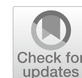
Folia Microbiologica (2023) 68:951–959
https://doi.org/10.1007/s12223-023-01069-5

ORIGINAL ARTICLE



COVID-19-associated pulmonary aspergillosis (CAPA): identification of *Aspergillus* species and determination of antifungal susceptibility profiles

Ali Ozturk[1] · Taylan Bozok[2] · Merve Erdogan[3] · Bashar MS. Ibrahim[4] · Tugce Simsek Bozok[5]

Received: 30 June 2022 / Accepted: 30 May 2023 / Published online: 9 June 2023
© Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i. 2023

Abstract

Among the co-infectious agents in COVID-19 patients, *Aspergillus* species cause invasive pulmonary aspergillosis (IPA). IPA is difficult to diagnose and is associated with high morbidity and mortality. This study is aimed at identifying *Aspergillus* spp. from sputum and tracheal aspirate (TA) samples of COVID-19 patients and at determining their antifungal susceptibility profiles. A total of 50 patients with COVID-19 hospitalized in their intensive care units (ICU) were included in the study. Identification of *Aspergillus* isolates was performed by phenotypic and molecular methods. ECMM/ISHAM consensus criteria were used for IPA case definitions. The antifungal susceptibility profiles of isolates were determined by the microdilution method. *Aspergillus* spp. was detected in 35 (70%) of the clinical samples. Among the *Aspergillus* spp., 20 (57.1%) *A. fumigatus*, six (17.1%) *A. flavus*, four (11.4%) *A. niger*, three (8.6%) *A. terreus*, and two (5.7%) *A. welwitschiae* were identified. In general, *Aspergillus* isolates were susceptible to the tested antifungal agents. In the study, nine patients were diagnosed with possible IPA, 11 patients were diagnosed with probable IPA, and 15 patients were diagnosed with *Aspergillus* colonization according to the used algorithms. Serum galactomannan antigen positivity was found in 11 of the patients diagnosed with IPA. Our results provide data on the incidence of IPA, identification of *Aspergillus* spp., and its susceptibility profiles in critically ill COVID-19 patients. Prospective studies are needed for a faster diagnosis or antifungal prophylaxis to manage the poor prognosis of IPA and reduce the risk of mortality.

Keywords *Aspergillus* spp. · Invasive pulmonary aspergillosis · COVID-19 · Antifungal susceptibility · SARS-CoV-2

Introduction

The outbreak of coronavirus 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a major threat to human health. Like SARS-CoV-1 and the Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 is responsible for lower respiratory tract infection and can cause acute respiratory distress syndrome (ARDS) (Song et al. 2020).

Bacterial and fungal infections are common complications of viral pneumonia, especially in critically ill patients. These infections cause an increased need for intensive care and an increased mortality rate (Antinor et al. 2020). In patients with COVID-19, the predisposing features of the patients' host environment allow coinfections to occur (Hoenigl et al. 2022).

Among the co-infectious agents in COVID-19 patients, *Aspergillus* species cause invasive pulmonary aspergillosis (IPA). IPA is difficult to diagnose and is associated

✉ Ali Ozturk
ali.81ozturk@gmail.com

Bashar MS. Ibrahim
basharibrahim@sdu.edu.tr

[1] Department of Medical Microbiology, Faculty of Medicine, Nigde Omer Halisdemir University, Nigde, Turkey

[2] Department of Medical Microbiology, Faculty of Medicine, Mersin University, Mersin, Turkey

[3] Department of Medical Microbiology, Faculty of Medicine, Sanko University, Gaziantep, Turkey

[4] Department of Pharmaceutical Microbiology, Suleyman Demirel University, Isparta, Turkey

