 3.41) | 0.51    |
| Malignancy                       | 11                       | 30                          | 3.01 (1.26, 7.22) | 0.013   |
| HIV infection                    | 2                        | 1                           | 0.42 (0.04, 4.75) | 0.48    |
| Viral Hepatitis                  | 4                        | 2                           | 0.40 (0.07, 2.32) | 0.31    |
| Transplant                       | 3                        | 3                           | 0.84 (0.16, 4.43) | 0.84    |
| Vascular and heart events        | 10                       | 16                          | 1.55 (0.61, 3.94) | 0.36    |
| Renal failure/ dialysis          | 20                       | 19                          | 0.62 (0.22, 1.76) | 0.37    |
| Sepsis                           | 17                       | 26                          | 1.67 (0.72, 3.87) | 0.23    |
| Pneumonia/Lung diseases          | 16                       | 12                          | 0.52 (0.21, 1.29) | 0.16    |
| **Underlying risk factors**      |                          |                             |               |         |
| CVC                              | 28                       | 39                          | 1.23 (0.47, 3.24) | 0.67    |
| Urinary catheterization          | 18                       | 28                          | 1.79 (0.77, 4.15) | 0.18    |
| Total Parenteral nutrition       | 4                        | 6                           | 1.32 (0.35, 5.05) | 0.68    |
| Immunosuppressive therapy        | 9                        | 13                          | 1.32 (0.50, 3.50) | 0.58    |
| Neutropenia                      | 4                        | 11                          | 2.75 (0.80, 9.42) | 0.11    |
| Intubation                       | 19                       | 28                          | 1.62 (0.70, 3.76) | 0.26    |

**Abbreviations:** DM: diabetes mellitus, CVC: central vein catheter
3.3. Treatment and outcome

The analysis of patients with candidemia showed that either selection of antifungal drug or duration of antifungal therapy was inadequate. A total of 60 episodes of candidemia (60/89; 67.4%) received antifungal therapy. Initial treatment was with uconazole in 33 patients (33/89; 37.1%), caspofungin in 29 patients (29/89; 32.6%), liposomal amphotericin B in 22 patients (22/89; 24.7%), and 14 patients (14/89; 15.7%) received other antifungal agents (e.g., voriconazole, clotrimazole, and nystatin) (Table 1). Meanwhile, 29 patients (29/89; 32.6%) did not receive any antifungal drug out of which 19 (19/29; 65.5%) patients expired. The crude mortality rate among 89 patients with candidemia was 47.2% (42/89). Mortality was similar among those infected with C. albicans (19/39; 48.7%) and C. glabrata (9/19; 47.4%), but lower in patients with C. parapsilosis (5/14; 35.7%) than other species. In the multivariate analyses of risk factors for BSIs mortality, intubation ($P = 0.001$) and urinary catheterization ($P = 0.03$) were independent risk factors for mortality (Table 3).

### Table 3

| Characteristics | 30-day outcome | Logistic Regression analysis |
|-----------------|----------------|-----------------------------|
|                 | Survival (n = 47) | Death (n = 42) | P-value | OR (95% CI) | P-value |
| Gender, Male (n, %) | 24 (51.1) | 18 (42.8) | 0.44 | 0.72 (0.31, 1.66) | 0.44 |
| Age (years, Mean ± SD) | 45.43 ± 25.39 | 54.24 ± 17.92 | 0.06 | 1.02 (0.99, 1.04) | 0.07 |
| Underlying diseases | | | | | |
| DM | 15 (31.9) | 12 (28.6) | 0.73 | 0.85 (0.34, 2.11) | 0.73 |
| Malignancy | 19 (40.4) | 22 (52.4) | 0.26 | 1.62 (0.70, 3.76) | 0.26 |
| Viral Hepatitis | 1 (2.1) | 5 (11.9) | 0.10 | 6.22 (0.70, 55.56) | 0.10 |
| Transplant | 3 (6.4) | 3 (7.1) | > 0.99 | 1.13 (0.22, 5.92) | 0.89 |
| Vascular and heart events | 14 (29.8) | 12 (28.6) | 0.90 | 0.94 (0.38, 2.36) | 0.90 |
| Renal failure/ dialysis | 16 (34.0) | 23 (54.8) | 0.79 | 0.87 (0.31, 2.46) | 0.79 |
| Sepsis | 19 (40.4) | 24 (57.1) | 0.12 | 1.97 (0.85, 4.57) | 0.12 |
| Pneumonia/Lung diseases | 12 (25.3) | 16 (38.1) | 0.20 | 1.80 (0.73, 4.43) | 0.21 |
| Risk factors | | | | | |
| CVC | 32 (68.1) | 35 (83.3) | 0.10 | 2.34 (0.85, 6.48) | 0.10 |
| Urinary catheterization | 19 (40.4) | 27 (64.3) | 0.03 | 2.65 (1.12, 6.26) | 0.03 |
| Total Parenteral nutrition | 5 (10.6) | 5 (11.9) | > 0.99 | 1.14 (0.30, 4.23) | 0.85 |
| Immunosuppressive therapy | 12 (25.5) | 10 (23.8) | 0.85 | 0.91 (0.35, 2.40) | 0.85 |
| Neutropenia | 11 (23.4) | 4 (9.5) | 0.08 | 0.34 (0.10, 1.18) | 0.09 |
| Intubation | 18 (38.3) | 31 (73.8) | 0.001 | 4.41 (1.80, 10.80) | 0.001 |

