ORIGINAL ARTICLE

# Cross-Sectional Study of Candidemia from Isfahan, Iran: Etiologic Agents, Predisposing Factors, and Antifungal Susceptibility Testing

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**Background:** Candidemia is a fatal invasive fungal infection that involves thousands of patients annually and is associated with high mortality rate and economic burden. The incidence of candidemia is increasing due to the use of invasive medical instruments and immunosuppressive drugs. The treatment of infection is problematic because of the increased resistance of clinical strains to antifungal drugs. The aim of the present study was to identify *Candida* species isolated from candidemia and determination of antifungal susceptibility patterns of clinical isolates. **Materials and Methods:** Three thousand eight hundred BACTEC bottles suspected to candidemia were evaluated from April 2019 to June 2020. For primary identification, a positive blood culture was subcultured onto the sabouraud glucose agar and CHROMagar™ Candida. For molecular identification, ITS1-5.8SrDNA-ITS2 region was amplified by ITS1 and ITS4 primers and *Msp*I restriction enzyme was applied to digest polymerase chain reaction amplicons. Minimum inhibitory concentration of seven antifungals was determined against clinical isolates by broth microdilution method in accordance with the Clinical and Laboratory Standards Institute M27-A3 and M27-S4 documents. **Results:** Forty-six out of 3800 suspected specimens were positive for candidemia (1.2%). The age range of the patients was between 11 days and 89 years, with a median age of 34.8 years. *Candida albicans* was found to be the most *Candida* species (58.7%), followed by *C. parapsilosis* complex (19.6%), *C. glabrata* complex (8.7%), *C. krusei* (6.5%), *C. famata* (4.3%), and *C. tropicalis* (2.2%). Resistance to amphotericin B, fluconazole, itraconazole, and voriconazole was detected in 13.6%, 11.3%, 6.8%, and 4.5% of clinical isolates, respectively. **Conclusion:** The incidence of non-*albicans Candida* species is increasing that must be highlighted. Since resistant *Candida* strains are found repeatedly, consecutive tracing of the species distribution and *in vitro* antifungal susceptibility of clinical isolates is recommended for better management of infections.

**Key words:** Antifungal susceptibility testing, candidemia, identification

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## INTRODUCTION

Candidemia is the most prevalent invasive fungal infection (IFI) among hospitalized patients, with an incidence of 0.4–1.5/1000 admissions and a mortality rate of 10%–50%, even with the antifungal therapy.[1,2] The incidence of IFI was shown to be increasing worldwide because of the use of invasive medical instruments such as urinary and venous catheters, antimicrobial therapy, parenteral nutrition, intensive care unit admission, surgery, and immunosuppressive therapies.[3,4] Epidemiological surveys have revealed that causative agents of infection have changed from *C. albicans* to the non-*albicans* species, such as *C. parapsilosis* complex, *C. glabrata* complex, *C. krusei* and *C. tropicalis*.[5] For example, in the North America ARTEMIS study, *C. glabrata* complex was the leading cause of non-*albicans* species isolated from candidemia and accounted for about 21.1% of cases, while in the countries

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of Latin America, *C. tropicalis* was the main cause of infection (13.2%).[6] Treatment of candidemia is problematic due to the increased resistance to antifungal drugs. Antifungal susceptibility profiles differ among *Candida* species and may affect the clinical outcomes for critically ill patients.[7] Non-selective treatment of candidemia without *in vitro* antifungal susceptibility assay, can lead to the development of drug-resistant *Candida* species by more intrinsically resistant species such as *C. glabrata* complex, *C. famata,* and *C. krusei.* The aim of the present study was to assess species distribution and antifungal susceptibility patterns of *Candida* species isolated from candidemia in Isfahan, the third-largest city of Iran.

## MATERIALS AND METHODS

### Subjects

From April 2019 to June 2020, 3800 BACTEC bottles suspected to candidemia were tested. The specimens were collected from three university hospitals (Al-Zahra, Seyed Al-Shohada, and Imam Hossein) in Isfahan, Iran.

#### Inclusion criteria

Patients with persistent fever despite adequate antibacterial therapy and patients with severe immunodeficiency disorders were included in the study.