[5] Department of Infectious Diseases and Clinical Microbiology, Mersin University Hospital, Mersin, Turkey

with high morbidity and mortality (Lai and Yu 2021). IPA has been increasingly detected as an infectious agent in critically ill patients who are hospitalized in the intensive care unit (ICU) in recent years. The incidence of COVID-19-associated pulmonary aspergillosis (CAPA) differs significantly according to the diagnostic criteria and the study design used. While some studies screened report the incidence of CAPA as low as 5%, several studies using a screening protocol have found up to 34% of ICU patients to have CAPA (Er et al. 2022). In this context, further studies are needed to determine the accurate incidence, optimize diagnoses, improve patient management, provide up-to-date epidemiological data, and investigate the risk of IPA in critical patients diagnosed with COVID-19. The aim of this study is to identify *Aspergillus* spp. isolates from respiratory tract samples of COVID-19 patients hospitalized in ICUs in Niğde Ömer Halisdemir Training and Research Hospital, Niğde, Turkey, by different methods and to determine their antifungal susceptibility profiles.

Methods

Patient group

A total of 50 adult (≥ 18 years old) patients with ARDS and COVID-19 were hospitalized in COVID-19 ICUs between 13 March and 25 December 2020 and were included in this study. All demographic, clinical, and microbiological data were obtained from the clinical records of the patients. The classifications of IPA in the patients included in the study were done by the ECMM/ISHAM consensus criteria (Koehler et al. 2021).

Microbiological analysis.

SARS-CoV-2 RT-PCR

A viral nucleic acid isolation kit (Bioeksen, Turkey) was used for the isolation of SARS-CoV-2 from oro-nasopharyngeal swab and tracheal aspirate (TA) samples. In accordance with the recommendations of the manufacturer, a sample of 10 μL (final volume) was used. RT-PCR kit (Bioeksen, Turkey) targeting N and ORF1ab genes of SARS-CoV-2 was used. Amplification was performed on the Qiagen Rotor-Gene Q 5plex HRM instrument (Qiagen, Hilden, Germany).

Phenotypic identification

Blood agar (5%), eosin-methylene-blue agar (EMB), chocolate agar, and Sabouraud dextrose agar (SDA) media were used for the cultivation of TA and sputum samples of LRT of COVID-19 patients sent to the microbiology laboratory of our hospital. After bacteriological evaluation, the SDA medium was incubated at 25 °C for at least seven days to monitor mold growth. Growing mold colonies were passaged on potato dextrose agar (PDA) medium. For the differentiation of *Aspergillus* spp. that grow in the PDA medium, macroscopic and microscopic methods were used. Microscopic examination was done by looking at the texture of the colony (colony size and color, surface appearance, and pigment formation) and the color of the mycelia. The growing mold was evaluated microscopically with lactophenol cotton blue. The species-level distinction was made by looking at the number of sterigmata, vesicle structure, arrangement of phialides (single or double row), and location, as well as the structure and color of conidiophores under the microscope (McClenny 2005; Bilgi and Kiraz 2019).

Molecular identification

DNA isolation

Heliosis DNA isolation kit (Metis Biotechnology, Turkey) was used to obtain nucleic acid from the isolates in accordance with the test kit procedure. The total amount of DNA obtained was measured in a DNA measuring device (NanoDrop, USA), and the presence of DNA was checked. DNAs were stored at -20 °C until use.

Amplification of DNA samples by PCR

PCR experiment was performed using internal transcribed spacer 2 (ITS2) ribosomal DNA primers. The amplification reaction was performed using primer set fITS7 5′-GTGARTCATCGAATCTTTG-3′ and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The amplification reactions were carried out for 25.0 μL total volume, using 2.0 μL DNA template, 12.5 μL 2 × Master Mix, 0.5 μL of each primer (10 μM), and 9.5 μL ddH$_2$O. The thermo-cycling conditions were adapted as an initial enzyme activation step at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s (touchdown PCR, starting from 10th cycle with 0.2 °C decreases), and elongation at 72 °C for 30 s.

