Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Clinical impact of Candida respiratory tract colonization and acute lung infections in critically ill patients with COVID-19 pneumonia

Mahzad Erami a, Omid Raiesi b, c, Mansooreh Momen-Heravi d, Muhammad Ibrahim Getso e, Mojtaba Fakhrehi f, Narges Mehti g, Mohammad Yarahmadi h, Sasan Amiri h, Vahid Raissi h, i, Seyed Jamal Hashemi a, i, *

a Department of Medical Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
b Department of Parasitology, School of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran
c Zoonotic Diseases Research Center, Ilam University of Medical Sciences, Ilam, Iran
d Department of Infectious Diseases, Kishan University of Medical Sciences, Kishan, Iran
e Department of Medical Microbiology and Parasitology, College of Health Sciences, Bayero University Kano, P.M.B 3011, Kano, Nigeria
f Kashan Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran
g Department of Medical Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
h Roozbeh hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

A R T I C L E   I N F O

Keywords:
COVID-19
SARS-CoV-2
Candida
Bronchoalveolar lavage
Mechanical ventilation
Antifungal agents

A B S T R A C T

Coronavirus disease 2019 (COVID-19), which is attributable to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been causing a worldwide health issue. Airways colonization by Candida spp. is prevalent among patients on automatic ventilation in intensive care units (ICUs). This research aimed to ascertain the risk factors and roles of Candida spp. respiratory tract colonization, and Candida lung infection during the progression of COVID-19 pneumonia in critically ill patients. In total, Candida spp. were recovered in 69 from 100 immunosuppressed patients with COVID-19. Bronchoscopy was used to collect the Bronchoalveolar lavage (BAL) specimens. For the identification of Candida spp. PCR sequencing was done using the ITS1 and ITS4 primers. The amplification of the HWP1 gene was conducted to identify the Candida albicans complex. The antifungal activities of fluconazole, itraconazole, voriconazole, amphotericin B and caspofungin against Candida spp. were evaluated using the Clinical and Laboratory Standards Institute M60. In 63.77% of the patients, Candida respiratory colonization at D0 and D14 had no impact on the severity of COVID-19. In comparison to C. albicans strains, Candida respiratory disorder with C. glabrata had influenced the severity of COVID-19 for critically ill patients following adjustment for the risk factors of COVID-19 (P < 0.05). Amphotericin B and caspofungin showed superior activity against all Candida spp. All antifungal agents showed 100% sensitivity against the two C. africana strains. Our observation on patients who used automatic ventilation, respiratory colonization by Candida spp. was not seen to influence the infection or death caused by COVID-19. Amphotericin B and caspofungin showed superior activity against all Candida spp. and were recommended for the treatment regime of pulmonary candidiasis associated with COVID-19 infection. Although “Candida pneumonia” is rarely being reported in critically ill patients, Candida airway colonization mainly by Candida albicans is common especially among patients with diabetes, malignancies, and kidney disorders.

1. Introduction

The novel coronavirus SARS-CoV-2, which emerged in Wuhan in November 2019, has increasingly spread causing a global pandemic that infected more than 494 million people, resulting in severe social and economic ramifications, and claimed more than 6,183,000 lives by April 6, 2022 [1]. Coronavirus disease 2019 (COVID-19), which is attributable to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been the cause of global health threats [2,3]. Bacterial and fungal co-infections are among various factors that play roles in morbidity and mortality in COVID-19 patients, particularly among those suffering from acute respiratory distress syndrome (ARDS). Furthermore, the wide use of corticosteroids and the irrational use antibiotics coupled with the

* Corresponding author. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. E-mail address: sjhashemi@tums.ac.ir (S.J. Hashemi).
tissue damage caused by SARS CoV-2, may facilitate invasion by commensal yeast causing deep seated invasive fungal infections. Patients with severe COVID-19 are at risk for healthcare-associated infections (HAIs), including *Candida* bloodstream infections. There have been reports on increasing incidence of candidemia in critically ill COVID-19 cases. High mortality rate is being reported among patients with COVID-19-associated candidemia (CAC). The mortality rate among patients with CAC reaches up to 83% despite antifungal therapy. The above highlights the clinical significance of severe COVID-19 that underscores the importance of rapid diagnosis and timely initiation of antifungal treatment [4–7]. Moreover, the undefined standard of pharmacological therapy for COVID-19, including the invasive nature and multi-drug treatment methods, as well as some pathological oral conditions can aggravate SARS-CoV-2, particularly in those patients with an immune-compromised system or a long-term usage of pharmacotherapies that expose them to increased risk for developing mucosal candidiasis [8]. Bronchial colonization by *Candida* spp. is prevalent among patients who use automatic ventilation in the intensive care unit (ICU). *Candida* colonization has been found in approximately 30% of people who used mechanical ventilation (MV) for longer than 48 h and in 50% of those diagnosed with ventilator-associated pneumonia (VAP) [9,10]. Isolation of *Candida* spp. via the respiratory tract is linked to longer periods of MV, ICU admission, and hospital stay, with attendant poorer outcomes [11–13]. Except for highly immunocompromised patients, who are prone to fungal pneumonia, *Candida* spp. in lower airways shall be interpreted with cautions as the causative agents of lung disease [14–18]. Colonization of the respiratory tract by *Candida* spp. can have a significant effect on the progression of COVID-19 pneumonia. Evaluation for secondary fungal infections in COVID-19 patients, as well as their initiating agents, is critical for effective management of COVID-19 infection. Additionally, understanding the antifungal susceptibility profile of *Candida* spp. would be essential in treatment of COVID-19 patients. This research aimed to evaluate antifungal susceptibility patterns and the role of *Candida* spp. respiratory tract colonization, risk factors, and *Candida* lung infection during the progression of COVID-19 pneumonia in critically ill patients.