#### Exclusion criteria

Patients who have taken antifungal drugs for the past week were excluded from the study.

A positive blood culture was subcultured onto the sabouraud glucose agar (Difco, Detroit, MI, USA) and CHROMagar™ Candida (Paris, France) for primary screening. This research was approved by the National Committee for Ethics in Biomedical Research (No. IR.IAU.SRB.REC.1397.171), and written informed consent was obtained from each patient.

### Molecular methods for identification of *Candida* species

#### DNA extraction

Genomic DNA of clinical isolates was extracted using boiling method.[8] Briefly, a loopful of fresh colonies were suspended in 100 µL of double distilled water (DDW) and boiled for 15–20 min and then centrifuged for 5 min at 8000 rpm; finally, the supernatant was kept at −20°C.

### Polymerase chain reaction

The ITS1-5.8SrDNA-ITS2 region was amplified by a polymerase chain reaction (PCR) mixture containing 5 µL of 10× reaction buffer, 0.4 mM dNTPs, 1.5 mM $MgCl_2$, 30 pmol of ITS1 primer (5'-TCC GTA GGT GAA CCT GCG G-3'), 30 pmol of ITS4 primer (5'-TCC TCC GCT TAT TGA TAT GC-3'), 2.5 U of Taq polymerase, and 2 µL of extracted DNA in a final volume of 50 µL. The PCR cycling conditions were as follows: an initial denaturation phase at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 7 min.

### Restriction fragment length polymorphism

At the next step, ITS amplicons were digested in a final volume of 15 µL containing 3 µL DDW, 1U of *Msp*I restriction enzyme (Fermentas, Vilnius, Lithuania), 1.5 µL buffer, and 10 µL PCR product at 37°C for 2 h.

### Electrophoresis

Aliquots of 5 µL of PCR and 12 µL of RFLP products were fractionated by electrophoresis on 1.5% and 2% agarose gel, respectively, and stained with SYBR Safe DNA gel stain (1:10,000 dilution in TBE) [Figure 1].

### Antifungal susceptibility testing

Minimum inhibitory concentration (MIC) was assessed by the Clinical and Laboratory Standards Institute (CLSI) M27-A3 and M27-S4 documents.[9,10] Fluconazole (FLC) (Pfizer Central Research, Sandwich, United Kingdom), amphotericin B (AMB) (Bristol-Myers-Squibb, Woerden, The Netherlands), itraconazole (ITR) (Janssen Research Foundation, Beerse, Belgium), posaconazole (POS) (Schering-Plough, Kenilworth, USA), caspofungin (CAS) (Merck Sharp and Dohme, Haarlem, The Netherlands), voriconazole (VOR) (Pfizer Central Research, UK), and luliconazole (LLCZ) (Sigma Chemical Co., St. Louis, MO, USA) were applied for preparation of the CLSI microdilution trays. The antifungal agents were diluted in the RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (Sigma Chemical Co., St. Louis, MO, USA) with L-glutamine and without bicarbonate. Final concentrations of antifungals were prepared as follows: 0.064–64 µg/ml for FLC, 0.016–64 µg/ml for POS, 0.016–16 µg/ml for ITR, CAS, AMB, VOR, and LLCZ. All identified *Candida* spp. were cultured on malt extract agar (MEA, Difco) and incubated at 35°C. The optical density (OD) was measured by a spectrophotometer, at a

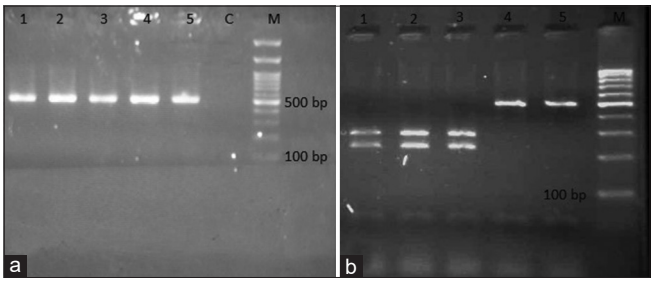


**Figure 1:** (a) Agarose gel electrophoresis of ITS-polymerase chain reaction amplicons of *Candida* species, lanes 1–3: *Candida albicans*, lanes 4, 5: *Candida parapsilosis* complex, lane C: Negative control, and lane M is 100 bp DNA size marker. (b) Agarose gel electrophoresis of ITS-polymerase chain reaction amplicons after digestion with *Msp*I. Lanes 1–3: *Candida albicans*, lanes 4, 5: *Candida parapsilosis* complex, and lane M is 100 bp DNA size marker

