Mycopathologia (2023) 188:9–20
https://doi.org/10.1007/s11046-022-00694-x

ORIGINAL ARTICLE

Assessment of Risk Factors and Clinical Outcomes in Hospitalized COVID-19 Patients with *Candida* spp. Co-infections: Species Distribution and Antifungal Susceptibility Patterns of Isolates

Somayeh Yazdanpanah · Mohammad Ahmadi · Zahra Zare · Hamed Nikoupour · Sara Arabsheybani · Ahmad Jabrodini · Esmaeel Eghtedarnejad · Parisa Chamanpara · Bita Geramizadeh · Mohammad Hossein Anbardar · Zahra Malekizadeh · Maryam Gashtasebi · Mehdi Mohsenzadeh · Mojtaba Shafiekhani · Kamiar Zomorodian

Received: 2 September 2022 / Accepted: 12 November 2022 / Published online: 10 December 2022

Abstract

Introduction Fungal co-infections are considered an important complication in hospitalized patients with SARS-CoV-2 that can be attributed to disease aggravation, increased mortality, and poor outcomes. This study was conducted to determine the species distribution and antifungal susceptibility patterns of *Candida* isolates from hospitalized COVID-19 patients in Shiraz, Iran, in addition to associated risk factors and outcomes of co-infections with *Candida* species.

Materials and Methods In this single-center study, a total of 106 hospitalized COVID-19 patients were evaluated for clinical characteristics and outcomes. Species identification was performed by ITS1-5.8S-ITS2 gene sequencing. Antifungal susceptibility testing to fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, amphotericin B, and

Handling Editor: Abdullah Mohammed Said Al-Hatmi.

S. Yazdanpanah · A. Jabrodini · E. Eghtedarnejad
Student Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

S. Yazdanpanah · A. Jabrodini · E. Eghtedarnejad · K. Zomorodian (✉)
Department of Medical Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
e-mail: zomorodian@sums.ac.ir

M. Ahmadi · Z. Zare · H. Nikoupour · S. Arabsheybani · B. Geramizadeh · M. H. Anbardar · Z. Malekizadeh · M. Gashtasebi · M. Shafiekhani (✉)
Shiraz Transplant Center, Abu-Ali Sina Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
e-mail: mojtabashafiekhani@gmail.com

P. Chamanpara
Department of Biostatistics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

M. Mohsenzadeh
Cellular and Molecular Research Center, Gerash University of Medical Sciences, Gerash, Iran

M. Shafiekhani
Department of Clinical Pharmacy, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

M. Shafiekhani
Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

K. Zomorodian
School of Medicine, Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

nystatin was determined according to the M27-A3/S4 CLSI protocol.

Results *Candida* species were recovered from 48% (51/106) of hospitalized COVID-19 patients. Statistical analysis showed that patients who had heart failure, bacterial co-infection, and were receiving empirical antifungal therapy had a higher risk of developing *Candida* co-infection. In total, 71 *Candida* isolates were recovered, of which *C. albicans* (69%) was the most prevalent isolate. The majority of the *Candida* isolates were susceptible to all classes of tested antifungal drugs.

Discussion Our results elucidate a high rate of *Candida* co-infections among hospitalized COVID-19 patients. Comorbidities such as heart failure, HTN, COPD, bacterial infections as well as therapeutic interventions including catheterization, mechanical ventilation, and ICU admission increased the risk of *Candida* spp. isolation from the bloodstream, respiratory tract and urine samples, which led to a higher in-hospital mortality rate. Additionally, obtained data clarified that empirical antifungal therapy was not as successful as anticipated.

Keywords Co-infection · COVID-19 · *Candida* · Antifungal susceptibility · Candidiasis

Introduction

Microbial co-infections in hospitalized patients with COVID-19 have been documented in many investigations from the onset of the global pandemic in Wuhan. Co-infections in SARS-CoV-2 infected individuals were caused by a variety of pathogens, and it was determined that these concomitant infections resulted in disease aggravation and poor outcomes [1–3].

In patients with SARS-CoV-2 infection, predisposing conditions such as mechanical ventilation and ICU admission in critically ill patients, the use of broad-spectrum antibiotics, immunosuppressive/anti-inflammatory treatments, catheterization, and underlying diseases all increase the risk of secondary infections [4, 5]. Among fungal pathogens, opportunistic fungi such as *Aspergillus* and *Candida* species cause fungal co-infections in COVID-19 patients [6, 7]. Since species of the genus *Candida* are normal inhabitants of such various sites and internal organs as skin, mucous membranes, respiratory tract, digestive system, and urinary tract, they can give rise to infections due to the presence of favorable conditions in COVID-19 patients [6, 8].

Iran has been one of the countries most afflicted by the COVID-19 pandemic in the Middle East, with 7,553,169 confirmed cases and 144,502 deaths as of October 16, 2022 [9]. Early diagnosis and management of concomitant fungal infections in COVID-19 patients with effective antifungal agents leads to improved clinical outcomes. As a result, clinicians can benefit from a better understanding of the epidemiological evidence of co-infections in COVID-19 patients, such as risk factors, species distribution, and susceptibility profiles of isolates, to manage and control super-infections. In view of these considerations, this study was conducted to assess the risk factors as well as clinical outcomes of *Candida* co-infections in patients with COVID-19 admitted to Abu-Ali Sina hospital in Shiraz, during the COVID-19 pandemic. Furthermore, the species distribution and antifungal susceptibility profiles of isolates recovered from COVID-19 patients were determined in this study.

Materials and Methods

Study Design and Participants

For this observational and single-center study, all patients with confirmed COVID-19 admitted to Abu-Ali Sina hospital in Shiraz, one of the largest hospitals in the south of Iran, were evaluated for *Candida* co-infections from September to November 2021. This center has 600 individual hospital beds, and the number of beds was increased during the COVID-19 pandemic. SARS-CoV-2 was diagnosed based on positive Real-time Polymerase Chain Reaction (PCR) tests for SARS-CoV-2 or according to the clinical guidelines definition for COVID-19 [10]. All yeast isolates were recovered from clinical samples of COVID-19 patients (blood, urine, tracheal aspirate) during their hospital stay. Moreover, no clinical intervention in antifungal treatment was included in this study.