2. Materials and methods

2.1. Study areas and subjects

This descriptive study was performed on COVID-19 patients who were diagnosed based on clinical symptoms, radiological signs, and positive molecular test results and admitted to Shabih Beheshti Hospital in Kashan, Iran. Bronchoscopy was used to collect the bronchoalveolar lavage (BAL) specimens. The collected specimens were initially subjected to microscopic examination using 10% KOH solution to detect budding yeasts or pseudohyphae. Parts of the specimens were cultured on Sabouraud’s Dextrose Agar (SDA) 2% (Merck, Denmark) and incubated at 35 °C for seven days. A few of the colonies grown on SDA were also mixed with sterile saline and 3% glycerol in 0.5 ml microtubes and stored at −70 °C [19–21].

The study included adult immunosuppressed patients with COVID-19 pneumonia who used invasive MV for more than four days. Other inclusion criteria were history of the regulation of immune status, even once; immunocompromised status; patients with neutropenia; use of corticosteroid at doses >2 mg/kg of dexamethasone; hospitalization in the ICU for more than four days; and use of invasive ventilation. Signs and symptoms of inflammation and other ICU-acquired complications were assessed regularly. The exclusion criteria were: Non-ICU patients with confirmed COVID-19, Age ≤18 year, and COVID-19 patients with Non-Invasive Ventilation. Verbal consent was obtained from patients before being enrolled in this study. The Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran has approved this study (ethics code: IR.TUMS.SPH.REC.1399.329).

2.2. Molecular identification of isolates

2.2.1. Extraction of genomic DNA

According to the manufacturer’s instructions, genomic DNA was extracted directly from BAL specimens using a high-purity polymerase chain reaction (PCR) template purification package (Roche, Germany). Briefly, 200 µl of specimens were mixed with 200 µl of binding buffer and 40 µl of proteinase K. The mixture was incubated at 70 °C for 10 min followed by the addition of 100 µl of isopropanol. A high-purity filter tube was inserted into a collection tube, and the setup was mixed using a vortex. The sample was pipetted into the upper buffer reservoir of the filter tube. The whole high-purity filter tube assembly was placed in a standard table-top centrifuge and centrifuged at ×8000 g for 1 min. The filter tube was then removed and the rest of the setup was discarded; keeping the collection tube containing the filtrate Subsequently, 500 µl inhibitor removal buffer was added to the supernatant and was centrifuged for 1 min at 8000×g. Finally, the supernatant was removed from the collection tube, 500 µl wash buffer was added to it, and centrifuged for 1 min at 8000×g.

The flow-through was scrapped, and the whole high purity assembly was centrifuged at full speed for another 30 s. The elution buffer was added, and the DNA was precipitated in 100 µl of 100 µl TE. Brief centrifugation (15,000 g for 1 min) was used to separate the cell debris, and 1 µl of the supernatant was used for the PCR. The extracted DNA was stored at −20 °C.

2.2.2. Amplification of internal transcribed spacers

We used the PCR to detect *Candida* spp. The PCR reaction was run in a cumulative volume of 25 µl, containing 1 µl of each of reverse and forward primers, 2 µl of prototype DNA, 12.5 µl of master mix (AmpliCon, Denmark), and water until it reached the final volume. The amplification was done using the internal transcribed spacers 1 (ITS1) and ITS4 primers based on the following protocol: 10 min of primary denaturation at 95 °C, 40 cycles of denaturation for 20 s at 95 °C, annealing for 20 s at 62 °C, an expansion for 20 s at 72 °C, and a final extension for 5 min at 72 °C. Eventually, the products were run on a 2% agarose gel. The *HWPI1* gene amplification using the paired primers *HWPI-F* (5’-GCTACATCCAGAGATCATCATC-3’) and *HWPI-R* (5’-GCACCTCAGTCGTAAGACGG-3’) was done as described previously for *Candida albicans* complex [14,22].