wavelength of 530 nm, and transmission of 75%–77%. Final inoculum sizes ranged from $2.5 \times 10^3$ to $5 \times 10^3$ CFU/ml. MIC results for all agents were determined visually following 24 h of incubation at 35°C, as we considered 100% growth inhibition for amphotericin B, and >50% growth inhibition for other antifungals.[9] *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) strains were used for quality controls.

### Statistical analysis

The MIC range, $MIC_{50}$, and $MIC_{90}$ were determined. The $MIC_{50}$ and $MIC_{90}$ values were considered as the minimum concentrations of antifungal agents being able to inhibit 50% and 90% of the growth of clinical *Candida* strains, respectively. Data were analyzed using the SPSS software version 23 (IBM, Chicago, USA). Correlation between antifungal susceptibility and species distribution was adjusted using Fisher's exact test and Mann–Whitney *U*-test. $p < 0.05$ was considered statistically significant.

## RESULTS

Forty-six out of 3800 suspected specimens were positive for candidemia (1.2%). The age range of the patients was between 11 days and 89 years, with a median age of 34.8 years. The male-to-female ratio of the patients was 26/20. The predisposing factors were recorded as antibiotic consumption ($n$ = 39, 84.8%), cancer ($n$ = 18, 39%), hemodialysis ($n$ = 4, 8.7%), preterm birth ($n$ = 3, 6.5%), surgery ($n$ = 2, 4.3%), the use of permanent central venous catheter system (port-a-cath) ($n$ = 1, 2.2%), thalassemia ($n$ = 1, 2.2%), peritoneal dialysis ($n$ = 1, 2.2%), and systemic lupus erythematosus ($n$ = 1, 2.2%). Three patients (6.5%) had coinfection with coronavirus disease 2019, and one of them passed away due to the acute respiratory distress syndrome. *Candida albicans* was found to be the most *Candida* species ($n$ = 27, 58.7%), followed by *C. parapsilosis* complex ($n$ = 9, 19.6%), *C. glabrata* complex ($n$ = 4, 8.7%), *C. krusei* ($n$ = 3, 6.5%), *C. famata* ($n$ = 2, 4.3%), and *C. tropicalis* ($n$ = 1, 2.2%). The MIC ranges of antifungal agents were as follows: *C. albicans*: AMB – 0.032–1 μg/mL, CAS – 0.008–16 μg/mL, VOR – 0.032–1 μg/mL, FLC – 0.125–4 μg/mL, POS – 0.032–0.5 μg/mL, ITR – 0.032–0.25 μg/mL, and LLCZ – 0.004–2 μg/mL; *C. parapsilosis*: AMB – 0.032–6 μg/mL, CAS – 0.25–4 μg/mL, VOR – 0.032–8 μg/mL, FLC – 0.25–64 μg/mL, POS – 0.032–8 μg/mL, ITR – 0.032–8 μg/mL, and LLCZ – 0.032–8 μg/mL; *C. glabrata*: AMB – 0.125–1 μg/mL, CAS – 0.032–0.125 μg/mL, VOR – 0.032 μg/mL, FLC – 1–32 μg/mL, POS – 0.25 μg/mL, ITR – 0.125–0.25 μg/mL, and LLCZ – 0.016–0.032 μg/mL; *C. krusei*: AMB – 0.125–1 μg/mL, CAS – 0.064–4 μg/mL, VOR – 0.008–0.125 μg/mL, FLC – 0.5–64 μg/mL, POS – 0.064–0.5 μg/mL, ITR – 0.125–0.5 μg/mL, and LLCZ – 0.004–0.25 μg/mL; *C. famata*: AMB – 0.064–0.125 μg/mL, CAS – 0.032–0.25 μg/mL, VOR – 0.064–0.125 μg/mL, FLC – 32–64 μg/mL, POS – 0.025–0.5 μg/mL, ITR – 0.25 μg/mL, and LLCZ – 0.5–1 μg/mL; and *C. tropicalis*: AMB – 0.064 μg/mL, CAS – 0.032 μg/mL, VOR – 0.25 μg/mL, FLC – 8 μg/mL, POS – 1 μg/mL, ITR – 1 μg/mL, and LLCZ – 1 μg/mL. Table 1 indicates interpretive guidelines for *in vitro* susceptibility testing of *Candida* species according to M27-S4, M60 documents, and Borman *et al.*,[10-12] and Tables 2-4 show the *in vitro* susceptibility patterns of *Candida* isolates, MIC of antifungal agents in details, and $MIC_{50}$, $MIC_{90}$, and geometric mean, respectively.

