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Clinical impact of *Candida* respiratory tract colonization and acute lung infections in critically ill patients with COVID-19 pneumonia

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ABSTRACT

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

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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)

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 a 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 *Shahid 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 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 *HWP1* gene amplification using the paired primers *HWP1*–F (5′- GCTACCACTTCAGAATCATCATC-3′) and *HWP1*-R (5′-GCACCTTCAGTCGTAGAGACG-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

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 *HWP1* 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 1 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 4 summarizes the MIC range and the MIC 90 of all antifungals. Amphotericin B and caspofungin showed superior activity against all *Candida* spp. For *C. albicans*: (isolates no:4,6–17,47-51,57,63) were resistant to voriconazole MIC≥ 16 μg/Ml; resistant to fluconazole MIC≥32 μg/Ml (isolates no: 6–17,25,26,48–51); to caspofungin MIC≥ 32 μg/Ml (isolates no: 2,8,16,17,57,63); and itraconazole MIC≥32 μg/Ml (isolates no: 2,6, 7–17,21-24,46–51,54,55) were seen. For *C. glabrata* resistant to voriconazole MIC≥ 8 μg/Ml (isolates no:3,5,18,19,21,58,66,68); fluconazole MIC≥ 32 μg/Ml (isolates no: all of isolates), caspofungin ≥8 μg/Ml (isolates no: 18,58) and itraconazole MIC≥32 μg/Ml (isolates no:3,5,18,19,21,58,59,66,68) were seen. All antifungal agents showed 100% sensitivity (range to 0.03–0.5) against Two *C. africana* strains. Table 5.

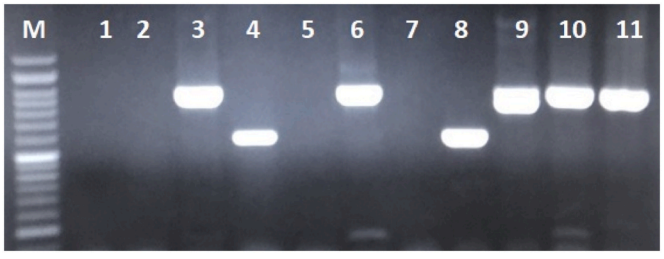

Fig. 1. Agarose gel of PCR amplification with *HWP1* gene; Lanes 3, 6, 9–11: *C. albicans* ~900 bp; Lanes 4, 8: *C. africana* ~600 bp.

Table 1
The major presenting symptoms in COVID-19 patients with *Candida* spp.

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	**679**
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%)
O	18 (26.1%)
Extension	
BAL	69 (100%)

Table 2
Characteristics of patients, clinical course, and outcome in *Candida* and non-*Candida* colonization cases.

Variable	*Candida* colonization (n = 69)	No *Candida* colonization (n = 31)	P-value[a]
COVID-19 infection	69/69 (100)	31/31 (100)	1
Age, yr, median (range)	61.1 (21–88)	56.6 (26–89)	0.67
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)	0.77
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

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)	6/12 (50)	2/31 (6.4)
Malignancy, n (%)	8/55 (14.5)	0/12 (0.0)	0/31 (0.0)
Cardiovascular disease, n (%)	5/55 (9.1)	2/12 (16.7)	1/31 (3.2)
Candidemia, n (%)	1/55 (1.8)	2/12 (16.7)	0/31 (0.0)
Urine culture, n (%)	10/55 (18.2)	7/12 (58.3)	5/31 (16.2)
Headache	53/55 (96.4)	12/12 (100)	24/31 (77.4)
Fever	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)
Dyspnea	55/55 (100)	12/12 (100)	27/31 (87.1)
WBC, mm^3, median	8.48 × 10^3/ mm3	9.9 × 10^3/mm3	10.7 × 10^3/ mm3
FBS, mg/dl, median (range)	189 (75–507)	164 (36–470)	101 (84–266)
BUN, mg/dl, median (range)	34.4 (8–100)	55.8 (11–103)	31.2 (12–82)
CRP, mg/dl, median (range)	96.1 (2–382)	109 (21–344)	85.6 (1–339)
ESR, mm/hr., median (range)	48.2 (10–107)	59.2 (15–109)	48.5 (6–109)
Interval from ICU admission to ICU discharge, median (range), d	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)

development of ventilator-associated pneumonia (VAP). Colonization can even change the antibiotic resistance patterns of pathogenic bacteria by polymicrobial biofilm formation [25,26]. Therefore, the significance of *Candida* colonization in RT remains controversial, and many clinical conditions need to be interpreted with caution. In this research, *C. albicans* (55; 79.7%) was the most common spp. followed by *C. glabrata* (12; 17.4%) and two (2.9%) *C. africana* (detected by the *HWP1* gene amplification) as the etiologic agents of pulmonary *Candida* colonization associated with COVID-19 infection. The co-infection of *C. albicans* and *C. glabrata* was seen in two cases. 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. From other reports, the rate of *Candida* spp. isolation in the RT is relatively high, especially in those with mechanical ventilation (MV) [25]. However, there are still controversies on whether *Candida* spp. can solely cause VAP due to the following reasons: (1) regardless of the causative pathogenic microorganism, the diagnosis of VAP is still challenging due to the lack of pathological evidence. The diagnostic criteria for a clinically suspected VAP are non-specific, and it is difficult to distinguish between colonization and infection [27]. (2) Generally, the understanding of the essence of bacterial and fungal co-existence in many 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 10^3 CFU/mL (protected specimen brush sample) or 10^4 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 concomitant 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 FUNGIBACT, 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* colonization 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 5
MIC interpretation of five antifungal drugs against *Candida* spp. recovered from COVID-19 patients.

Antifungal agents		C. albicans N = 55%	C. glabrata N = 12%	C. africana N = 2%
Amphotericin B	S	51 (92.7)	10 (83.4)	2 (100)
	R	4 (7.3)	2 (16.6)	0 (0)
Itraconazole	S	30 (54.5)	3 (25)	2 (100)
	R	25 (45.5)	9 (75)	0 (0)
Voriconazole	S	35 (63.6)	4 (33.4)	2 (100)
	R	20 (36.4)	8 (66.6)	0 (0)
Fluconazole	S	37 (67.3)	0 (0)	2 (100)
	R	18 (32.7)	12 (100)	0 (0)
Caspofungin	S	49 (89.1)	10 (83.4)	2 (100)
	R	6 (10.1)	2 (16.6)	0 (0)

S: susceptible; R: resistance.

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

Species (n)		Amphotericin B μg/mL	Voriconazole μg/mL	Itraconazole μg/mL	Fluconazole μg/mL	Caspofungin μg/mL
C. albicans	MICs Range	0.03–1	0.03–16	0.03–32	0.125–32	0.03–32
	MIC90	0.03	16	16	32	0.03
C. glabrata	MICs Range	0.03–1	0.03–16	0.03–32	32	0.125–16
	MIC90	0.5	8	2	32	0.125

interaction. However, in a murine model, Ader 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 regime of pulmonary candidiasis associated with COVID-19 infection. Various degrees of resistance to voriconazole, itraconazole and fluconazole were seen in *C. albicans* and *C. glabrata* strains. Antifungal agents showed 100% sensitivity against the two *C. africana* strains. In another study, 100%, 30%, and 40% of the *Candida auris* isolates were resistant to FCZ, combination of FCZ and voriconazole, and combination of FCZ and AMB, respectively, and only one *Candida glabrata* isolate was resistant against echinocandin [8,36–42].

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, Mehri N; Critical revision of the manuscript: Raiesi O, Getso M; Procedure: Erami M, Raiesi O.

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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 Raissi:** 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.

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