2.3. Antifungal susceptibility assay

The Clinical and Laboratory Standards Institute (CLSI) M60 approach was used to assess the minimum inhibitory concentrations (MIC) of fluconazole, itraconazole, voriconazole, caspofungin, and amphotericin B. Antifungal agent powders were bought from Sigma, USA. The serial dilution of routine antifungals was prepared in concentrations ranging from 0.0125 to 32/64 mg/ml, depending on the drug. The 100 µl of each agent was dispensed in a 96-well microplate. Growth and negative controls were included. The negative control was prepared using the 200 µl of RPMI1640 medium. The plates were incubated at 35 °C for 24 h. *Candida parapsilosis* ATCC 22019 was

| Abbreviation used |
|--------------------|
| Coronavirus disease 2019 (COVID-19) |
| Minimum inhibitory concentration (MIC) |
| Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) |
| Intensive care unit (ICU) |
| Bronchoalveolar lavage (BAL) |
| Mechanical ventilation (MV) |
| Ventilator-associated pneumonia (VAP) |
checked for quality control. It should be mentioned that each test was carried out twice [16,23].

2.4. Statistical analysis

Statistical analysis was carried out using SPSS software (version 16.0). Descriptive test was performed to describe the demographic characteristics, and chi-square test was performed to demonstrate any statistically significant relationship between the variables explored in this study. The MICs range and MICs 90 of all antifungals were calculated.

3. Results

*Candida* colonization was confirmed in 69 (69%) of the 100 COVID-19 patients under MV. Of these, 37/69 (53.6%) patients were males; the mean age of all patients at presentation was 61.1 years (range = 21–88 years). Based on the PCR sequencing results, *C. albicans* (55; 79.7%) was the most common spp. followed by, *C. glabrata* (12; 17.4%). The co-infection of *C. albicans* and *C. glabrata* was seen in two cases (2.9%). In this research, two (2.9%) *Candida africana* were detected by the *HWPI* gene amplification (Fig. 1), and no *Candida dubliniensis* was found.

On the first day of admission, D0, all 69 patients using MV had *Candida* spp. airway colonization, while there was no substantial difference in the cause for ICU entry (P > 0.05). Moreover, at D0, *C. albicans* was responsible for 79.7% of *Candida* respiratory tract colonization. In 63.77% of patients, *Candida* respiratory colonization had no impact on the severity of COVID-19 (P > 0.05) between D0 and D14. In comparison to *C. albicans* strains, *Candida* respiratory tract colonization with *C. glabrata* had influenced the severity of COVID-19 in critically ill patients following adjustment for the risk factors of COVID-19 (P < 0.05).

The most common underlying diseases among patients with *Candida* colonization included diabetes (28 cases), malignancy (8 cases), kidney disorders (11 cases), cardiovascular diseases (7 cases), and one case each of pregnancy and hyperthyroidism. Whereas patients with *Candida* colonization had diabetes (40.6%) and kidney disorders (16%) as their main underlying diseases, headache (97.1%), fever (85.5%), myalgia (91.6%), arthralgia (49.3%), gastrointestinal symptoms (71%), and dyspnea (100%) were most frequent symptoms at presentation depending on patients’ status of *Candida* colonization. Table 1.

The clinical course and disease outcome of patients with and without *Candida* colonization is being been demonstrated in Table 2 (see Table 3).

Table 1

| Number of patients Characteristic, no (%) | 69 |
|------------------------------------------|----|
| Age at the time of diagnosis-years*     | 61.1 (range = 21–88 years) |
| Sex                                      | No |
| Male                                     | 32 (46.4%) |
| Female                                   | 37 (53.6%) |
| Total COVID-19 patients                 | 69 |
| ICU patients                             | 100 (14.2%) |
| Mechanical ventilation (MV) with colonization | 69/100 (69%) |
| Underlying cause of immunosuppression    |    |
| Malignancy                               | 8 (11.6%) |
| Diabetes Mellitus                        | 28 (40.6%) |
| Kidney disorder                         | 11 (16%) |
| Hyperthyroidism                          | 1 (1.4%) |
| Pregnancy                                | 1 (1.4%) |
| Cardiovascular disease                  | 7 (10.1%) |
| Signs and symptoms                      |    |
| Headache                                 | 67 (97.1%) |
| Fever                                    | 59 (85.5%) |
| Myalgia                                  | 63 (91.6%) |
| Arthralgia                               | 34 (49.3%) |
| Gastrointestinal                        | 49 (71%) |
| Dyspnea                                  | 69 (100%) |
| Blood group                              |    |
| A                                        | 26 (37.7%) |
| AB                                       | 5 (7.2%) |
| B                                        | 20 (29%) |
| D                                        | 18 (26.1%) |
| Extension                                |    |
| BAL                                      | 69 (100%) |