The amplification products as the single-band were validated by agarose gel electrophoresis run at 50 V for 15 min. PCR products were purified and sequenced by ABI 3730XL sequencer using the BigDye Terminator v3.1 Cycle Sequencing Kit. The species identification was done based on the NCBI n-blast search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as well as considering the colony morphologies. The phylogenetic tree was constructed using Qiagen CLC workbench program, including outgroup sequences obtained from the NCBI database.

Antifungal susceptibility profiles of isolates

Susceptibility testing of all isolates to antifungal agents was performed by liquid microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines (CLSI 2008). Amphotericin B (AmB), itraconazole (ITR), voriconazole (VOR), and caspofungin (CSP) were used in the experiment. Serial dilutions were made between 0.03–16 µg/mL for AmB, ITR, and VOR and 0.06–32 µg/mL for CSP. Inoculated microplates were incubated at 35 °C for 48 h. The MIC ranges and MIC_{90} (MIC value inhibiting 90% of the isolates) of the tested *Aspergillus* isolates were also determined. The MIC values obtained were compared with the proposed CLSI epidemiological cutoff values (ECVs) to determine the susceptibility profiles of the isolates (Espinel-Ingroff and Turnidge 2016).

Detection of *Aspergillus* antigen by ELISA

In order to diagnose IPA infection early and accurately in COVID-19 patients, blood samples were collected from 35 patients with *Aspergillus* overgrowth, and serum galactomannan (GM) was investigated serologically in only one single serum sample that was taken from the patients. Platelia™ Aspergillus antigen ELISA kit (BioRad, France) was used for this. According to the manufacturer's recommendation, a serum sample was considered positive when the optical density (OD) value was ≥ 0.5.

Statistical analysis

The analysis of the obtained data was performed using the SPSS 20.0 package program (IBM, Armonk, NY, USA), and the chi-square test was used.

Ethical approvement

The study protocol was approved by the Niğde Ömer Halisdemir University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (protocol number: 2021/32).

Results

Demographic findings of the patient group

Demographic characteristics of the patient groups included in the study are given in Table 1. Of the patients included in our study, 21 (42%) were females and 29 (58%) were males. The median hospital stay of the patients was 16 (IQR = 12–22) day (between 9 and 50 days), and their median age was 72.5 (IQR = 48.25–79) (age range: 37–87 years). *Aspergillus* spp. growth was detected in 35 (70%) of the clinical samples taken from the patients. Included patients were classified according to ECMM/ISHAM criteria (Koehler et al. 2021). Accordingly, in the patients included in the study, nine patients (18%) were diagnosed with possible IPA, 11 (22%) patients were diagnosed with probable IPA, and 15 (30%) patients were diagnosed with *Aspergillus* colonization. For the remaining 15 patients (30%), IPA was not diagnosed because they did not meet the criteria of ECMM/ISHAM. There was no patient diagnosed with proven IPA in the study.

Patients with confirmed IPA (20; 40%) had various types of underlying conditions. Among them are corticosteroid therapy, dyspnea, *Aspergillus* positive culture of LRT specimens, abnormal chest computed tomography (CT) scan (compatible with COVID-19), and worsening respiratory failure despite appropriate antibiotic therapy and respiratory support. Of the patients with *Aspergillus* positive culture of LRT samples, 29 (82.8%) had hypertension (HT), 20 (57.1%) chronic obstructive pulmonary disease (COPD), 19 (54.3%) diabetes mellitus (DM), two (5.7%) chronic kidney disease (CKD), three (5.6%) heart failure, three (5.6%) asthma, and one (2.8%) atrial fibrillation (Table 1).

A total of 30 (60%) patients included in the study died. Among them, 25 were IPA patients (seven possible, 10 probable, and eight with *Aspergillus* colonization).

Among the patients with *Aspergillus* growth, the presence of IPA was found to be significantly higher in patients with COPD ($n = 17$; 85%; $p < 0.0001$). No significant difference was found in patients with HT.