**Table 1: Interpretive guidelines for *in vitro* susceptibility testing of *Candida* species**

| Antifungal agent | *Candida* species | MIC breakpoints (μg/mL) | | | |
|---|---|---|---|---|---|
| | | S | I | SDD | R |
| Fluconazole | *C. albicans* | ≤2 | - | 4 | ≥8 |
| | *C. glabrata* | - | - | ≤32 | ≥64 |
| | *C. parapsilosis* | ≤2 | - | 4 | ≥8 |
| | *C. krusei* | - | - | - | - |
| | *C. tropicalis* | ≤2 | - | 4 | ≥8 |
| Voriconazole | *C. albicans* | ≤0.12 | 0.25-0.5 | - | ≥1 |
| | *C. glabrata*[a] | - | - | - | - |
| | *C. parapsilosis* | ≤0.12 | 0.25-0.5 | - | ≥1 |
| | *C. krusei* | ≤0.5 | 1 | | ≥2 |
| | *C. tropicalis* | ≤0.12 | 0.25-0.5 | - | ≥1 |
| Itraconazole | *C. albicans* | ≤0.125 | - | 0.25-0.5 | ≥1 |
| | *C. glabrata* | ≤0.125 | - | 0.25-0.5 | ≥1 |
| | *C. parapsilosis* | ≤0.125 | - | 0.25-0.5 | ≥1 |
| | *C. krusei* | ≤0.125 | - | 0.25-0.5 | ≥1 |
| | *C. tropicalis* | ≤0.125 | - | 0.25-0.5 | ≥1 |
| Posaconazole[b] | *C. albicans* | - | - | - | - |
| | *C. glabrata* | - | - | - | - |
| | *C. parapsilosis* | - | - | - | - |
| | *C. krusei* | - | - | - | - |
| | *C. tropicalis* | - | - | - | - |
| Luliconazole[c] | *C. albicans* | - | - | - | - |
| | *C. glabrata* | - | - | - | - |
| | *C. parapsilosis* | - | - | - | - |
| | *C. krusei* | - | - | - | - |
| | *C. tropicalis* | - | - | - | - |
| Caspofungin | *C. albicans* | ≤0.25 | 0.5 | - | ≥1 |
| | *C. glabrata* | ≤0.12 | 0.25 | - | ≥0.5 |
| | *C. parapsilosis* | ≤2 | 4 | - | ≥8 |
| | *C. krusei* | ≤0.25 | 0.5 | - | ≥1 |
| | *C. tropicalis* | ≤0.25 | 0.5 | - | ≥1 |
| Amphotericin B[b] | *C. albicans* | - | - | - | ≥1 |
| | *C. glabrata* | - | - | - | ≥1 |
| | *C. parapsilosis* | - | - | - | ≥1 |
| | *C. krusei* | - | - | - | ≥1 |
| | *C. tropicalis* | - | - | - | ≥1 |

[a]For *C. glabrata* and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome; [b]For posaconazole and Amphotericin B, Epidemiological cutoff values (ECVs) have been replaced for *Candida* species with no breakpoints; [c]For luliconazole, there is no breakpoints and EVC for *Candida* species, *C. albicans=Candida albicans; C. glabrata=Candida glabrata; C. parapsilosis=Candida parapsilosis; C. krusei=Candida krusei; C. tropicalis=Candida tropicalis*; S=Susceptible; I=Intermediate; SDD=Susceptible dose dependent; R=Resistant

### Data analysis

Fisher's exact test showed that the association between the MIC and *Candida* species was not statistically significant ($p = 0.82$).