Table 2

| Variables                              | Candida colonization (n = 69) | No Candida colonization (n = 31) | P-value |
|---------------------------------------|-----------------------------|---------------------------------|---------|
| COVID-19 infection                    | 69/69 (100)                 | 31/31 (100)                     | 1       |
| Age, yr, median (range)               | 61.1 (21–88)                | 56.6 (26–99)                    | 0.07    |
| Sex, F, n (%)                         | 37/69 (53.6)                | 13/31 (41.9)                    | 0.24    |
| Blood group, A, n (%)                 | 26/69 (37.7)                | 8/31 (25.8)                     | 0.18    |
| Systemic corticosteroid use, n (%)    | 46/69 (66.6)                | 19/31 (61.3)                    | 0.37    |
| Interval from ICU admission to ICU discharge, median (range), d | 13.1 (5–35) | 10.9 (3–14) | 0.21 |
| ICU patients                          | 69/69 (100)                 | 31/31 (100)                     | 1       |
| Mechanical ventilation, n (%)         | 69/69 (100)                 | 22/31 (70.9)                    | 0.09    |
| Candidemia                            | 3/69 (4.3)                  | 0/31 (0.0)                      | 0.05    |
| Urine culture                         | 17/69 (24.6)                | 5/31 (16.2)                     | 0.19    |
| Mortality, n (%)                      | 45/69 (65.2)                | 19/31 (61.3)                    | 0.61    |

a Fischer’s exact test; Mann-Whitney test for continuous data.

4. Discussion

Although microbial colonization is an important factor in the development of secondary infections, *Candida* pneumonia— as a secondary infection following airways colonization —is seldom reported even in the intensive care unit (ICU). Thus, the common consensus is that anti-*Candida* therapy is rarely necessary in most cases and it should be managed as airways colonization in which *Candida* spp. are being isolated [24]. Some studies have reported that *Candida* colonization in respiratory tracts (RT) might be an independent risk factor for the
Many microbial laboratories do not conduct further analysis when fast-growing Candida spp. are being isolated from RT samples. Further, only filamentous fungi isolation was being reported by some institutions [28]. (3) It is widely accepted that the cutoff counts of pathogenic bacteria for VAP diagnosis is 10³ CFU/mL (protected specimen brush sample) or 10⁴ CFU/mL (bronchoalveolar lavage fluid sample), but such consensus has not yet been reached for Candida; Candida pneumonia must be diagnosed by histopathology [27]. Thus, reporting Candida pneumonia is generally quite rare in the ICU, and the guidelines for the management of Candida spp. of both the IDSA and ESCMID do not recommend commencement of antifungal treatment without clear histological evidence of infection [24,29]. Reports of clinical studies from some centers have highlighted the isolation rate of Candida from the RT of ICU patients using MV to be as high as 50% with a prolonged median hospital stay (59.9 vs. 38.6 days, p = 0.006) or even increased the hospital mortality (34.2 vs. 21.0%, p = 0.003) [30]. Moreover, it might be associated with persistent immunosuppression and inflammation [31]. Candida airways colonization and its consecu- tive secretory inflammation may worsen the host’s cellular immune function, especially in immunosuppressed hosts with severe monocyte and lymphocyte dysfunction that results in a decreased effective clearance of bacteria and fungi and may increase the incidence of VAP [32]. A report of a longitudinal cohort analysis published more than 10 years ago found that Candida spp. bronchial colonization was an independent risk factor for the establishment of Pseudomonas aeruginosa VAP (9 vs. 4.8% in non-colonized patients, P = 0.048). Likewise, the results of a retrospective single-center case-control study indicated that antifungal treatment of patients with Candida airway colonization was able to inhibit P. aeruginosa VAP [17]. Findings from recent research have revealed that Candida airway colonization was independently related to Acinetobacter baumannii VAP [18]. In another prospective cohort study, the FUNGBACT, that examined 146 patients under MV for more than 96 h. After adjusting for the immune index mHLA-DR, the findings revealed that there was no correlation between airway Candida coloni- zation and the incidence of VAP [HR: 0.98; 95% CI (0.59–1.65), p = 0.95] [33].

The co-occurrence of viral and fungal species is possible and both organisms can detect and react to a variety of diffusible signaling molecules created in the niches in which they co-exist. Increased host tissue damage and inflammation may result from fungi and COVID-19 cases is shallow. Many microbiology laboratories do not conduct further analysis when fast-growing Candida spp. are being isolated from RT samples. Further, only filamentous fungi isolation was being reported by some institutions [28]. (3) It is widely accepted that the cutoff counts of pathogenic bacteria for VAP diagnosis is 10³ CFU/mL (protected specimen brush sample) or 10⁴ CFU/mL (bronchoalveolar lavage fluid sample), but such consensus has not yet been reached for Candida; Candida pneumonia must be diagnosed by histopathology [27]. Thus, reporting Candida pneumonia is generally quite rare in the ICU, and the guidelines for the management of Candida spp. of both the IDSA and ESCMID do not recommend commencement of antifungal treatment without clear histological evidence of infection [24,29]. Reports of clinical studies from some centers have highlighted the isolation rate of Candida from the RT of ICU patients using MV to be as high as 50% with a prolonged median hospital stay (59.9 vs. 38.6 days, p = 0.006) or even increased the hospital mortality (34.2 vs. 21.0%, p = 0.003) [30]. Moreover, it might be associated with persistent immunosuppression and inflammation [31]. Candida airways colonization and its consecu- tive secretory inflammation may worsen the host’s cellular immune function, especially in immunosuppressed hosts with severe monocyte and lymphocyte dysfunction that results in a decreased effective clearance of bacteria and fungi and may increase the incidence of VAP [32]. A report of a longitudinal cohort analysis published more than 10 years ago found that Candida spp. bronchial colonization was an independent risk factor for the establishment of Pseudomonas aeruginosa VAP (9 vs. 4.8% in non-colonized patients, P = 0.048). Likewise, the results of a retrospective single-center case-control study indicated that antifungal treatment of patients with Candida airway colonization was able to inhibit P. aeruginosa VAP [17]. Findings from recent research have revealed that Candida airway colonization was independently related to Acinetobacter baumannii VAP [18]. In another prospective cohort study, the FUNGBACT, that examined 146 patients under MV for more than 96 h. After adjusting for the immune index mHLA-DR, the findings revealed that there was no correlation between airway Candida coloni- zation and the incidence of VAP [HR: 0.98; 95% CI (0.59–1.65), p = 0.95] [33].