### Abbreviations:
DM: diabetes mellitus, CVC: central vein catheter

3.4. Antifungal susceptibility testing

Table 4 depicts the MIC ranges, MIC$_{50}$, MIC$_{90}$, geometric means (GM) MIC, and MIC modes of eight antifungal drugs against 152 Candida isolates recovered from 89 episodes of candidemia. In terms of MIC$_{50}$ and MIC$_{90}$, echinocandins demonstrated the highest MIC against C. parapsilosis (1 µg/ml and 4 µg/ml for caspofungin, 2 µg/ml for micafungin, 2 µg/ml and 4 µg/ml for anidulafungin) which was higher than against other NAC. However, we did not detect resistance to caspofungin, micafungin, and anidulafungin in any of the C. tropicalis, C. guilliermondii, C. lusitaniae, C. krusei, and C. kefyr. Among common species, C. albicans showed high susceptibilities to fluconazole (95.8%), while fluconazole susceptibility was lower in NAC species, particularly in C. tropicalis (S = 35.7%). Accordingly, C. glabrata exhibited the highest MIC$_{50}$ for fluconazole (16 µg/ml). The activity of all the four azoles was low against NAC species, but C. albicans has a lower MIC$_{50}$ (0.5, 0.06, 0.015, and 0.03 µg/ml) and MIC$_{90}$ (1, 0.12, 0.03, and 0.06 µg/ml) for fluconazole, itraconazole, voriconazole, and posaconazole, respectively.
Table 4

*In vitro* activities of eight antifungal agents tested for 152 *Candida* isolates obtained from 89 patients with candidemia