The patient data was extracted from the electronic medical record system of the hospital considering

several variables, such as demographic characteristics, comorbidities, COVID-19 severity, and management such as antivirals, corticosteroids, antibacterial and immunomodulators, bacterial co-infections, empirical/definitive antifungal therapy, length of hospitalization, and length of ICU stay. In addition, relative laboratory results for the patients who participated in the study were collected. Moreover, risk factors associated with yeast co-infections and also clinical outcomes were investigated. Informed consent was obtained from all individual participants included in the study, which was approved by the ethics committee of the Shiraz University of Medical Sciences (IR.SUMS.REC.1401.210).

Fungal Culture and Isolation

To detect fungal strains, all clinical samples obtained from confirmed COVID-19 patients (blood, urine, tracheal aspirate) were cultured on Sabouraud Dextrose Agar (SDA, HiMedia, India), and HiCrome *Candida* Differential Agar (HiMedia,India) in the Department of Microbiology of Abu-Ali Sina Medical Center.

Species Identification by Sequencing the rDNA Region

DNA extraction of pure colonies was performed according to the method as previously described [11]. Definitive species identification of isolates were performed by PCR-sequencing method following amplification of ITS region [12].The ITS region sequence for each isolate was assembled and used for BLAST explores (http://blast.ncbi.nlm.nih.gov/Blast.cgi?). Sequence data of ITS region were deposited in NCBI and GenBank, and accession numbers of sequences are available for all isolates as follows: ON 312540-69, ON312571-99, ON479767-77, ON514607.

Antifungal Susceptibility Testing (AFST)

Minimum Inhibitory concentration (MIC) values were determined using broth microdilution method according to the M27-A3/S4 protocol documented by Clinical and Laboratory Standards Institute (CLSI) [13]. All clinical isolates were evaluated for susceptibility to antifungals including Fluconazole (FLZ, Sigma,USA), Posaconazole (PSZ,Sigma,Germany), Voriconazole (VRZ, Pfizer, New York, USA), Itraconazole (ITR,Sigma,USA), Caspofungin (CSP,sigma,USA), Nystatin (NYS, Sigma, Germany), and also amphotericin B AMB,Sigma,Germany). RPMI 1640 (Sigma, St. Louis, Missouri,USA) was prepared as directed by the manufacturer, and buffered to pH 7.0 using 0.165 N-morpholino propanesulfonic acid (MOPS) (Sigma, USA). Pure isolates were grown on SDA by incubation for 24 h at 35 °C. Following the growth of isolates, the inoculum suspensions were made by suspending the colonies in NaCl, and the turbidity adjusted to 0.5 McFarland at 530 nm.Then, prepared suspension was diluted to 0.5–2.5 10^3 cells/ml in RPMI 1640 media. Two-fold serial dilutions of antifungals were made in 96-well plates, and 100 µlit of yeast inoculum was added to each well with in equal volume. Finally, the plates were incubated for 24 h at 35 °C.

Interpretation of data was performed using clinical breakpoints that described in former CLSI documents [14, 15], and new editions of defined breakpoints [16, 17].When the clinical breakpoint was not available, the epidemiological cutoff value (ECV) was used. MIC was defined as the lowest concentration of antifungal agents that inhibits the growth of yeast isolates by 50% for CSP, FLZ, VRZ, ITR, and PSZ in comparison to the controls (drug-free wells). Moreover, the lowest concentration that resulted in any visible growth of isolates (100% inhibition) was considered as the MIC for NYS and AMB. The final concentrations of the antifungal agents were 0.032–16 µg/ml for AMB, ITR, PSZ, VRZ, and NYS, 0.125–64 µg/ml for FLZ, and 0.015–8 µg/ml for CSP. *C. krusei* (ATCC 6258) was included as a reference strain for quality control.

Statistical Analysis

Data were analyzed using SPSS statistical software version 18.00 (SPSS Inc.IBM.USA). Continuous variables are shown as median and interquartile range (IQR). Also, continuous data with normal distribution were expressed either as means ± standard deviation. Categorical variables were reported as numbers and percentages. Statistical differences between groups were analyzed using the exact Chi-square and/or Fisher's exact test, and the χ^2 test or Mann–Whitney U

test. p-value < 0.05 was considered statistically significant.

To highlight the risk factors associated with *Candida* co-infections, univariable and multivariable logistic regression models were used. Factors with a p-value < 0.2 were retained for multivariate analysis, and those demonstrating statistical significance (p-value < 0.05) on multivariate analysis were considered verifiable risk factors.

Results

A total number of 106 patients were included in this study (51 patients with *Candida* spp. positive culture and 55 patients without *Candida* spp. positive culture). All the statistical data analyzed in this study is shown in Table 1.

Data are median (IQR) or n (%), *p- values were calculated by Mann–Whitney U test, χ^2 test, or Fisher's exact test, as appropriate. ICU:Intensive Care Unit, MV:Mechanical Ventilation, CVC: Central Venous Catheter, DM:Diabetese mellitus, HTN:Hypertension, ACS: Acute coronary syndrome, COPD: Chronic obstructive pulmonary disease, LDH: lactate dehydrogenase.

The mean age of total patients was 61 ± 16 years, ranging from 18 to 99 years. Of the total patients, 64 (60.4%) were male and 42 (39.6%) were female. Our results showed about 48% of hospitalized COVID-19 patients during this study period had urinary tract, respiratory system, and bloodstream co-infections with *Candida* species. According to statistical analysis of the data, there was a significant difference between patients with and without *Candida* spp. in terms of age (66 vs. 57 years; *p-value*: 0.003),whereas no significant difference was found in sex distribution between the two groups of patients. In patients with *Candida* positive culture, the median time from hospital admission to positive fungal culture was 6 days, ranging from 2 to 36 days (IQR: 4–11). As far as the time of staying in hospital in patients is concerned, there was not found a statistical difference in length of hospitalization between two groups (p-value: 0.9).