In 22 patients, corticosteroid treatment ≥ 40 mg/day prednisone equivalent was administered IV. All patients received broad-spectrum antibiotic therapy (such as teicoplanin, meropenem, piperacillin – tazobactam, and tigecycline). Antifungal therapy (liposomal AmB and VOR) was used as prophylaxis in three IPA patients (two possible and one probable IPA), and 30 (60%) of the patients were intubated and received mechanical ventilation support.

Phenotypic characterization

When TA and sputum samples of a total of 50 patients were evaluated with the traditional method, *Aspergillus* was recorded in only 35 patients. Among the *Aspergillus* spp., 20 (57.1%) *A. fumigatus* species complex, six (17.1%) *A. flavus* species complex, three (8.6%) *A. niger* species complex, three (8.6%) *A. terreus* species complex, and three (8.6%) *Aspergillus* spp. were identified from the clinical samples of 50 patients (34 (68%) were TA and 16 (32%) were sputum). Of the patient samples that were classified according to IPA identification algorithm, 20 (57.1%) were TA and 15 (42.8%) were sputum. The clinical specimens and *Aspergillus* species are shown in Table 2.

Table 1 The demographic, clinical, and mycological characterization of COVID-19 patients

	IPA according to ECMM/ISHAM criteria			
Characteristics	Possible ($n = 9$)	Probable ($n = 11$)	Colonization ($n = 15$)	Non-IPA ($n = 15$)
Gender (male/female)	5/4	6/5	9/6	11/4
Median age (IQR)	82 (74–85)	73 (72.5–80)	76 (69.5–79.5)	43 (39.5–46.5)
The median length of stay (day)	19	14	14	16
Risk factors				
Hypertension (HT)	4 (44.4%)	8 (72.7%)	7 (46.7%)	10 (66.7%)
Chronic obstructive pulmonary disease (COPD)	8 (88.9%)	9 (81.8%)	3 (20%)	-
Asthma	2 (22.2%)	1 (9.1%)	-	-
Chronic kidney disease (CKD)	2 (22.2%)	-	-	-
Diabetes mellitus (DM)	3 (33.3%)	3 (27.3%)	2 (13.3%)	11 (73.3%)
Heart failure	2 (22.2%)	1 (9.1%)	-	-
Atrial fibrillation	1 (11.1%)	-	-	-
Dyspnea	4 (44.4%)	7 (63.6%)	-	6 (40%)
Respiratory failure despite appropriate antibiotic therapy and respiratory support	5 (55.6%)	4 (36.4%)	-	-
Steroid therapy (≥ 40 mg/day)	9 (100%)	11 (100%)	2 (13.3%)	-
COVID-19 therapy	9 (100%)	10 (90.9%)	15 (100%)	12 (80%)
Mechanical ventilation	9 (100%)	11 (100%)	2 (13.3%)	8 (53.3%)
Prophylactic antifungal therapy	2 (22.2%)	1 (9.1%)	-	-
Broad-spectrum antibiotic therapy	9 (100%)	11 (100%)	15 (100%)	13 (86.7%)
Outcome	7 (77.8%)	10 (90.9%)	8 (53.3%)	5 (33.3%)
Diagnosis				
Abnormal chest CT scan[a]	9 (100%)	11 (100%)	1 (6.7%)	-
Positive RT-PCR[b]	9 (100%)	11 (100%)	15 (100%)	15 (100%)
Aspergillus positive culture	9 (100%)	11 (100%)	15 (100%)	-
Range of serum galactomannan positive	-	11(0.8 –9.4)	-	-

[a]Compatible with COVID-19

[b]SARS-CoV-2 RT-PCR

Molecular identification

By sequencing the ribosomal DNA ITS region, which we used as a reference method in our study, 20 of the *A. fumigatus* samples, six *A. flavus*, four *A. niger*, three *A. terreus*, and two *A. welwitschiae* species were identified. Two phenotypically unidentified *Aspergillus* isolates were identified by DNA sequence as *A. welwitschiae* (Table 3 and Fig. 1).