| Species (n) | Antifungal | MIC Range (µg/ml) | MIC<sub>50</sub> | MIC<sub>90</sub> | GM  | Mode | % S/WT* |
|------------|------------|-------------------|------------------|------------------|-----|------|--------|
| *C. albicans* (72) | FLU | 0.125-64 | 0.5 | 1.0 | 0.539 | 0.5 | 95.8 |
| ITR | 0.016-16 | 0.06 | 0.12 | 0.082 | 0.06 | 93.1 |
| VRC | 0.008-8 | 0.015 | 0.03 | 0.014 | 0.008 | 97.2 |
| AMB | 0.25-1 | 0.25 | 0.85 | 0.367 | 0.25 | 100 |
| CAS | 0.03-8 | 0.06 | 0.25 | 0.084 | 0.06 | 91.7 |
| MFG | 0.008-4 | 0.045 | 0.22 | 0.018 | 0.08 | 91.7 |
| ANI | 0.015-1 | 0.12 | 0.12 | 0.093 | 0.125 | 93 |
| PSO | 0.008-8 | 0.03 | 0.06 | 0.033 | 0.03 | 91.7 |
| *C. glabrata* (33) | FLU | 1–64 | 16 | 32 | 15.667 | 32 | 0 |
| ITR | 0.12-4 | 1 | 1.6 | 0.825 | 1 | 100 |
| VRC | 0.03-2 | 0.5 | 1 | 0.478 | 0.5 | 21.2 |
| AMB | 0.25-1 | 0.5 | 1 | 0.533 | 0.5 | 100 |
| CAS | 0.03-1 | 0.06 | 0.5 | 0.109 | 0.06 | 81.8 |
| MFG | 0.007-0.5 | 0.15 | 0.15 | 0.019 | 0.016 | 93.9 |
| ANI | 0.015-1 | 0.06 | 0.125 | 0.056 | 0.03 | 93.9 |
| PSO | 0.06-2 | 1 | 2 | 0.824 | 1 | 81.8 |
| *C. parapsilosis* (22) | FLU | 0.25-64 | 1 | 23.6 | 1.286 | 1 | 77.3 |
| ITR | 0.06-2 | 0.125 | 0.85 | 0.178 | 0.125 | 100 |
| VRC | 0.015-1 | 0.03 | 0.25 | 0.039 | 0.016 | 86.4 |
| AMB | 0.06-1 | 0.25 | 1 | 0.341 | 0.25 | 100 |
| CAS | 0.06-8 | 1 | 4 | 0.937 | 1 | 86.4 |
| MFG | 0.15-8 | 2 | 2 | 1.381 | 2 | 95.5 |
| ANI | 0.03-8 | 2 | 4 | 1.411 | 2 | 91 |
| PSO | 0.06-2 | 0.06 | 0.85 | 0.109 | 0.06 | 86.4 |
| *C. tropicalis* (14) | FLU | 1–64 | 6 | 64 | 5.656 | 1 | 35.7 |
| ITR | 0.06-16 | 0.75 | 16 | 1.047 | 0.25 | 50 |
| VRC | 0.03-16 | 0.75 | 16 | 0.703 | 1 | 14.3 |
| AMB | 0.5-2 | 0.5 | 1.5 | 0.672 | 0.5 | 100 |
| CAS | 0.03–0.25 | 0.12 | 0.25 | 0.100 | 0.06 | 100 |
| MFG | 0.007–0.15 | 0.03 | 0.105 | 0.030 | 0.03 | 100 |
| ANI | 0.015–0.12 | 0.06 | 0.12 | 0.066 | 0.06 | 100 |
| PSO | 0.06-1 | 0.375 | 1 | 0.285 | 0.5 | 35.7 |
| *C. lusitaniae* (4) | FLU | 1–4 | 2.5 | ND | 2 | ND | 100 |
| ITR | 0.12-1 | 0.5 | ND | 0.416 | 0.5 | 100 |
| VRC | 0.015–0.25 | 0.0375 | ND | 0.042 | 0.016 | 100 |
| AMB | 0.25-1 | 0.5 | ND | 0.5 | 0.5 | 100 |

*Abbreviations.* GM, geometric mean; S: susceptible; WT: wild-type; AMB: amphotericin B; FLU: fluconazole; ITR: itraconazole; VRC: voriconazole; POS: posaconazole; AFG: anidulafungin; MFG: micafungin.