## DISCUSSION

Bloodstream infections with the genus of *Candida* are a consequential concern that involves thousands of individuals annually and is associated with economic burden.[13] Although *C. albicans* is the most common species, changing in the frequency of etiologic agents of candidemia has revealed a remarkable increase in the incidence of NAC species with intrinsic resistance to azoles such as FLC.[5] NAC species affect mortality rate among patients, which is higher for *C. krusei* and *C. tropicalis* and lower for *C. parapsilosis* complex.[14] There are geographical discrepancies for etiologic agents of candidemia; for instance, in Europe and North America, *C. glabrata* complex is a more prevalent species of candidemia than in other areas, while in Latin America, *Candida* bloodstream infections are more generally caused by *C. tropicalis* and *C. parapsilosis* complex with low incidence of *C. glabrata* complex.[15] In the past, 68%–91% of clinical *Candida* species were *C. albicans*, and 5% included *C. tropicalis*, *C. parapsilosis* complex, and *C. glabrata* complex.[16] Regrettably, the majority of the drug-resistant clinical *Candida* strains are reported from developing countries, where many patients with bloodstream fungal infections are treated with azoles, such

**Table 2: *In vitro* susceptibility patterns of *Candida* species isolates from candidemia**

| *Candida* species | Antifungal pattern | Antifungal agents | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | AMB | CAS | VOR[b] | FLC | POS[a] | ITR | LLCZ[a] |
| *C. albicans* | S | 26 | 25 | 25 | 23 | - | 23 | - |
| | I/(SDD) | 0 | 1 | 1 | (2) | - | (3) | - |
| | R | 1 | 1 | 1 | 2 | - | 1 | - |
| *C. parapsilosis* | S | 6 | 8 | 7 | 6 | - | 6 | - |
| | I/(SDD) | 0 | 1 | 1 | (1) | - | (2) | - |
| | R | 3 | 0 | 1 | 2 | - | 1 | - |
| *C. glabrata* | S | 3 | 4 | - | 3 | - | 1 | - |
| | I/(SDD) | 0 | 0 | - | (1) | - | (3) | - |
| | R | 1 | 0 | - | 0 | - | 0 | - |
| *C. krusei*[c] | S | 2 | 3 | 3 | - | - | 2 | - |
| | I/(SDD) | 0 | 0 | 0 | - | - | (1) | - |
| | R | 1 | 0 | 0 | - | - | 0 | - |
| *C. famata*[d] | S | - | - | - | - | - | - | - |
| | I/(SDD) | - | - | - | - | - | - | - |
| | R | - | - | - | - | - | - | - |
| *C. tropicalis* | S | 1 | 1 | 0 | 0 | - | 0 | - |
| | I/(SDD) | 0 | 0 | (1) | 0 | - | 0 | - |
| | R | 0 | 0 | 0 | 1 | - | 1 | - |
| Total | S | 38 | 41 | 35 | 32 | - | 32 | - |
| | I/(SDD) | 0 | 2 | 3 | (4) | - | (9) | - |
| | R | 6 | 1 | 2 | 5 | - | 3 | - |

[a]Posaconazole and luliconazole have no breakpoint in the new version of CLSI; [b]For *C. glabrata* and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome; [c]The breakpoints of fluconazole has not been described for *C. krusei*, because it is intrinsically resistant to fluconazole; [d]All antifungals have no breakpoints for uncommon species such as *C. famata. C. albicans=Candida albicans; C. parapsilosis=Candida parapsilosis; C. glabrata=Candida glabrata; C. krusei=Candida krusei; C. famata=Candida famata; C. tropicalis=Candida tropicalis;* S=Susceptible; I=Intermediate; R=Resistant; AMB=Amphotericin B; CAS=Caspofungin; VOR=Voriconazole; FLC=Fluconazole; POS=Posaconazole; ITR=Itraconazole; LLCZ=Luliconazole; SDD=Susceptible dose dependent