Compared to patients without *Candida* positive culture, a higher percentage of patients with *Candida* co-infections were admitted in the ICU (47.1% vs 27.3%; p-value:0.03), used mechanical ventilation (47.1% vs 25.5; p-value:0.02) and used urinary catheter (54.9% vs 30.9%; p-value:0.013). Also, the median time of urinary catheterization in patients with *Candida* spp. positive culture was 5 days, with a significant difference in comparison to patients without *Candida* spp. positive culture (p-value: 0.036).Although hypertension (HTN) and diabetes mellitus (DM) were the most common comorbidities among the study population, there was not a statistically significant association between the presence of *Candida* co-infection and HTN/DM in COVID-patients. In regards to other underlying conditions evaluated in this study, significant differences were observed in the presence of chronic obstructive pulmonary disease (COPD), heart failure, and solid organ transplantation. Among patients with COVID-19, those with *Candida* positive cultures showed a higher proportion of bacterial co-infection (60.8% vs. 32.7; p-value: 0.004). Moreover, in 24 of 45 patients with positive culture of *Candida* spp. from respiratory samples, isolation of bacterial species was also reported. *Enterococcus* and *Acinetobacter* spp. were the most commonly identified bacteria.

Regarding treatments, 93.4% of patients received antibiotics, 96.2% received high doses of corticosteroids, 82.1% received antiviral treatments, and 47.2% received antifungals as empirical therapy. Toclizumab, a recombinant anti-interleukin-6 receptor (IL-6R) monoclonal antibody, was prescribed for 19.8% of patients. Moreover, fluconazole was the most common antifungal agent used as empirical therapy in hospitalized patients with COVID-19 (31/50).

Antifungal treatment was administered to 34/51 patients with a *Candida* positive culture. The most commonly prescribed antifungal agents were as follows: fluconazole(12/34), combination therapy with caspofungin and fluconazole (6/34), liposomal amphotericin B (4/34), and nystatin (3/34). Although a higher proportion of in-hospital mortality was observed in patients with *Candida* positive cultures than in patients without *Candida* positive cultures, no significant difference in mortality rate was observed between the two groups (35.3% vs 20%; p-value = 0.07).

Regression analysis demonstrates that odds for *Candida* co-infection were higher in patients who admitted in ICU, had mechanical ventilatory supports, urinary catheterization, bacterial infection and patients with severe /critical diseases (Table 2). With

Table 1 Demographic characteristics, clinical data, laboratory findings, treatments and outcomes of COVID-19 patients (N = 106)

	Total (106)	Patients with *Candida* positive culture (51)	Paients without *Candida* positive culture (55)	p-*value*
Demographics characteristics				
Age, years (mean ± SD)	61 ± 16	66 ± 15	57 ± 11	0.003
Gender				
Male	64 (60.4%)	28 (54.9%)	36 (65.4%)	0.26
Female	42 (39.6%)	23 (45.1%)	19 (34.5%)	
Clinical interventions				
Nasal cannula oxygenation	90 (85.7%)	44 (86.3%)	46 (83.6%)	0.73
ICU admission	39 (36.8%)	24 (47.1%)	15 (27.3%)	0.03
MV	38 (35.8%)	24 (47.1%)	14 (25.5%)	0.02
CVC	36 (34%)	16 (31.4%)	20 (36.4%)	0.58
Urinary catheterization	45 (42.5%)	28 (54.9%)	17 (30.9%)	0.01
Comorbid and clinical conditions				
Hepatic failure	16 (15.2%)	5 (10%)	11 (20%)	0.15
Renal failure	32 (30.2%)	18 (35.3%)	14 (25.5%)	0.27
Heart failure	35 (33.0%)	26 (50.9%)	9 (16.4%)	< 0.001
DM	46 (43.4%)	25 (49.0%)	21 (38.2%)	0.26
HTN	69 (65.1%)	38 (74.5%)	31 (56.4%)	0.05
ACS	9 (8.5%)	4 (7.8%)	5 (9.1%)	0.81
COPD	15 (14.2%)	12 (23.5%)	3 (5.5%)	0.008
Malignancy	8 (7.5%)	5 (9.8%)	3 (5.5%)	0.39
Neutropenia	2 (1.9%)	0	2 (3.6%)	0.16
Hemodialysis	23 (21.9%)	10 (19.6%)	13 (24.1%)	0.58
Solid organ Transplantation	23 (21.7%)	5 (9.8%)	18 (32.7%)	0.004
Septic shock	28 (26.4%)	17 (33.3%)	11 (20.0%)	0.12
Bacterial co-infection	49 (46.2%)	31 (60.8%)	18 (32.7%)	0.004
Disease severity				
Mild/moderate	69 (65.1%)	28 (54.9%)	41 (74.5%)	0.03
Severe/critical	37 (34.9%)	23 (45.1%)	14 (25.5%)	
Treatments				
Antibacterial treatment	99 (93.4%)	49 (96.1%)	50 (90.9%)	0.44
Empirical antifungal treatments	50 (47.2%)	30 (58.8%)	20 (36.4%)	0.02
Anti-viral treatments	87 (82.1%)	40 (78.4%)	47 (85.5%)	0.34
IL-6 antagonist	21 (19.8%)	11 (21.6%)	10 (18.2%)	0.66
High dose corticosteroids	102 (96.2%)	47 (92.2%)	55 (100%)	0.05
Outcomes				
In-hospital mortality	29 (27.4%)	18 (35.3%)	11 (20%)	0.07
Duration of hospitalization, days	12 (8–17)	12 (8–16)	11 (8–18)	0.9
Duration of ICU stay, days	0 (0–6)	8 (5–14)	9 (5–23)	0.61
Duration of MV,days	0 (0–5)	8 (4–13)	7 (5–23)	0.98
Duration of CVC, days	0 (0–4)	1 (0–8)	4 (0–22)	0.19
Duration of urinary catheterization	0 (0–9)	5 (0–10)	0 (0–6)	0.036