* Percentage of susceptible wild-type isolates based on the clinical breakpoint values or epidemiological cutoff values.
| Species (n) | Antifungal | MIC Range (µg/ml) | MIC<sub>50</sub> | MIC<sub>90</sub> | GM | Mode | % S/WT* |
|------------|------------|-------------------|------------------|------------------|----|------|---------|
| CAS        | 0.25–0.5   | 0.375             | ND               | 0.353            | ND | 100  |
| MFG        | 0.06–0.25  | 0.185             | ND               | 0.145            | 0.25| 100  |
| ANI        | 0.125–0.25 | 0.25              | ND               | 0.210            | 0.25| 100  |
| PSO        | 0.03–0.12  | 0.03              | ND               | 0.084            | 0.125| 25   |
|            |            |                   |                  |                  |     |      |         |
| C. guiliermondii (3) | FLU | 2                 | 0.25             | 0.25             | 2   | 100  |
|            | ITR | 0.25              | 0.25             | ND               | 0.25| 100  |
|            | VRC | 0.06              | 0.185            | ND               | 0.06| 100  |
|            | AMB | 0.25–0.5          | 0.25             | ND               | 0.314| 100 |
|            | CAS | 0.25              | 0.25             | ND               | 0.25| 100  |
|            | MFG | 0.5               | 0.5              | ND               | 0.5 | 100  |
|            | ANI | 0.5–1             | 0.5              | ND               | 0.629| 100 |
|            | PSO | 0.12–0.25         | 0.12             | ND               | 0.153| 100 |
| C. dubliniensis (2) | FLU | 0.25–0.5          | ND               | ND               | ND  | 100  |
|            | ITR | 0.016             | ND               | ND               | ND  | 100  |
|            | VRC | 0.016             | ND               | ND               | ND  | 100  |
|            | AMB | 0.125–0.25        | ND               | ND               | ND  | 100  |
|            | CAS | 0.06              | ND               | ND               | ND  | 100  |
|            | MFG | 0.008             | ND               | ND               | ND  | 100  |
|            | ANI | 0.12              | ND               | ND               | ND  | 100  |
|            | PSO | 0.03              | ND               | ND               | ND  | 100  |
| C. krusei (1) | FLU | 8                 | ND               | ND               | ND  | 100  |
|            | ITR | 1                 | ND               | ND               | ND  | 100  |
|            | VRC | 1                 | ND               | ND               | ND  | 100  |
|            | AMB | 1                 | ND               | ND               | ND  | 100  |
|            | CAS | 0.5               | ND               | ND               | ND  | 100  |
|            | MFG | 0.125             | ND               | ND               | ND  | 100  |
|            | ANI | 0.06              | ND               | ND               | ND  | 100  |
|            | PSO | 0.125             | ND               | ND               | ND  | 100  |
| C. kefyr (1) | FLU | 0.5               | ND               | ND               | ND  | 100  |
|            | ITR | 0.25              | ND               | ND               | ND  | 100  |
|            | VRC | 0.06              | ND               | ND               | ND  | 100  |
|            | AMB | 0.5               | ND               | ND               | ND  | 100  |
|            | CAS | 2                 | ND               | ND               | ND  | 100  |
|            | MFG | 0.5               | ND               | ND               | ND  | 100  |
|            | ANI | 0.5               | ND               | ND               | ND  | 100  |
|            | PSO | 0.016             | ND               | ND               | ND  | 100  |

**Abbreviations:** GM, geometric mean; S: susceptible; WT: wild-type; AMB: amphotericin B; FLU: fluconazole; ITR: itraconazole; VRC: voriconazole; POS: posaconazole; AFG: anidulafungin; MFG: micafungin.

* Percentage of susceptible wild-type isolates based on the clinical breakpoint values or epidemiological cutoff values.

4. Discussion
We focused on candidemia patients and found that *C. albicans* was the most prevalent of the candidemia episodes (43.8%). Among the NAC species, *C. glabrata* was the predominant species, followed by *C. parapsilosis, C. tropicalis, and C. lusitaniae*. In most previous studies around the world, *C. albicans* was the most common species isolated from candidemia, which is consistent with our study [13]. Despite this, in a systematic review, *C. parapsilosis* (30.8%) was the leading agent of candidemia in Iran [23]. Similar to previous studies, *C. glabrata* is the most common NAC species in this study [24–26]. However, in other studies, *C. parapsilosis* has been the most prevalent NAC species [27–30]. Moreover, the increasing mortality rate associated with the increased frequency of NAC might be linked to the failure in clinical cure rate resulting from either acquired or intrinsic resistance to the few antifungal drugs available to treat candidemia. For example, echinocandins are regarded as the first-line drug for the treatment of *C. glabrata* BSI. However, the increasing reports on BSI caused by *C. glabrata* isolates resistant to both fluconazole and the echinocandins are alarming. It has been shown that *C. lusitaniae* can rapidly acquire multidrug resistance traits (MDR) during the course of antifungal treatment with fluconazole, amphotericin B, and caspofungin [31]. *C. kefyr* can cause serious infection in patients with hematologic malignancies and recently, resistance to amphotericin B have been reported in *C. kefyr* isolates [32]. *C. norvegensis*, is also shown to be azole resistant [33]. While 12.5% of the NAC species in this study were fluconazole-resistant, only 4.2% of the *C. albicans* species were resistant to fluconazole. Our study confirmed that primary fluconazole resistance is uncommon in *C. albicans*. The majority of *C. albicans* are sensitive to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and echinocandins in vitro, especially in patients without a history of exposure to antifungal agents [34]. A recent study looked at the impact of the new CLSI breakpoints and demonstrated that applying revised fluconazole breakpoints increased the rate of fluconazole resistance in *C. albicans, C. tropicalis, and C. parapsilosis* [35]. The occurrence of fluconazole resistance in *C. tropicalis* has been previously reported as 5.0–7.2% from two reports from the ARTEMIS study over 12 years [36, 37]. The high rate of azole non-susceptible *C. tropicalis* in this study was similar to other studies from Asia [38, 39]. Also among the fluconazole-resistant *C. tropicalis* isolates in our study, six were resistant to voriconazole. Many candidemia studies revealed a significant increase in azole-resistant *C. tropicalis* blood isolates and some reported pan-azole and amphotericin B resistant isolates [40]. An extensive candidemia study in India showed that *C. tropicalis* and *C. auris* isolates also carry MDR trait [41]. The susceptible dose-dependent (SDD) C. glabrata isolate is defined as fluconazole MIC ≤ 32 mg/l, since 2012. Overall, 96.7% of *C. glabrata* isolates were categorized as SDD (MIC<sub>90</sub> = 16 mg/l, MIC<sub>90</sub> = 32 mg/l) that is consistent with studies from the Asian-Pacific region [42]. As demonstrated in the current study, no resistance to any of the antifungal agents was observed in *C. guiliermondii, C. lusitaniae, C. kefyr*, and *C. krusei* (except for the intrinsic fluconazole resistance in *C. krusei*). Furthermore, resistance to echinocandins was very low, except for *C. parapsilosis*, which exhibited higher MICs than those of other *Candida* species.