The co-occurrence of viral and fungal species is possible and both organisms can detect and react to a variety of diffusible signaling molecules created in the niches in which they co-exist. Increased host tissue damage and inflammation may result from fungi and COVID-19

### Table 3
Characteristics of patients, clinical findings, signs and symptoms, laboratory findings, and outcome in patients colonized with C. albicans, patients colonized with C. glabrata, and non-colonized patients.

| Variable                        | Candida albicans colonization (n = 55) | Candida glabrata colonization (n = 12) | No Candida colonization (n = 31) |
|---------------------------------|--------------------------------------|--------------------------------------|---------------------------------|
| Colonization                    | 55/69 (79.7)                         | 12/69 (17.4)                         | 31/31 (100)                     |
| Age, yr, median (range)         | 56.1 (21–88)                         | 67.9 (44–83)                         | 56.6 (26–89)                    |
| Sex, F, n (%)                   | 30/55 (54.5)                         | 6/12 (50)                            | 13/31 (41.9)                    |
| Diabetes Mellitus, n (%)        | 21/55 (38.2)                         | 6/12 (50)                            | 4/31 (12.9)                     |
| Kidney disorder, n (%)          | 5/55 (9.1)                           | 2/12 (16.7)                          | 1/31 (3.2)                      |
| Malignancy, n (%)               | 8/55 (14.5)                          | 0/12 (0.0)                           | 2/31 (6.4)                      |
| Cardiovascular disease, n (%)   | 5/55 (9.1)                           | 2/12 (16.7)                          | 1/31 (3.2)                      |
| C. albicans, n (%)              | 1/55 (1.8)                           | 2/12 (16.7)                          | 0/31 (0.0)                      |
| C. glabrata, n (%)              | 10/55 (18.2)                         | 7/12 (58.3)                          | 5/31 (16.2)                     |
| Urine culture, n (%)            | 53/55 (96.4)                         | 12/12 (100)                          | 24/31 (77.4)                    |
| Headache                        | 46/55 (83.6)                         | 12/12 (100)                          | 27/31 (87.1)                    |
| Myalgia                         | 51/55 (92.7)                         | 11/12 (91.7)                         | 19/31 (61.3)                    |
| Arthralgia                      | 25/55 (45.4)                         | 9/12 (75)                            | 7/31 (22.6)                     |
| Gastrointestinal                | 43/55 (78.2)                         | 5/12 (41.7)                          | 13/31 (41.9)                    |
| Dyspepsia                       | 55/55 (100)                          | 12/12 (100)                          | 27/31 (87.1)                    |
| WBC, mm³, median                | 8.48 ± 10³/μmol/mm³                 | 9.9 ± 10³/μmol/mm³                  | 10.7 ± 10³/μmol/mm³            |
| FBS, mg/dl, median (range)      | 189 (75–507)                         | 164 (36–470)                         | 101 (84–266)                    |
| CRP, mg/dl, median (range)      | 34.4 (8–100)                         | 55.8 (11–103)                        | 31.2 (12–82)                    |
| ESR, mm/hr., median (range)     | 96.1 (1–2382)                        | 109 (21–344)                         | 85.6 (1–339)                    |
| Interval from ICU admission to ICU discharge (median, range) | 10.2 (5–30) | 16.8 (7–35) | 10.9 (3–14) |
| Mortality, n (%)                | 33/55 (60)                           | 11/12 (91.7)                         | 19/31 (61.3)                    |