Table 1 continued

	Total (106)	Patients with *Candida* positive culture (51)	Paients without *Candida* positive culture (55)	p-*value*
Laboratory findings				
White blood cell count, $\times 10^3$ per microliter	12 (7.25–20)	15 (11–23)	12 (4–16)	0.005
Neutrophil (%)	84 (79–90)	86.5 (81–92)	82 (76.75–89.25)	0.1
Serum Ferritin (ng/mL)	1218.50 (490.75–2476.50)	1219 (359–3001)	1218 (630–2373)	0.84
LDH (U/L)	735 (499–1236)	960 (564–1560)	643 (458–953)	0.005
D-dimer (μg/mL)	3.6 (1.49–13.48)	3.34 (1.43–18.80)	3.75 (1.54–11.02)	0.80
C-Reactive Protein (mg/mL)	128 (64–256)	128 (64–256)	128 (48–256)	0.1

Table 2 Risk factors associated with isolation of *Candida* spp. in hospitalized COVID-19 patients (N = 106)

	Univariable OR (95% CI)	*p*-value	Multivariable OR (95% CI)	*p*-value
Demographics characteristics				
Age,years[a]	1.03 (1.01–1.06)	0.005	–	–
Clinical interventions				
ICU admission	2.37 (1.05–5.32)	0.003	–	–
MV	2.6 (1.14–5.9)	0.02	–	–
Urinary catheterization	2.72 (1.22–6.02)	0.01	–	–
Comorbid and clinical conditions				
Heart failure	5.31 (2.15–13.8)	< 0.001	7.37 (2.4–22.5)	< 0.001
HTN	2.26 (0.99–5.16)	0.05	–	–
COPD	5.33 (1.4–20.19)	0.014	–	–
Solid organ transplantation	0.22 (0.07–0.65)	0.007	–	–
Bacterial co-infection	3.18 (1.43–7.06)	0.004	4.97 (1.8–13.6)	0.002
Disease severity[b]	2.4 (1.06–5.46)	0.036		
Treatments				
Empirical antifungal therapy	2.5 (1.14–5.46)	0.02	3.17 (1.1–8.4)	0.02
Laboratory findings				
Neutrophil (%)	1.04 (0.99–1.09)	0.07	–	–
C-Reactive protein	1 (0.99–1)	0.09	–	–

[a]Per 1 unit increase

[b]Severe/Critical versus Mild/Moderte disease

OR Odds Ratio, *CI* Confidence Interval, *ICU* Intensive Care Unit, *MV* Mechanical Ventilation, *HTN* Hypertension, *COPD* Chronic Pulmonary Obstructive Diseas

regards to underlying conditions, HTN, COPD, and heart failure are associated with an increased risk of the development of *Candida* co-infection among patients with COVID-19. Moreover, patients who had severe /critical SARS-CoV-2 infection were at a greater risk for *Candida* co-infections.

In univariable analysis, the odds of in-hospital mortality was higher in patients with positive *Candida* culture (RR: 2.18, 95% confidence interval: 0.9, 5.2). Moreover, a multiple logistical regression showed that heart failure, bacterial co-infection, and prescribed antifungals as empirical treatment were significant

Table 3 Species distribution of *Candida* isolates from different clinical specimens

Candida isolates	Tracheal aspirate	Urine	Blood	Total
C.albicans	31	16	2	49
C.glabrata	10	4	–	14
C.tropicalis	5	2	–	7
C.dubliniensis	1	–	–	1
Total	47	22	2	71

risk factors for *Candida* co-infection among hospitalized COVID-19 patients.

Overall, 67 episodes of *Candida* positive cultures were documented from different clinical samples (45 teracheal aspirate samples, 20 urine samples, and 2 blood samples) collected from 106 COVID-19 patients during the study period. With regards to the categorization of fungal infections according to European Organisation for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG), two patients had proven invasive candidiasis [18]. Moreover, 45 patients were identified with *Candida* airway colonization and 20 patients with candiduria. Also, 4 samples (2 urine and 2 teracheal aspirate) presented mixed infection. Of the patients with *Candida* positive culture, 11 patients showed multiple episodes of *Candida* infections during their hospitalization. Totally, 71 *Candida* spp. were isolated from 51 COVID-19 patients with *Candida* positive culture as follows: *C. albicans* (*n*:49, 69%), *C.glabrata* (*n*:14, 19.7%), *C. tropicalis* (n: 7, 9.9%), and *C. dubliniensis* (n:1, 1.4%) (Table 3).

The categorization of all *Candida* isolates in this study was based on obtained MICs according to recommended breakpoints by CLSI as shown in Table 4. Moreover, *in-vitro* antifungal susceptibility patterns of all isolates are shown in Table 5 in detail.

All *Candida* isolates presented low MIC values for CSP (MIC range: 0.03–0.12) that classified as susceptible according to M-60 CLSI breakpoints. Resistant to itraconazole were observed with a high proportion among *Candida* species regarding breakpoints documented by M27-S3. According to the M59 CLSI document, all *C. albicans* isolates were wild-type (WT) for AMB. Among non-*albicans Candida* species, one *C.glabrata* isolate had a non-wild-type (NWT) phenotype for AMB. In regards to another polyene drug, nystatin, all *Candida* isolates were categorized as susceptible. Although no resistant isolate was found among all *Candida* species against posaconazole, few isolates were resistant to other azoles tested in this study. One *C. albicans* isolate was cross-resistant to VRZ (≥ 1 μg/ml) and ITZ (≥ 1 μg/ml), and also two *C.albicans* were cross-resistant toFLZ (≥ 8 μg/ml), VRZ (≥ 1 μg/ml) and ITZ (≥ 1 μg/ml). Cross-resistant species against FLZ (≥ 8 g/ml) and ITZ (≥ 1 g/ml) were also found in one *C.albicans* and one *C.tropicalis* isolate.