**Table 3: The minimum inhibitory concentration of antifungal agents among *Candida* species isolated from candidemia**

| Antifungal agents | Minimum inhibitory concentration (μg/mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤0.016 | 0.032 | 0.064 | 0.128 | 0.256 | 0.512 | 1 | 2 | 4 | 8 | 16 | ≥32 |
| AMB | 0 | 5 | 10 | 8 | 9 | 8 | 5 | 0 | 1 | 0 | 0 | 0 |
| CAS | 1 | 24 | 4 | 3 | 5 | 1 | 2 | 3 | 2 | 0 | 1 | 0 |
| VOR | 1 | 35 | 2 | 3 | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| FLC | 0 | 0 | 0 | 2 | 8 | 7 | 9 | 6 | 4 | 1 | 0 | 9 |
| POS | 0 | 4 | 10 | 10 | 10 | 4 | 6 | 0 | 0 | 1 | 0 | 1 |
| ITR | 0 | 14 | 10 | 8 | 10 | 1 | 2 | 0 | 0 | 1 | 0 | 0 |
| LLCZ | 10 | 11 | 2 | 7 | 2 | 5 | 4 | 4 | 0 | 1 | 0 | 0 |

AMB=Amphotericin B; CAS=Caspofungin; VOR=Voriconazole; FLC=Fluconazole; POS=Posaconazole; ITR=Itraconazole; LLCZ=Luliconazole

**Table 4: Minimum inhibitory concentration range, minimum inhibitory concentration 50, minimum inhibitory concentration 90, and geometric mean of the antifungal agents used in the present study**

| *Candida* species | MIC range (μg/mL) | $MIC_{50}$ (μg/mL) | $MIC_{90}$ (μg/mL) | Geometric mean |
|---|---|---|---|---|
| *C. albicans* | AMB (0.032-1) | 0.125 | 0.5 | 0.15 |
| | CAS (0.008-16) | 0.032 | 0.125 | 0.04 |
| | VOR (0.032-1) | 0.032 | 0.125 | 0.04 |
| | FLC (0.125-4) | 1 | 4 | 0.92 |
| | POS (0.032-0.5) | 0.125 | 1 | 0.20 |
| | ITR (0.032-0.25) | 0.064 | 0.25 | 0.06 |
| | LLCZ (0.004-2) | 0.032 | 0.5 | 0.06 |
| *C. parapsilosis* | AMB (0.032-6) | 0.25 | 6 | 0.30 |
| | CAS (0.25-4) | 1 | 4 | 0.92 |
| | VOR (0.032-8) | 0.032 | 9 | 0.07 |
| | FLC (0.25-64) | 1 | 64 | 2.16 |
| | POS (0.032-8) | 0.125 | 8 | 0.17 |
| | ITR (0.032-8) | 0.125 | 9 | 0.16 |
| | LLCZ (0.032-8) | 1 | 8 | 0.50 |
| *C. glabrata* | AMB (0.125-1) | 0.25 | 1 | 0.29 |
| | CAS (0.032-0.125) | 0.064 | 0.125 | 0.07 |
| | VOR (0.032) | 0.032 | 0.032 | 0.03 |
| | FLC (1-32) | 2 | 32 | 4 |
| | POS (0.25) | 0.25 | 0.25 | 0.21 |
| | ITR (0.125-0.25) | 0.25 | 0.25 | 0.21 |
| | LLCZ (0.016-0.032) | 0.016 | 0.032 | 0.02 |
| *C. krusei* | AMB (0.125-1) | 0.25 | 1 | 0.31 |
| | CAS (0.064-4) | 0.25 | 4 | 0.40 |
| | VOR (0.008-0.125) | 0.032 | 0.125 | 0.03 |
| | FLC (0.5-64) | 0.5 | 64 | 2.51 |
| | POS (0.064-0.5) | 0.064 | 0.5 | 0.12 |
| | ITR (0.125-0.5) | 0.125 | 0.5 | 0.19 |
| | LLCZ (0.004-0.25) | 0.125 | 0.25 | 0.05 |
| *C. famata* | AMB (0.064-0.125) | 0.064 | 0.125 | 0.09 |
| | CAS (0.032-0.25) | 0.032 | 0.25 | 0.09 |
| | VOR (0.064-0.125) | 0.064 | 0.125 | 0.09 |
| | FLC (32-64) | 32 | 64 | 45.25 |
| | POS (0.025-0.5) | 0.25 | 0.5 | 0.35 |
| | ITR (0.25) | 0.25 | 0.25 | 0.25 |
| | LLCZ (0.5-1) | 0.5 | 1 | 0.70 |
| *C. tropicalis* | AMB (0.064) | N/A | N/A | N/A |
| | CAS (0.032) | N/A | N/A | N/A |
| | VOR (0.25) | N/A | N/A | N/A |
| | FLC (8) | N/A | N/A | N/A |
| | POS (1) | N/A | N/A | N/A |
| | ITR (1) | N/A | N/A | N/A |
| | LLCZ (1) | N/A | N/A | N/A |