### Table 4
MIC range and MIC 90 of five antifungals against Candida species.

| Species | Antifungal agents | C. albicans N = 55 | C. glabrata N = 12 | C. africana N = 2% |
|---------|-------------------|--------------------|-------------------|-------------------|
|         | Amphotericin B μg/mL | 10 (83.4) | 2 (100) | 2 (100) |
|         | R (7.3) | 2 (16.6) | 0 (0) | 0 (0) |
|         | Itraconazole μg/mL | 3 (25) | 2 (100) | 2 (100) |
|         | R (45.5) | 9 (75) | 0 (0) | 0 (0) |
|         | Voriconazole μg/mL | 4 (33.4) | 2 (100) | 2 (100) |
|         | R (20.36) | 6 (66.6) | 0 (0) | 0 (0) |
|         | Fluconazole μg/mL | 8 (66.6) | 0 (0) | 0 (0) |
|         | R (20.18) | 12 (100) | 0 (0) | 0 (0) |
|         | Caspofungin μg/mL | 10 (83.4) | 2 (100) | 2 (100) |
|         | R (61.0) | 2 (16.6) | 0 (0) | 0 (0) |

S: susceptible; R: resistance.
interaction. However, in a murine model, Adar et al. found that animals colonized by direct tracheal inoculation of live Candida spp. with a protocol developed to acquire Candida spp. colonization without epithelial injury was immune to P. aeruginosa pneumonia [34]. Besides, in a cohort investigation, usage of nebulized amphotericin B in people with Candida spp. airway colonization who used mechanical ventilation did not affect the incidence rate of VAP or ICU mortality despite the increase in the rate of Candida spp. decolonization. Furthermore, micafungin treatment of people with multiple Candida spp. colonization, new sepsis of unknown etiology, and multiple organ failure could not decrease the incidence rate of VAP in comparison with the placebo [35]. Regarding the results, amphotericin B and caspofungin showed superior activity against all Candida spp. and were recommended for the treatment regimen of pulmonary candidiasis associated with COVID-19 infection. Various degrees of resistance to voriconazole, itraconazole and fluconazole were seen in Candida spp. and were recommended for the treatment of VAP in comparison with the placebo [35].

5. Conclusion

In this study, the use of automatic ventilation, respiratory colonization, or infection with Candida spp. was not recognized to influence variables of the infection or death caused by COVID-19. Although “Candida pneumonia” is rarely being reported in critically ill patients, Candida airway colonization mainly by Candida albicans is common especially among patients with diabetes, malignancies, and kidney disorders. In this study, amphotericin B and caspofungin showed superior activity against all Candida spp.

Authors’ contribution

Study concept and design and technical supervision: Erami M, Hashemi SJ; Obtaining the specimens from patients and interpretation: Erami M, Heravi M, Yarahmadi M, Amiri S, Fakhrehi M, Raissi V; Acquisition of data and drafting of the manuscript: Raiesi O, Getso M; Procedure: Erami M, Raiesi O.

Financial support

This work was supported by the Tehran University of Medical Sciences, Tehran, Iran (grant number: 9711353002).

CRediT authorship contribution statement

Mahzad Erami: Investigation, Data curation, Conceptualization. Omid Raiesi: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. Mansooreh Momen-Heravi: Methodology, Investigation, Data curation. Muhammad Ibrahim Getso: Writing – review & editing, Writing – original draft. Mojtaba Fakhrehi: Formal analysis, Data curation. Narges Mehri: Methodology, Investigation, Data curation. Mohammad Yarahmadi: Formal analysis, Data curation. Sasan Amiri: Data curation. Vahid Raiesi: Data curation. Seyed Jamal Hashemi: Visualization, Validation, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Seyed Jamal Hashemi reports financial support was provided by Tehran University of Medical Sciences.