Antifungal susceptibility testing

The MIC_{90} and MIC ranges of AmB, ITR, VOR, and CSP tested against 35 *Aspergillus* isolates are presented in Table 4. Overall, the lowest MIC values (0.03 μg/mL) for all 35 isolates tested in the study were seen in the ITR, followed by AMB and CSP (0.06 μg/mL) and VOR (0.125 μg/L). As shown in Table 4, the tested antifungal agents showed good activity (MICs ≤ ECV)

Table 2 The clinical sample type and isolated *Aspergillus* spp. in the patients

IPA identifying	Sample type (n)						
	TA[a]	Sputum	*A. fumigatus*	*A. flavus*	*A. terreus*	*A. niger*	*A. welwitschiae*
Possible	9	-	7	1	-	1	-
Probable	8	3	8	2	1	-	-
Colonization	3	12	5	3	2	3	2
Total	20	15	20	6	3	4	2

[a]Tracheal aspirate

Table 3 The phenotypic and genotypic characterization of *Aspergillus* isolates

Isolate no.	Isolate code	IPA diagnosis	Phenotypical identification	Genotypical identification	
				Aspergillus spp.	Accession no.
1	AO512021	Probable	*A. fumigatus*	*A. fumigatus*	OK631790
2		Probable	*A. fumigatus*	*A. fumigatus*	ON584277
3		Colonization	*A. fumigatus*	*A. fumigatus*	ON584278
4		Possible	*A. fumigatus*	*A. fumigatus*	ON584279
5		Probable	*A. fumigatus*	*A. fumigatus*	ON584280
6		Possible	*A. fumigatus*	*A. fumigatus*	ON584281
7		Probable	*A. fumigatus*	*A. fumigatus*	ON584282
8		Probable	*A. fumigatus*	*A. fumigatus*	ON584283
9		Possible	*A. fumigatus*	*A. fumigatus*	ON584284
10		Colonization	*A. fumigatus*	*A. fumigatus*	ON584285
11		Possible	*A. fumigatus*	*A. fumigatus*	ON584286
12		Probable	*A. fumigatus*	*A. fumigatus*	ON584287
13		Colonization	*A. fumigatus*	*A. fumigatus*	ON584288
14		Possible	*A. fumigatus*	*A. fumigatus*	ON584289
15		Colonization	*A. fumigatus*	*A. fumigatus*	ON584290
16		Possible	*A. fumigatus*	*A. fumigatus*	ON584291
17		Probable	*A. fumigatus*	*A. fumigatus*	ON584292
18		Colonization	*A. fumigatus*	*A. fumigatus*	ON584293
19		Possible	*A. fumigatus*	*A. fumigatus*	ON584294
20		Probable	*A. fumigatus*	*A. fumigatus*	ON584295
21	AO512021 21	Colonization	*A. terreus*	*A. terreus*	ON606023
22	AO512021 22	Probable	*A. terreus*	*A. terreus*	ON606024
23	AO512021 23	Colonization	*A. terreus*	*A. terreus*	ON606025
24	AO512021 24	Probable	*A. flavus*	*A. flavus*	OK631791
25	AO512021 25	Colonization	*A. flavus*	*A. flavus*	ON606026
26	AO512021 26	Probable	*A. flavus*	*A. flavus*	ON606027
27	AO512021 27	Colonization	*A. flavus*	*A. flavus*	ON606028
28	AO512021 28	Colonization	*A. flavus*	*A. flavus*	ON606029
29	AO512021 29	Possible	*A. flavus*	*A. flavus*	ON606030
30	AO512021 30	Colonization	*A. niger*	*A. niger*	ON606031
31	AO512021 31	Colonization	*A. niger*	*A. niger*	ON606032
32	AO512021 32	Colonization	*A. niger*	*A. niger*	ON606033
33	AO512021 33	Possible	*Aspergillus* spp.	*A. niger*	ON606034
34	AO512021 34	Colonization	*Aspergillus* spp.	*A. welwitschiae*	OK631792
35	AO512021 35	Colonization	*Aspergillus* spp.	*A. welwitschiae*	ON606035