The increased frequency of NAC may also be attributable to the improved diagnostic technique, allowing NAC species to be characterized with methods that are more sensitive. In this study, all *Candida* isolates were identified using the DNA sequencing method to assess the exact epidemiological pattern of species distribution. The mean age of the patients in this study was 49.6 years. Most of these patients were over forty years old (68.5%). The mean age in other studies varies from 40 to 65 years. Candidemia patients (usually with underlying conditions, such as diabetes, cancer, pulmonary and heart complications) mostly are admitted to ICUs. The overall crude mortality of 47.2% in our study is similar to that reported by other investigators from Iran [43–45] but considerably higher than the 26% quoted by Chen *et al.* [46]. Sixty patients took at least one antifungal drug and the mortality rate for these patients was 38.3% (23/60; 38.3%). However, the mortality rate among patients who did not receive antifungal drugs was 65.5% (19/29; 65.5%). This suggests that early diagnosis and timely antifungal administration can dramatically reduce the rate of mortality. We found that mortality was highest among ICU patients (65.7%) which is unsurprising, given the severity of underlying illness in this population. Evidence supports the fact that patients admitted in the ICU have higher mortality rates than those in other wards [47]. The major underlying diseases were malignancy, sepsis, renal failure/dialysis, and HTN. Diabetes mellitus, cardiovascular diseases, and pulmonary disorders were other underlying diseases. Consistently, similar underlying condition were documented in studies conducted in Turkey [48], China [9], and Australia [49]. Multivariate analyses of risk factors for BSIs caused by *C. albicans* and NAC species showed that malignancy was an independent risk factor for candidemia (*P* = 0.013). In the multivariate analyses of risk factors for BSIs mortality, intubation (*P* = 0.001) and urinary catheterization (*P* = 0.03) were independent risk factors for mortality. In a prospective study performed in the ICU of a tertiary care hospital in Athens, the authors noted that the administration of glucocorticoids, presence of CVCs, and candiduria were independent risk factors for candidemia caused by NAC species [49].

The current study had some limitations. First, in this study, two main medical centers in the capital of Iran had been selected; however, information from other centers has not been included in this study to obtain insights into the epidemiological status of candidemia in Tehran. Second, the results of the multivariate analyses might be influenced by the sample size and the number of variables included in the models.

In conclusion, Candidemia, with a shift in species distribution towards non-*albicans Candida* species, remains a lethal disease. The results of this study provide important information regarding the distribution of *Candida* spp in patients with candidemia in Tehran, the capital of Iran for which there is a paucity of data regarding the epidemiology, risk factors, and antifungal susceptibility patterns of these species. Accurate knowledge of predisposing factors and epidemiological patterns can be an effective step in disease management. In this study, *C. albicans* is reported to be the most common species causing candidemia; however, an increasing frequency of NAC species could pose a serious challenge to treatment due to different antifungal susceptibility patterns. This report shows that candidemia is a significant source of morbidity in Tehran.