Discussion

The presence of fungal co-infections is considered an important complication in COVID-19 hospitalized patients by association with increased mortality [19–21]. COVID-19-Associated Candidiasis has been studied in different parts of the world with a range of 0.7–23.5% [4]. According to obtained data, almost half of hospitalized patients with COVID-19 have shown at least one positive culture of urinary, respiratory, or blood specimens for *Candida* species during their stay in hospital. To the best of our knowledge, this is the first comprehensive study on COVID-19-associated candidiasis (CAC) that included all clinical samples collected from patients with COVID-19 in Iran. It should be noted that the inclusion of both invasive and non-invasive candidiasis cases in this study led to a considerable difference in our reported rates compared to earlier studies.

As we know, *Candida* spp. are a part of the human microbiome that resides in different sites. So, dysregulation of the immune system following infection with SARS-CoV-2 leaves patients vulnerable to *Candida* superinfections. In general, previous evidence supports our findings that ICU-admission, mechanical ventilation, and urinary catheterization represent important risks for the development of candidiasis [4, 22–28].

Moreover, underlying conditions in COVID-19 patients, including DM, hypertension, and organ failure, in addition to a number of various antimicrobial and immunosuppressive medications, may be related to fungal co-infections [21, 29–32]. However, in the present work we identified HTN, COPD, hepatic and heart failures as risk factors for CAC that are in consistent with other previous studies in Iran and other parts of the world [25, 27, 28, 33].

Table 4 MIC interpretation of tested antifungal drugs for *Candida* spp. recovered from COVID-19 patients

Candida species	Antifungal drugs						
	Susceptibility profile	FLZ	ITZ	VRZ	CSP	PSZ	AMB	NYS
C. albicans (n = 49)	≤ ECV	47	39	27	49	32	49	
	> ECV	2	10	22	0	17	0	
	S	39	29	34	49		NA	49
	SDD/I	6	10	8	0		NA	0
	R	4	10	7	0		NA	0
C. glabrata (n = 14)	≤ ECV	12	14	12	14	13	13	
	> ECV	2	0	2	0	1	1	
	S	0	7		14		NA	14
	SDD/I	14	5		0		NA	0
	R	0	2		0		NA	0
C. tropicalis (n = 7)	≤ ECV	6	6	5	7	6	7	
	> ECV	1	1	2	0	1	0	
	S	6	4	5	7		NA	7
	SDD/I	0	2	2	0		NA	0
	R	1	1	0	0		NA	0
C.dubliniensis (n = 1)	≤ ECV	1	0	1	1	1	1	
	> ECV	0	1	0	0	0	0	
	S		1		1		NA	1
	SDD/I		0		0		NA	0
	R		0		0		NA	0

ECV Epidemiological cut-off value, *S* Susceptible, *SDD/I* Susceptible-dose-dependant/Intermediate, *R* Resistant, *FLZ* fluconazole, *ITZ* itraconazole, *VRZ* voriconazole, *CSP* caspofungin, *PSZ* posaconazole, *AMB* amphotericin B, *NYS* nystatin, *NA* not applicable, *MIC* ≤ *ECV* wild-type, *MIC* > *ECV* non-wild-type

Similar to other investigations, our results revealed that more severe conditions of pneumonia associated with SARS-Cov-2 infection increased the probability of fungal co-infections [25, 34]. Certainly, such therapeutic approaches in severe cases of COVID-19 patients as steroids, mechanical ventilation, broad-spectrum antimicrobial therapy, and ICU admission make them more susceptible to candidiasis, which has been previously reported in the Iranian population [27, 28, 35]. However, further studies are needed to investigate the possibility that *Candida* infection may increase the severity of the disease in patients with COVID-19.

Despite the fact that isolation of *Candida* spp. from respiratory samples occurs particularly in patients on mechanical ventilation, the link between this colonization and pneumonia is still controversial. Because the definitive diagnosis of *Candida* pneumonia is confirmed by examining the invasive form of the fungus in biopsy tissue, that is a limited diagnostic procedure. Since the lung is the main affected organ in COVID-19 patients, *Candida* colonization following immunosuppressive treatments and antibacterial medications can lead to overgrowth and morphogenesis of *Candida* spp. due to induced defects in cell-mediated immunity of the respiratory epithelium [36–39].

According to our research findings, patients with bacterial co-infection were at higher risk for *Candida* infections. Also, a high proportion of COVID-19 patients received antibacterial treatment, which is in agreement with the previous literature [5, 40]. Aligned to these results, it could be explained that overprescribing of broadspectrum antibiotics in COVID-19 patients causes an imbalance of normal flora and overgrowth of *Candida,* which could result in *Candida* co-infections [41]. Furthermore, the coexistence of bacterial and fungal infections could contribute to increased pathogenicity, host damage, and inflammation [42]. Our findings showed that mortality among COVID-19 patients with *Candida* co-infection was higher in comparison to COVID-19 alone, which is in agreement with many previous studies [25, 34, 43–45].

Efficacy of empirical or preemptive antifungal therapy in high-risk patients has been studied in a number of researches [46, 47]. In nearly half of our

Table 5 *In-vitro* antifungal susceptibility patterns of *Candida* spp. recovered from COVID-19 patients using micro broth dilution method