against *A. fumigatus* isolate. While two *A. flavus* isolates were susceptible to all antifungal agents (MIC_{90} range of 0.125– 4 µg/mL; $MIC_{90} \leq$ ECV), four isolates were resistant to AMB with MICs of > 4 µg/mL according to the proposed ECVs. For *A. niger* isolates, AMB, ITR, VOR, and CSP antifungals showed good activity with MIC values lower than the proposed ECV values. Two *A. terreus* isolates were susceptible to ITR, VOR, and CSP ($MIC_{90} \leq$ ECV). On the other hand, one isolate was resistant to ITR, and two isolates were resistant to AmB (MIK_{90} 16 > ECV). Two *A. welwitschiae* isolates were found to be sensitive to all tested agents with 1– 0.125 µg/mL MIC_{90} values.

Serum GM test

In the study, serum GM antigen positivity was found in 11 of the patients, and these patients were considered as probable IPA. The group of patients with *Aspergillus* colonization was negative for serum GM. The non-IPA patient group was not included in the serum GM test because they did not meet the evaluation criteria of ECMM/ISHAM (Koehler et al. 2021).

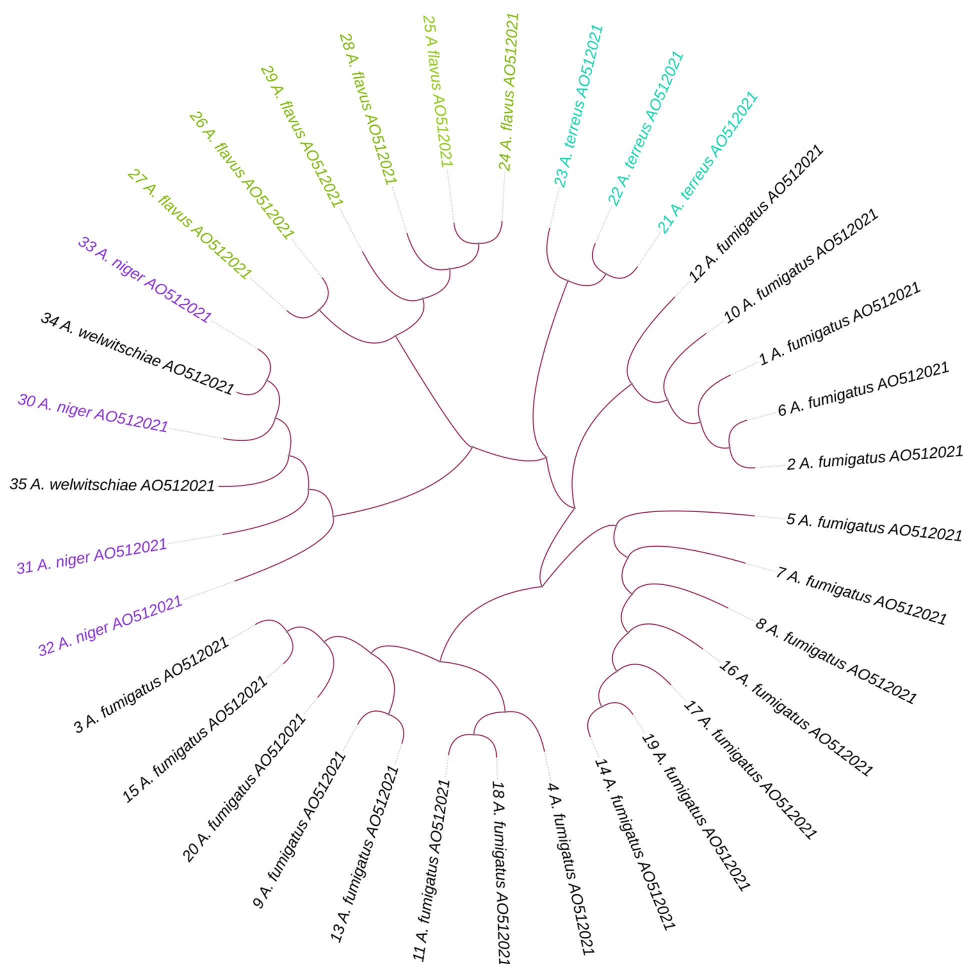


Fig. 1 Phylogenetic tree of the *Aspergillus* isolates using the maximum likelihood method based on the combined sequences of the ITS region

Discussion

Serious viral pulmonary infections such as COVID-19 are associated with an increased risk of superinfection, including IPA, especially in those with hematological malignancies and in immunocompromised patients. Emerging fungal infection such as aspergillosis is profoundly identified in critically ill patients (Chong and Neu 2021; Al-Tawfiq et al. 2021).

CAPA is defined as IPA in most of the critically ill COVID-19 patients. Consequently, a question arises about the burden of IPA among these patients (Gouzien et al. 2021). Risk factors identified in IPA patients associated with COVID-19 include advanced age, lymphopenia, chronic respiratory diseases, corticosteroid therapy, antimicrobial therapy, mechanical ventilator, or cytokine storm (Lai and Yu 2021; Hoenigl et al. 2022). In a study, secondary infections developed in 65.96% of patients with