**Abbreviations**

BSIs: bloodstream infections; NAC: non-*albicans Candida*; HIV: human immunodeficiency virus; CVC: central venous catheter; TPN: total parenteral nutrition; SDA: Sabouraud dextrose agar; CBPs: clinical breakpoints; ECVs: epidemiological cutoff values; ICU: intensive care unit; MDR: multidrug resistance; SDD: susceptible dose-dependent; DM: diabetes mellitus; CVC: central vein catheter; GM, geometric mean; S: susceptible; WT: wild-type; AMB: amphotericin B; FLU: fluconazole; ITR: itraconazole; VRC: voriconazole; POS: posaconazole; AFG: anidulafungin; MFG: micafungin
Declarations

Ethics approval and consent to participate

The Ethics Committee of Tehran University of Medical Science, Tehran, Iran (IR.TUMS.SPH.REC.1396.4195) approved the procedures to be used in this study. Besides, in line with the principles of research ethics, written informed consent was obtained from individual patients. Informed consent from parent and/or legal guardian/next of kin of minor participants and deceased patients with age less than 18 years and deceased (dead) patients data have been taken and approved by ethics committee. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

NOT APPLICABLE.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Conflict of interest:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Authors contribution

SKH, MK, SJH, and MRS designed the study. Material preparation, data collection and analysis were performed by SKH, MK, KA, FA, AL, SHM, AM, MM, NP, NA, and SSHF. The first draft of the manuscript was written by MK, SKH, AA, HB, and SR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Tortorano AM, Dho G, Prigittano A, Breda G, Grancini A, Emmi V et al. Invasive fungal infections in the intensive care unit: a multicentre, prospective, observational study in Italy (2006–2008). Mycoses. 2012;55(1):73-9.
2. Kourkoumpetis T, Manolakaki D, Velmahos G, Chang Y, Alam HB, De Moya MM et al. Candida infection and colonization among non-trauma emergency surgery patients. Virulence. 2010;1(5):359-66.
3. Meletiadis J, Arabatzis M, Bompola M, Tsiveriotis K, Hini S, Petinaki E et al. Comparative evaluation of three commercial identification systems using common and rare bloodstream yeast isolates. Journal of clinical microbiology. 2011;49(7):2722-7.
4. Kanafani ZA. Emerging opportunistic yeast infections. The Journal of Invasive Fungal Infections. 2011;5(2):56.
5. Kullberg BJ, Arendrup MC. Invasive candidiasis. The New England journal of medicine. 2016;374(8):794.
6. Miceli MH, Díaz JA, Lee SA. Emerging opportunistic yeast infections. The Lancet infectious diseases. 2011;11(2):142-51.
7. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. Journal of Antimicrobial Chemotherapy. 2018;73(suppl_1):i4-i13.
8. Gamacho-Montero J, Díaz-Martín A, García-Cabrera E, de Pipaón MR, Hernández-Caballero C, Aznar-Martín J et al. Risk factors for fluconazole-resistant candidemia. Antimicrobial agents and chemotherapy. 2010;54(8):3149-54.
9. Yang Z-T, Wu L, Liu X-Y, Zhou M, Li J, Wu J-Y et al. Epidemiology, species distribution and outcome of nosocomial Candida spp. bloodstream infection in Shanghai. BMC infectious diseases. 2014;14(1):241.
10. Gudlaugsson Q, Gillespie S, Lee K, Berg JV, Hu J, Messer S et al. Attributable mortality of nosocomial candidemia, revisited. Clinical Infectious Diseases. 2003;37(9):1172-7.
11. Morgan J, Meltzer MI, Plikaytis BD, Sofair AN, Huie-White S, Wilcox S et al. Excess mortality, hospital stay, and cost due to candidemia: a case-control study using data from population-based candidemia surveillance. Infection Control & Hospital Epidemiology. 2005;26(6):540-7.
12. Weems Jr JJ. Candida parapsilosis: epidemiology, pathogenicity, clinical manifestations, and antimicrobial susceptibility. Clinical Infectious Diseases. 1992;14(3):756-66.
13. Faglas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans Candida spp among candidemia isolates from inpatients in various parts of the world: a systematic review. International Journal of Infectious Diseases. 2010;14(11):e954-e66.