References

[1] M. Mohseni, V. Raisi, Y. Sharifan, K. Barkiro, S. Amir, M.S. Mohseni, et al., Therapeutic status of famotidine in COVID-19 patients: a review, Infect. Disord. - Drug Targets (2022), https://doi.org/10.2174/18717265266620201725511.
[2] Z.Y. Zu, M.D. Jiang, P.P. Xu, W. Chen, Q.Q. Ni, G.M. Lu, et al., Coronavirus disease 2019 (COVID-19): a perspective from China, Radiology 296 (2020) E15–E25.
[3] Z. Tabanejad, S. Darvich, Z. Borjan Boroujeni, S.S. Asadi, M. Mesty, O. Raiesi, et al., Seroepidemiological study of novel coronavirus disease (CoVid-19) in Tehran, Iran, Infection Epidemiology and Microbiology 7 (2021), 0.
[4] B Kayaslan, A Kaya Kalem, D Asliturk, B Kaplan, G Donertas, I Hasanoglu, et al., Incidence and risk factors of COVID-19 associated candidemia (CAI) in ICU patients, Microbes 2022 (2021) 1–9, https://doi.org/10.1101/2021.11.13.4341.
[5] B, Kayaslan, F. Eser, A. Kaya Kalem, Z. Bilgic, D. Asliturk, I. Hasanoglu, et al., Characteristics of candidemia in COVID-19 patients; increased incidence, earlier occurrence and higher mortality rates compared to non-COVID-19 patients, Microbes 64 (2021) 1083–1091.
[6] M. Nucci, G. Barreiros, L.F. Guimarães, V.A. Deriquêmed, A.C. Castêseiras, S. A. Nour, Increased incidence of candidemia in a tertiary care hospital with the COVID-19 pandemic, Microbes 64 (2021) 152–156.
[7] A. Arastehfar, T. Shahan, H. Zarrinfar, M. Roudbarly, M. Ghazanfari, M.-T. Hedaya, et al., Candidemia among Iranian patients with severe COVID-19 admitted to Kous, Journal of Fungi 7 (2021) 280.
[8] M. Salehi, K. Ahmadkia, S. Mahmoudi, K. Kalantari, S. Jamalolghahamishkhali, A. Izadi, et al., Orpharyngeal candidiasis in hospitalised COVID-19 patients from Iran: species identification and antifungal susceptibility pattern, Mycoses 63 (2020) 771–778.
[9] O. Epelbaum, R. Chasan, Candidemia in the intensive care unit, Clin. Chest Med. 38 (2017) 493–509.
[10] H.-H. Wu, Y.-T. Chen, C.-J. Shih, Y.-T. Lee, S.-C. Kuo, T.-L. Chen, Association between recent use of antibiotics and development of nosocomial infections and nosocomial salmonellosis: a nested case-control study, Clin. Infect. Dis. 59 (2014) 1554–1558.
[11] K.M. Hillman, P.J. Bristow, T. Chey, K. Daffurn, T. Jacques, S.L. Norman, et al., Duration of life-threatening antecedents prior to intensive care admission, Intensive Care Med. 28 (2002) 1629–1634.
[12] T. Shokohi, M.H. Soti, Z.S. Pouri, M. Hedayati, S. Mayahi, Identification of Candida species using PCR-RFLP in cancer patients in Iran, Indian J. Med. Microbiol. 28 (2010) 147–151.
[13] H. Khadami, L. Masooei, S. Farahy, A.A. Delbandi, O. Raiesi, A. Farzanezeg, et al., Antifungal Activity of caprylic acid, nystatin, and fluconazole and their in vitro interactions against candida isolates from neonatal oral thrush, Assay Drug Dev. Technol. 18 (2020) 195–201.
[14] O. Romeo, G. Grisio, First molecular method for discriminating between Candida africana, Candida albicans, and Candida dubliniensis by using hwp1 gene, Diagn. Microbiol. Infect. Dis. 62 (2008) 235–238.
[15] A. Fattahi, L. Zein, H. Mousaomi-Ali, S. Sayyahfar, M. Kalani, M.R. Khourugami, et al., Candidemia and its risk factors in neonates and children, Archives of Pediatric Infectious Diseases 8 (2020) 1–5.
[16] P. Wayne, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard-M2-91. CLSI Document M2-A9, 2017.
[17] I.M. Green, I. Margoni, S.P. Nair, H. Petridis, Adhesion of methicillin-resistant Staphylococcus aureus and Candida albicans to parylene-C-coated polymethyl methacrylate, Int. J. Frostbath. 32 (2019) 193–195.
[18] M. Dadar, R. Tiwari, K. Karthik, S. Chakraborty, Y. Shabali, K. Dhamma, Candida albicans-Biology, molecular characterization, pathogenicity, and advances in diagnosis and control-An update, Microb. Pathog. 117 (2021) 128–138.
[19] Z.B. Boroujeni, S. Shamsaei, M. Yarahmadi, M.I. Getso, A.S. Khorrashad, L. Haghighi, et al., Distribution of invasive fungal infections: molecular epidemiology, etiology, clinical conditions, diagnosis and risk factors: a 3-year experience with 490 patients under intensive care, Microb. Pathog. 152 (2021), 104616.
[20] O. Raiesi, S.J. Hashemi, M.M. Ardabali, K. Ahmadkia, M.I. Getso, F. Pakdel, et al., Molecular identification and clinical features of fungal rhinosinusitis: a 3-year experience with 108 patients, Microb. Pathog. 158 (2021), 105018.
[21] O. Raiesi, S.J. Hashemi, M.I. Getso, P. Ardil, M.M. Ardabali, V. Raisi, et al., First report of chronic invasive fungal rhinosinusitis in a patient with ovarian cancer caused by Didymella pedieae and successful treatment with voriconazole: a case report, Current Medical Mycology 7 (2021) 55.
[22] S. Shamshami, M. Falahati, S. Farahy, O. Raiesi, L. Haghighi, H.E. Farahani, et al., Acute invasive fungal rhinosinusitis: molecular identification and update in management of frozen section biopsy, Microb. Pathog. 159 (2021), 105125.
[23] Performance Standards for Antifungal Susceptibility Testing of Yeasts, 2017. CLSI supplement M60-A6. 1 st ed. 1–2, https://clsi.org/media/1895/m60ed6_sample.pdf.
[24] P.G. Pappas, C.A. Kauffman, T.H. Andes, C.J. Chancy, R.A. Marr, L. Ostrosky-Zeichner, et al., Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America, Clin. Infect. Dis. 62 (2016) e1–e50.
[25] M. Hatem, A. Favon, F. Dalle, A. Pechnot, S. Prin, J.-P. Quenet, et al., Candida spp. airway colonization could promote antibiotic-resistant bacteria selection in patients with suspected ventilator-associated pneumonia, Intensive Care Med. 38 (2012) 1272–1279.
[26] R.A. Gabrielik, K.P. Rumbah, Biofilm models of polymicrobial infection, Future Microbiol. 10 (2015) 1997–2015.
[27] A.C. Kalil, M.L. Metersky, M. Klompas, J. Muscedere, D.A. Sweeney, L.B. Palmer, et al., Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society, Clin. Infect. Dis. 63 (2016) e61–e111.