Table 4 Antifungal susceptibility profiles of *Aspergillus* species

Antifungal agents[a]	MIC[b]	*A. fumigatus*	*A. flavus*	*A. niger*	*A. terreus*	*A. welwitschiae*
AmB	MIC_{90}	1	8	1	16	1
	MIC range	0.06–1	2–8	0.25–1	4–16	0.5–1
ITR	MIC_{90}	0.5	0.5	2	16	1
	MIC range	0.03–0.5	0.5–0.25	0.5–2	0.5–16	0.25–1
VOR	MIC_{90}	1	0.5	1	2	0.5
	MIC range	0.25–1	0.5–1	0.25–1	0.5–2	0.125–0.5
CSP	MIC_{90}	0.5	0.5	0.25	0.125	1
	MIC range	0.125–0.5	0.125–0.25	0.125–0.25	0.06–0.25	0.125–1

[a]*AmB* amphotericin B, *ITR* itraconazole, *VOR* voriconazole, *CSP* caspofungin, [b]*MIC* minimum inhibitory concentration (μg/mL)

COVID-19 infection (De Bruyn et al. 2022). In another study, 20 (13.3%) of 150 patients diagnosed with COVID-19 had secondary infections. The rate of secondary infection in ICU patients (72%) was found to be significantly higher than in patients in general service (1.6%) (Arıcı et al. 2022).

In a study conducted at San Salvatore Hospital in Pesaro, Italy, out of a total of 89 patients with COVID-19 in the ICU, 68 (76.4%) developed a secondary infection. Bacteria constituted most of the isolates (94.6%) in the study, followed by fungi with 5.4% (Caiazzo et al. 2022). In a multicenter study conducted in France, IPA was diagnosed in 129 (25.1%) of 366 COVID-19 patients admitted to the ICU (Dellière et al. 2020).

The increased incidence of IPA in people with severe respiratory virus infection has raised concerns that it may also occur in patients with acute respiratory failure due to COVID-19 infection. In particular, this infection causes an inflammatory environment that allows pulmonary damage and fungal infection (White et al. 2021).

In parallel with the increase in the number of immunocompromised patients, it has been reported that the incidence of IA increases in patients hospitalized in intensive care, transplant, and burn units (Weber et al. 2009). Although *Aspergillus* species are less seen in intensive care patients, they are increasingly detected as infectious agents. The presence of COPD, steroid use, and multiple organ failure in non-neutropenic ICU patients facilitate the development of IPA (Sánchez Martín et al. 2022).