*C. albicans=Candida albicans; C. parapsilosis=Candida parapsilosis; C. glabrata=Candida glabrata; C. krusei=Candida krusei; C. famata=Candida famata; C. tropicalis=Candida tropicalis*; N/A=Not applicable; MIC=Minimum inhibitory concentration; AMB=Amphotericin B; CAS=Caspofungin; VOR=Voriconazole; FLC=Fluconazole; POS=Posaconazole; ITR=Itraconazole; LLCZ=Luliconazole

as FLC, as the first treatment choice.[17] The lack of precise identification of fungi in these countries by the novel and appropriate methods such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), can be further worsen and lead to unselective use of antifungal agents. A multicenter survey from Brazil[18] targeting the vulnerable population of candidemia showed that catheterization, chronic renal failure, and parenteral nutrition are the main risk factors for candidemia; whereas, we revealed antibiotic consumption (84.8%), malignancies (39%), and hemodialysis (8.7%) as leading predisposing factors in our investigation. Similar to our results, Hii *et al*.[19] in Taiwan reported that more than 40% of patients had different underlying diseases containing malignancy and diabetes mellitus. In another research performed in Turkey, CVP catheterization, the length of hospitalization, parenteral nutrition, and chronic renal failure were also considered to be independent predisposing factors.[20] In this regard, they

showed that being female gender is another risk factor for candidemia, while bloodstream *Candida* infection was similar in males and females in the present survey. In agreement with our findings, Yapar *et al*.[21] demonstrated that the use of antibacterial agents can remarkably increase the risk of candidemia among patients, and almost all patients had taken antibiotics prior to *Candida* infection. Although former studies revealed a mortality rate of 50% for candidemia,[22,23] the mortality rate was 19.6% in the present report. Similar to our study, Mirhendi *et al*.[24] reported *C. albicans* and *C. parapsilosis* as the most common etiologic agent and NAC species of candidemia in Tehran, Iran, respectively. Contrary to the results of our study, they presented a uniform activity of AMB against all *Candida* isolates with no MICs equal or above the breakpoint of 1 mg/L; whereas, 13.6% of *Candida* species were resistant to AMB in the present investigation. Furthermore, they showed that VOR and FLC had 100% activity against nearly all *Candida* species, but we found 4.5% and 11.3% of resistant isolates to VOR and FLC, respectively. In this regard, 100% of *C. famata* isolates were resistant to FLC. In agreement with Ghahri *et al*.,[25] all *Candida* species but one of *C. albicans* (MIC = 16 μg/mL) were susceptible to CAS. They revealed that VOR was the most potent antifungal agent against isolated *Candida* species; nevertheless, our findings indicated that 4.5% of *Candida* species were resistant to VOR. Three out of 46 strains (6.5%) were resistant to ITR in the present study (MIC ≥1), close to the outcomes that Xiao *et al*. reported (7.8%).[26] In the case of LLCZ, breakpoints or epidemiological cutoff values (ECVs) have not been described for *Candida* species; however, 19.6% of isolates had MIC ≥1 for LLCZ.

## CONCLUSION

The incidence of NAC species is increasing that must be considered significant. *C. parapsilosis* was the most common *Candida* species causing non-*albicans* candidemia in our survey, and some *C. parapsilosis* were resistant to FLC, VOR, AMB, and ITR among the clinically NAC species. This can represent a regional phenomenon and points to the value of careful selection of empiric therapy. Consecutive tracing of the species distribution and *in vitro* antifungal susceptibility of clinical isolates is essential for better management of infection.

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### Conflicts of interest

There are no conflicts of interest.

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