[28] J. Barenfanger, P. Arakere, R.D. Cruz, A. Imran, C. Drake, J. Lawhorn, et al., Improved outcomes associated with limiting identification of Candida spp. in respiratory secretions, J. Clin. Microbiol. 41 (2003) 5645–5649.

[29] I. Martin-Loeches, M. Antonelli, M. Cuenca-Estrella, G. Dimopoulos, S. Einav, J. De Waele, et al., ESICM/ESCMID task force on practical management of invasive candidiasis in critically ill patients, Intensive Care Med. 45 (2019) 789–805.

[30] M.-S. Delisle, D.R. Williamson, M.M. Perreault, M. Albert, X. Jiang, D.K. Heyland, The clinical significance of Candida colonization of respiratory tract secretions in critically ill patients, J. Crit. Care 23 (2008) 11–17.

[31] Y. Huang, Y. Jiao, J. Zhang, J. Xu, Q. Cheng, Y. Li, et al., Microbiological and prognostic factors of ventilator-associated pneumonia: a multicenter retrospective study in Shanghai, Clin. Infect. Dis. 67 (2018) S146–S152.

[32] M.-S. Delisle, D.R. Williamson, M. Albert, M.M. Perreault, X. Jiang, A.G. Day, et al., Impact of Candida species on clinical outcomes in patients with suspected ventilator-associated pneumonia, Can. Respir. J. Can. Thorac. Soc. 18 (2011) 131–136.

[33] J.-F. Timsit, C. Schwebel, L. Styfálova, M. Cornet, P. Poirier, C. Forrestier, et al., Impact of bronchial colonization with Candida spp. on the risk of bacterial ventilator-associated pneumonia in the ICU: the FünGIBACT prospective cohort study, Intensive Care Med. 45 (2019) 834–843.

[34] F. Ader, S. Jawhara, S. Nseir, E. Kipnis, K. Faure, F. Vuotto, et al., Short term Candida albicans colonization reduces Pseudomonas aeruginosa-related lung injury and bacterial burden in a murine model, Crit. Care 15 (2011) 1–9.

[35] P.J. Vaan der Geest, E.I. Dieters, B. Rijnders, J.A. Groeneveld, Safety and efficacy of amphotericin-B deoxycholate inhalation in critically ill patients with respiratory Candida spp. colonization: a retrospective analysis, BMC Infect. Dis. 14 (2014) 1–9.

[36] S. Antinori, C. Bonazzetti, G. Gubertini, A. Capetti, C. Fagani, V. Morena, et al., Tocilizumab for cytokine storm syndrome in COVID-19 pneumonia: an increased risk for candidemia? Autoimmun. Rev. 19 (2020) 102564, https://doi.org/10.1016/j.autrev.2020.102564.

[37] I. Ventoulis, T. Sarmourli, P. Amoiridou, P. Mantzana, M. Exindari, G. Gioula, et al., Bloodstream infection by Saccharomyces cerevisiae in two COVID-19 patients after receiving supplementation of Saccharomyces in the ICU, Journal of Fungi 6 (2020) 98.

[38] L. White, R. Dhillon, A. Cordey, H. Hughes, F. Faggian, S. Soni, et al., A National Strategy to Diagnose COVID-19 Associated Invasive Fungal Disease in the ICU, 2020.

[39] B. Posteraro, R. Torelli, A. Vella, P.M. Leone, G. De Angelis, E. De Carolis, et al., Pan-echinocandin-resistant Candida glabrata bloodstream infection complicating COVID-19: a fatal case report, Journal of Fungi 6 (2020) 163.

[40] A.M. Al-Hatmi, J. Mohsin, A. Al-Hurazi, F. Khamis, COVID-19 associated invasive candidiasis, J. Infect. 82 (2020) 45–46.

[41] A. Chowdhury, B. Tarai, A. Singh, A. Sharma, Multidrug-resistant Candida auris infections in critically ill coronavirus disease patients, India, April–July 2020, Emerg. Infect. Dis. 26 (2020) 2694.

[42] O. Raiesi, H. Shahbandoust, M. Getso, V. Raisi, A.A. Rezaei, Candida auris: a new emerging fungal monster, Archives of Clinical Infectious Diseases 14 (2019),