Candida spp. (71)	Antifungal drug	Range	GM[a]	MIC50	MIC90	MIC[b] (μg/ml)									
						0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
C.albicans (49)	Fluconazole	0.12–16	0.91	0.5	4			1	10	15	7	6	6	2	2	
	Itraconazole	0.03–4	0.17	0.12	1	4	14	11	9	1	7		3			
	Voriconazole	0.03–8	0.11	0.03	2.2	18	9	7	4	4	1		4	2		
	Posaconazole	0.03–1	0.07	0.06	0.3	16	16	6	6	3	2					
	Caspofungin	0.03–0.06	0.03	0.03	0.06	40	9									
	Nystatin	0.06–8	1.16	2	4		1		13	6	2	8	18	1		
	Amphotericin B	0.06–2	0.37	0.5	1		1	4	16	20	6	2				
C.glabrata (14)	Fluconazole	0.12–32	1.55	1	6.8			1		4	3		4	1		1
	Itraconazole	0.03–1	0.16	0.12	0.8	2	3	2	3	2	2					
	Voriconazole	0.03–1	0.09	0.06	0.4	6	2	2	2	1	1					
	Posaconazole	0.03–2	0.08	0.06	0.3	4	4	4		1		1				
	Caspofungin	0.03–0.12	0.03	0.03	0.12	11	1	2								
	Nystatin	0.25–8	0.8	0.3	6.8				7	1	1	1	2	2		
	Amphotericin B	0.06–2	0.4	0.5	1		1		4	3	5	1				
C.tropicalis (7)	Fluconazole	0.25–8	0.74	NA	NA				2	3		1		1		
	Itraconazole	0.06–2	0.18	NA	NA		2	2	2			1				
	Voriconazole	0.03–0.5	0.15	NA	NA	2	1	2	1	1						
	Posaconazole	0.03–0.25	0.08	NA	NA	1	3	2	1							
	Caspofungin	0.03–0.06	0.03	NA	NA	6	1									
	Nystatin	0.5–4	1.64	NA	NA					2	1	1	3			
	Amphotericin B	0.25–1	0.55	NA	NA				2	2	3					
C.dubliniensis (1)	Fluconazole	–	–	–	–				1							
	Itraconazole	–	–	–	–		1									
	Voriconazole	–	–	–	–		1									
	Posaconazole	–	–	–	–	1										
	Caspofungin	–	–	–	–	1										
	Nystatin	–	–	–	–							1				
	Amphotericin B	–	–	–	–					1						

[a]*GM* Geometric Mean, [b]*MIC* Minimum Inhibitory Concentration, *NA* not applicable

research population, antifungal agents were provided as empirical therapy, despite the fact that there is no treatment guideline for fungal co-infections in COVID-19-positive individuals. Excessive use of empiric antifungal regimens not only has not been proven to be safe or beneficial but also could lead to resistance and therapeutic failures in patients who have previously been exposed, in addition to imposing an economic burden. Obviously, drug-drug interactions, toxicity, and adverse side effects of antifungals used in COVID-19 patients should all be considered [38].

Based on recent studies, *C.albicans* is known as the most prevalent species responsible for candidiasis in COVID-19 patients [48–51]. However, the identification of *C. auris*, a multi-drug resistant species, has been reported in previous studies with a high mortality rate [25]. In accordance with the findings presented in

previous reports, *C. albicans* was the major species for candidiasis in COVID-19 patients in this study. Moreover, the species distribution of isolates from CAC with the highest proportion of *C.albicans* and *C.glabrata* is in accordance with previous reports from different countries[31, 34, 49, 51–54]. Although it is known that *C.albicans* accounts for half the isolates recovered from different types of *Candida* infections, it should be noted that non-*albicans Candida* species have emerged as prominent species in recent years [55]. Importantly, outbreaks of *C.auris* infections have been reported among COVID-19 patients in some regions of the world [25, 56, 57].

Although many reports have been published, there are a few studies about the susceptibility of fungal isolates from COVID-19 patients. Also, there have been few studies about the susceptibility patterns of *Candida* species isolated from non-invasive candidiasis. In total, resistance to azole drugs was observed among *Candida* species recovered from different samples in our study in spite of their high level of susceptibility to all tested antifungals. As reported from previous studies in Shiraz, the current study found azole-resistant *Candida* species among clinical isolates [58, 59]. Moreover, assessment of the azole susceptibility profiles of *Candida* isolates showed that *C. albicans* had cross-resistant profile to azoles. [38]Also, the resistant rate among *C.glabrata* was low in agreement with previous reports from Iran [60, 61]. Of note, itraconazole resistance among *Candida* species has been reported in previous studies[62–65]. Collectively, various degrees of resistance to azole antifungals have been declared previously in many reports from Iran [66, 67]. As a consequence, the presence of azole-resistant species could be a result of high background usage of azoles as empirical or first-line choice treatments and repeated exposure in our reviewed medical center.

Although posaconazole has no defined breakpoint in CLSI documents, it totally showed low MIC values for *Candida* species in the current study, which is consistent with the other studies [67, 68]. Considering the potent activity of posaconazole against *Candida* species, administration of this agent is proposed for the treatment of *Candida* infections. Additionally, the efficacy of posaconazole for treatment of mucormycosis, as a post-COVID-19 fungal infection, makes this azole antifungal an efficacious choice for treatment of other mycoses than yeast infections. In this survey, the most potent antifungal agent was caspofungin, with low MIC values for all species in accordance with prior research [32, 65, 69]. From the perspective of availability and cost-effectiveness, echinocandins appear to be an appropriate drug of first choice to define the most effective approach to the management of candidiasis in our country.

Some limitations must be acknowledged. In this study,we analyzed the available information for a small research population in one center that follow-up of patients after discharge was not included. The unavailability of new antifungals such as Isavuconazole in our country precludes the possibility of evaluating their efficacy in therapeutic procedures.

In conclusion, the results of our study declared some fundamental issues for patients infected with SARS-CoV-2 that had *Candida* co-infections. The main finding of this study reveals a high rate of *Candida* co-infections among hospitalized COVID-19 patients. In addition, comorbidities such as heart failure, HTN, COPD, bacterial infections as well as therapeutic interventions including catheterization, mechanical ventilation, and ICU admission increased the risk of infection by *Candida* spp. in COVID-19 patients. The isolation of *Candida* species from urinary/respiratory tracts and the bloodstream led to poor clinical outcomes, which presented as a higher in-hospital mortality rate. Also, obtained data clarified that empiric antifungal therapy did not achieve expected effectiveness. Finally, our results could assist infectious disease specialists with a better understanding of COVID-19-associated candidiasis. Definitely, selection of the appropriate antifungal therapies considering the species distribution and susceptibility patterns of causative agents can result in more effective therapeutic interventions and desired outcomes.