14. Kollef M, Micek S, Hampton N, Doherty JA, Kumar A. Septic shock attributed to Candida infection: importance of empiric therapy and source control. Clinical infectious diseases. 2012;54(12):1739-46.

15. Grim SA, Berger K, Teng C, Gupta S, Layden JE, Janda WM et al. Timing of susceptibility-based antifungal drug administration in patients with Candida bloodstream infection: correlation with outcomes. Journal of antimicrobial chemotherapy. 2011;67(3):707-14.

16. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clinical infectious diseases. 2012;54(8):1110-22.

17. Puig-Asensio M, Padilla B, Gamacho-Montero J, Zaragoza Q, Aguado J, Zaragoza R et al. Epidemiology and predictive factors for early and late mortality in Candida bloodstream infections: a population-based surveillance in Spain. Clinical Microbiology and Infection. 2014;20(4):0245-054.

18. Pincus DH. Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of Rapid Microbiological Methods Bethesda, MD: Parenteral Drug Association. 2006:1-32.

19. Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J Clin Microbiol. 2006;44(3):693-9. doi:10.1128/jcm.44.3.693-699.2006.

20. Clinical, Institute LS. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard-Third Edition M27-A3. 2008;28.

21. Clinical, Institute LS. Reference method for broth dilution antifungal susceptibility testing of yeasts: fourth informational supplement M27-S4. CLSI Wayne, PA, USA; 2012.

22. CLSI. Performance standard for antifungal susceptibility testing of yeast, 1st ed. CLSI supplement M59. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

23. Vaezi A, Fakhim H, Khodavaisy S, Alizadeh A, Nazari M, Soleimani A et al. Epidemiological and mycological characteristics of candidemia in Iran: A systematic review and meta-analysis. Journal de mycologie medicale. 2017;27(2):146-52.

24. Takakura S, Fujihara N, Saito T, Kudo T, Linuma Y, Ichiyama S. National surveillance of species distribution in blood isolates of Candida species in Japan and their susceptibility to six antifungal agents including voriconazole and micafungin. Journal of Antimicrobial Chemotherapy. 2004;52(3):283-9.

25. Das I, Nightingale P, Patel M, Jumaa P. Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK. International Journal of Infectious Diseases. 2011;15(11):e759-e63.

26. Zeppelin MB-v, Kunz L, Rüechel R, Reichard U, Weig M, Groß U. Epidemiology and antifungal susceptibilities of Candida spp to six antifungal agents: results from a surveillance study on fungaemia in Germany from July 2004 to August 2005. Journal of antimicrobial chemotherapy. 2007;60(2):424-8.

27. González GM, Elizondo M, Ayala J. Trends in species distribution and susceptibility of bloodstream isolates of Candida collected in Monterrey, Mexico, to seven antifungal agents: results of a 3-year (2004 to 2007) surveillance study. Journal of clinical microbiology. 2008;46(9):2902-5.

28. Cortés JA, Reyes P, Gómez C, Buitrago G, Leal AL. Fungal bloodstream infections in tertiary care hospitals in Colombia. Revista iberoamericana de micología. 2011;28(2):74-8.

29. Guinea J, Zaragoza Ó, Escobedo P, Martín-Mazuelos E, Péman J, Sánchez-Deus F et al. Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. Antimicrobial agents and chemotherapy. 2014;58(3):1529-37.

30. Tadec L, Talarmín JR, Gastinnette T, Bretonnière C, Miegeville M, Le Pape P et al. Epidemiology, risk factor, species distribution, antifungal resistance and outcome of Candidemia at a single French hospital: a 7-year study. Mycoses. 2016;59(5):296-303.

31. Asner SA, Giulieri S, Diezi M, Marchetti O, Sanglard D. Acquired multidrug antifungal resistance in Candida lusitaniae during therapy. Antimicrobial agents and chemotherapy. 2015;59(12):7715-22.

32. Dufresne SF, Marr KA, Sydoren H, Staab JF, Karp JE, Lu K et al. Epidemiology of Candida kefyr in patients with hematologic malignancies. Journal of clinical microbiology. 2015;53(2):424-8.