Author Contributions SY: Conceptualization,Investigation, Writing—original draft, Writing—review and editing. PC: Formal analysis. MA, ZZ, AJ, EE, ZM, MG: Investigation. HN, SA, BG, MHA, MM: Validation, Data curation. MS: Supervision, Writing—review and editing, Project administration. KZ: Project administration,Supervision, Resources, Funding acquisition, review and editing. All authors read and approved the final manuscript.

Funding This work was supported by the Research Council of Shiraz University of Medical Sciences [Grant No. 26040].

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

1. Zhou F, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62.
2. Silva DL, et al. Fungal and bacterial coinfections increase mortality of severely ill COVID-19 patients. J Hosp Infect. 2021;113:145–54.
3. Shafiekhani M, et al. Bacterial and fungal co-infections with SARS-CoV-2 in solid organ recipients: a retrospective study. Virol J. 2022;19(1):1–7.
4. Arastehfar A, et al. COVID-19-associated candidiasis (CAC): an underestimated complication in the absence of immunological predispositions? J Fungi. 2020;6(4):211.
5. Chen N, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507–13.
6. Chen X, et al. The microbial coinfection in COVID-19. Appl Microbiol Biotechnol. 2020;104(18):7777–85.
7. Song G, Liang G, Liu W. Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from China. Mycopathologia. 2020;185(4):599–606.
8. Rolling T, Hohl TM, Zhai B. Minority report: The intestinal mycobiota in systemic infections. Curr Opin Microbiol. 2020;56:1–6.
9. World health organization (WHO). *WHO coronavirus (COVID-19) Dashboard [Internet]*. Available from: https://covid19.who.int/.
10. Pascarella G, et al. COVID-19 diagnosis and management: a comprehensive review. J Intern Med. 2020;288(2):192–206.
11. Jafari M, et al. Exoenzyme activity and possibility identification of Candida dubliniensis among Candida albicans species isolated from vaginal candidiasis. Microb Pathog. 2017;110:73–7.
12. Mirhendi H, et al. Differentiation of Candida albicans and Candida dubliniensis using a single-enzyme PCR-RFLP method. Jpn J Infect Dis. 2005;58(4):235.
13. Wayne, P., *Clinical and Laboratory Standards Institute: Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard.* CLSI document M27-A3 and Supplement S, 2008;3:6–12.
14. Wayne, P., *Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard.* CLSI document M27-A2, 2002.
15. Wayne P. Reference method for broth dilution antifungal susceptibility testing of yeasts. Clin Lab Standards Inst. 2008;3:M27–33.
16. Clinical and L.S. Institute, *Performance standards for antifungal susceptibility testing of yeasts.* CLSI supplement M60, 2017.
17. Clinical and L.S. Institute, *Epidemiological cutoff values for antifungal susceptibility testing*. 2016, Clinical and Laboratory Standards Institute Wayne, PA.
18. De Pauw B, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. Clin Infect Dis. 2008;46(12):1813–21.
19. Falces-Romero I, et al. Isolation of Aspergillus spp. in respiratory samples of patients with COVID-19 in a Spanish tertiary care hospital. Mycoses. 2020;63(11):1144–8.
20. Zhou P, et al. Bacterial and fungal infections in COVID-19 patients: a matter of concern. Infect Control Hosp Epidemiol. 2020;41(9):1124–5.
21. Norberg C, et al. Candida infections associated with COVID-19: an underestimated risk. WJPPS. 2021;10(9):48–64.
22. Clancy CJ, Nguyen MH. Coronavirus disease 2019, superinfections, and antimicrobial development: what can we expect? Clin Infect Dis. 2020;71(10):2736–43.
23. Chen G, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Investig. 2020;130(5):2620–9.
24. Huang C, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.
25. Chowdhary A, et al. Multidrug-resistant Candida auris infections in critically Ill coronavirus disease patients, India, April–July 2020. Emerg Infect Dis. 2020;26(11):2694.
26. Bishburg E, et al. Fungemia in covid-19 ICU patients, a single medical center experience. J Med Virol. 2021;93(5):2810–4.
27. Salehi M, et al. Opportunistic fungal infections in the epidemic area of COVID-19: a clinical and diagnostic perspective from Iran. Mycopathologia. 2020;185(4):607–11.
28. Nazari T, et al. COVID-19-associated fungal infections in Iran: A systematic review. PLoS ONE. 2022;17(7): e0271333.
29. Seagle EE, et al. The landscape of candidemia during the COVID-19 pandemic. Clin Infect Dis. 2021;74(5):802–11.
30. Ezeokoli OT, Gcilitshana O, Pohl CH. Risk factors for fungal co-infections in critically ill COVID-19 patients, with a focus on immunosuppressants. J Fungi. 2021;7(7):545.
31. Antinori S, et al. Tocilizumab for cytokine storm syndrome in COVID-19 pneumonia: an increased risk for candidemia? Autoimmun Rev. 2020;19(7): 102564.
32. Kordalewska M, et al. Antifungal drug susceptibility and genetic characterization of fungi recovered from COVID-19 patients. J Fungi. 2021;7(7):552.
33. Herc E et al. Characteristics and outcomes of COVID-19 patients with fungal infections. In: Open Forum Infectious Disease. Oxford University Press 2020.
34. Segrelles-Calvo G, et al. Candida spp. co-infection in COVID-19 patients with severe pneumonia: Prevalence study and associated risk factors. Respir Med. 2021;188:106619.
35. Vinayagamoorthy K, Pentapati KC, Prakash H. Prevalence, risk factors, treatment and outcome of multidrug resistance Candida auris infections in Coronavirus disease (COVID-19) patients: a systematic review. Mycoses. 2022;65(6):613–24.
36. Pittet D, et al. Candida colonization and subsequent infections in critically ill surgical patients. Ann Surg. 1994;220(6):751.
37. Shoham S, Levitz SM. The immune response to fungal infections. Br J Haematol. 2005;129(5):569–82.

38. Silva LN, et al. Fungal infections in COVID-19-positive patients: a lack of optimal treatment options. Curr Top Med Chem. 2020;20(22):1951–7.
39. Benelli JL, et al. Fungal bloodstream co-infection by trichosporon asahii in a COVID-19 critical patient: case report and literature review. Mycopathologia. 2022;187(4):397–404.
40. Goncalves Mendes Neto A, et al. Bacterial infections and patterns of antibiotic use in patients with COVID-19. J Med Virol. 2021;93(3):1489–95.
41. Riad A et al. Oral candidiasis in non-severe COVID-19 patients: call for antibiotic stewardship. Oral Surg. 2020;15(3):465–466.
42. Morales DK, Hogan DA. Candida albicans interactions with bacteria in the context of human health and disease. PLoS Pathog. 2010;6(4): e1000886.
43. Cultrera R, et al. Co-infections in critically ill patients with or without COVID-19: a comparison of clinical microbial culture findings. Int J Environ Res Public Health. 2021;18(8):4358.
44. Cruz AB, LeRose J, Chopra T. Comparison of Outcomes in Candidemia Between COVID-19 and Non–COVID-19 Patients. Antimicrob Steward Healthc Epidemiol. 2021;1(S1):s58–s58.
45. Kayaaslan B, et al. Characteristics of candidemia in COVID-19 patients; increased incidence, earlier occurrence and higher mortality rates compared to non-COVID-19 patients. Mycoses. 2021;64(9):1083–91.
46. Whitney L, et al. Effectiveness of an antifungal stewardship programme at a London teaching hospital 2010–16. J Antimicrob Chemother. 2019;74(1):234–41.
47. Morrissey C, et al. Consensus guidelines for the use of empiric and diagnostic-driven antifungal treatment strategies in haematological malignancy, 2014. Intern Med J. 2014;44(12b):1298–314.
48. Ventoulis I, et al. Bloodstream infection by Saccharomyces cerevisiae in two COVID-19 patients after receiving supplementation of Saccharomyces in the ICU. J Fungi. 2020;6(3):98.
49. White PL, et al. A national strategy to diagnose coronavirus disease 2019–associated invasive fungal disease in the intensive care unit. Clin Infect Dis. 2021;73(7):e1634–44.
50. Posteraro B, et al. Pan-echinocandin-resistant Candida glabrata bloodstream infection complicating COVID-19: a fatal case report. J Fungi. 2020;6(3):163.
51. Al-Hatmi AM, et al. COVID-19 associated invasive candidiasis. J Infect. 2021;82(2):e45–6.
52. White PL et al. Candidemia in Coronavirus Disease 2019: A Link to Disease Pathology or Increased Clinical Pressures? Clin Infect Dis. 2020;73(9):e2839–2841
53. Arastehfar A, et al. Candidemia among Iranian patients with severe COVID-19 admitted to ICUs. J Fungi. 2021;7(4):280.
54. Salehi M, et al. Oropharyngeal candidiasis in hospitalised COVID-19 patients from Iran: species identification and antifungal susceptibility pattern. Mycoses. 2020;63(8):771–8.
55. Kullberg BJ, Arendrup MC. Invasive candidiasis. N Engl J Med. 2015;373(15):1445–56.
56. Villanueva-Lozano H, et al. Outbreak of Candida auris infection in a COVID-19 hospital in Mexico. Clin Microbiol Infect. 2021;27(5):813–6.
57. Almeida JND, et al. Emergence of Candida auris in Brazil in a COVID-19 intensive care unit. J Fungi. 2021;7(3):220.
58. Arastehfar A, et al. Clinical and microbiological features of candiduria in critically ill adult patients in Shiraz, Iran (2016–2018): Deviations from international guidelines and fluconazole therapeutic failure. Med Mycol. 2021;59(6):600–7.
59. Arastehfar A, et al. Epidemiology of candidemia in Shiraz, southern Iran: A prospective multicenter study (2016–2018). Med Mycol. 2021;59(5):422–30.
60. Arastehfar A, et al. Low level of antifungal resistance in Iranian isolates of Candida glabrata recovered from blood samples in a multicenter study from 2015 to 2018 and potential prognostic values of genotyping and sequencing of PDR1. Antimicrob Agents Chemother. 2019;63(7):e02503-e2518.
61. Amanloo S, et al. Drug susceptibility profile of Candida glabrata clinical isolates from Iran and genetic resistant mechanisms to caspofungin. Rev Iberoam Micol. 2018;35(2):88–91.
62. Badiee P, et al. Susceptibility of Candida species isolated from immunocompromised patients to antifungal agents. East Mediterr Health J. 2011;17(5):425–30.
63. Haddadi P et al. Yeast colonization and drug susceptibility pattern in the pediatric patients with neutropenia. Jundishapur J Microbiol. 2014;7(9): e11858
64. Badiei P et al. Molecular identification and in-vitro susceptibility of *Candida albicans* and *C. dubliniensis* isolated from immunocompromised patients. 2009.
65. Erami M, et al. Clinical impact of Candida respiratory tract colonization and acute lung infections in critically ill patients with COVID-19 pneumonia. Microb Pathog. 2022;166: 105520.
66. Badiee P, et al. Antifungal susceptibility testing of Candida species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates. BMC Infect Dis. 2017;17(1):1–8.
67. Mahmoudabadi AZ, Rezaei-Matehkolaei A, Ghanavati F. The susceptibility patterns of *Candida* species isolated from urine samples to posaconazole and caspofungin. Jundishapur J Microbiol. 2015;**8**(3).
68. Castanheira M, et al. Isavuconazole and nine comparator antifungal susceptibility profiles for common and uncommon Candida species collected in 2012: application of new CLSI clinical breakpoints and epidemiological cutoff values. Mycopathologia. 2014;178(1):1–9.
69. Cataldo MA, et al. Incidence of bacterial and fungal bloodstream infections in COVID-19 patients in intensive care: An alarming "collateral effect." J Glob Antimicrob Resist. 2020;23:290.