33. Sugita T, Takeo K, Ohkusu M, Virtudazo E, Takashima M, Asako E et al. Fluconazole resistant Pathogens Candida inconspicua and C. norvegensis: DNA Sequence Diversity of the rRNA Intergenic Spacer Region, Antifungal Drug Susceptibility, and Extracellular Enzyme Production. Microbiology and immunology. 2004;48(10):761-6.

34. Pfaffer M, Diekema D, Gibbs D, Newell V, Meis J, Gould I et al. Results from the ARTEMIS DISK Global Antifungal Surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of Candida species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. Journal of clinical microbiology. 2007;45(6):1735-45.

35. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal Susceptibility Testing: Current Approaches. Clinical Microbiology Reviews. 2020;33(3).

36. Pfaffer M, Diekema D, Gibbs D, Newell V, Ellis D, Tullio V et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. Journal of clinical microbiology. 2010;48(4):1366-77.
37. Pfaller M, Diekema D, Rinaldi MG, Barnes R, Hu B, Veselov A et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-year analysis of susceptibilities of Candida and other yeast species to fluconazole and voriconazole by standardized disk diffusion testing. Journal of clinical microbiology. 2005;43(12):5848-59.

38. Huang Y-T, Liu C-Y, Liao C-H, Chung K-R, Sheng W-H, Hsueh P-R. Antifungal susceptibilities of Candida isolates causing bloodstream infections at a medical center in Taiwan, 2009-2010. Antimicrobial agents and chemotherapy. 2014;58(7):3814-9.

39. Xiao M, Fan X, Chen SC-A, Wang H, Sun Z-Y, Liao K et al. Antifungal susceptibilities of Candida glabrata species complex, Candida krusei, Candida parapsilosis species complex and Candida tropicalis causing invasive candidiasis in China: 3 year national surveillance. Journal of Antimicrobial Chemotherapy. 2015;70(3):802-10.

40. Arastehfar A, Daneshnia F, Hafez A, Khodavaisy S, Najafzadeh M-J, Charsizadeh A et al. Antifungal susceptibility, genotyping, resistance mechanism, and clinical profile of Candida tropicalis blood isolates. Medical Mycology. 2019.

41. Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive care medicine. 2015;41(2):285-95.

42. Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M et al. Antifungal susceptibility of invasive Candida bloodstream isolates from the Asia-Pacific region. Sabouraudia. 2016;54(5):471-7.

43. Salehi M, Ghomi Z, Mirshahi R, Manshadi SAD, Rezahosseini O. Epidemiology and Outcomes of Candidemia in a Referral Center in Tehran. Caspian journal of internal medicine. 2019;10(1):73.

44. Kord M, Salehi M, Khodavaisy S, Hashemi SJ, Ghazvini RD, Rezaei S et al. Epidemiology of yeast species causing bloodstream infection in Tehran, Iran (2015–2017); superiority of 21-plex PCR over the Vitek 2 system for yeast identification. Journal of Medical Microbiology. 2020;69(5):712-20.

45. Ahangarkani F, Shokohi T, Rezaei MS, Ilkit M, Mahmoodi Nesheli H, Karami H et al. Epidemiological features of nosocomial candidaemia in children, infants, and neonates: A multicentre study in Iran. Mycoses. 2020.

46. Chen S, Slavin M, Nguyen Q, Marriott D, Playford EG, Ellis D et al. Active surveillance of candidemia, Australia. Emerging infectious diseases. 2006;12(10):1508.

47. Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, Viscoli C. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. PloS one. 2011;6(9).

48. Yapar N, Pullukcu H, Avkan-Oguz V, Sayin-Kutlu S, Ertugrul B, Sacar S et al. Evaluation of species distribution and risk factors of candidemia: a multicenter case-control study. Medical mycology. 2011;49(1):26-31.

49. Keighley C, Chen SC, Marriott D, Pope A, Chapman B, Kennedy K et al. Candidaemia and a risk predictive model for overall mortality: a prospective multicentre study. BMC infectious diseases. 2019;19(1):445.

50. Dimopoulos G, Ntziora F, Rachiotis G, Armaganidis A, Falagas ME. Candida albicans versus non-albicans intensive care unit-acquired bloodstream infections: differences in risk factors and outcome. Anesthesia & Analgesia. 2008;106(2):523-9.