In addition, negative pressure application in the COVID-19 ICU may be the source of air pollution by *Aspergillus* spp., which increases the risk of opportunistic infection (Ichai et al. 2020). Despite a large number of case reports and extensive studies, *Aspergillus* spp. can cause devastating inflammatory and invasive pathology in individuals with severe influenza, and culture results are mistakenly reported as respiratory tract colonization by many clinicians. It is always difficult to distinguish between respiratory tract colonization and potential disease caused by *Aspergillus* spp. The definition of IPA is still difficult, especially in patients with severe COVID-19 infection in the ICU. Therefore, the focus has been on the development of new algorithms based on symptoms compatible with *Aspergillus* spp. cultures, host factors, and abnormal imaging to improve the process of obtaining information about the disease agent and prognosis (Marr et al. 2021; White et al. 2021; Castro-Fuentes et al. 2022). Current studies particularly highlight the need to monitor COVID-19 patients who develop ARDS and remain in intensive care for IPA (Chiumello et al. 2022).

In this study, we aimed to collect data on the incidence of IPA, risk factors, identification, and susceptibility profile of *Aspergillus* agents in patients hospitalized in the COVID-19 ICUs of our hospital who developed ARDS. Clinical, radiological, and mycological criteria in ECMM/ISHAM logarithm were used for IPA definitions. EORTC definitions were not included in our study due to the absence of immunocompromised individuals (such as acute myeloid leukemia, solid organ transplantation, and neutropenia) in our hospital among ICU patients.

In our study, 11 patient were considered probable IPA according to the results of *Aspergillus* culture positivity, serum GM, and abnormal CT scan of the lungs in addition to the lesions attributed to typical COVID-19. The incidence of possible and probable IPA in the whole population of the study was determined to be 18% and 22%, respectively. These results were found to be compatible with similar studies (Salas et al. 2022).

Our results show that in patients having SARS-CoV-2-associated pneumonia and in patients without immunosuppression are more susceptible to increased risk of IPA (40% overall). Due to the difficulty of obtaining samples for histopathological confirmation in these critically ill patients, a proven diagnosis of IPA could not be made.

The serum GM test was negative in 30% of the patients, and mycological evidence was not detected as *Aspergillus* species did not grow in the respiratory samples. The isolation of *Aspergillus* species from respiratory specimens in the absence of pneumonia symptoms in 30% of our cases was thought to represent colonization. Based on these results, we cannot rule out that damaged pulmonary epithelium is an indicator of colonization with *Aspergillus* hyphae prior to the development of active IPA. Some publications indicate that colonization is an important risk factor for the development of IPA (Arastehfar et al. 2020).

Isolation of *Aspergillus* species from respiratory specimens in critically ill patients is significantly associated with both the diagnosis of the underlying disease (such as COPD) and corticosteroid therapy (Townsend and Martin-Loeches 2022). In our study, results were obtained supporting that the presence of COPD comorbidity in patients with *Aspergillus* growth in the LRT sample would be a risk factor for the development of IPA.

In the current study, *Aspergillus* positive cultures were obtained in 35 of 50 respiratory samples. The most frequently isolated species was *A. fumigatus*, followed by *A. flavus*, *A. niger*, *A. terreus*, and *A. welwitschiae* (Table 2). In our study, when the results of the conventional and molecular methods were compared, it was found that although 91.4% agreement was observed, the conventional method could not identify three isolates (8.6%). The susceptibility of *Aspergillus* isolates identified was evaluated in vitro against AmB, ITR, VOR, and CSP. Overall, the tested antifungal agents showed good activity against the isolates. Limited literature available on CAPA, which suggests that serum GM will not be the best marker to distinguish between IA and colonization, and that perhaps serum GM testing from a

bronchoalveolar lavage (BAL) sample should be performed (Verweij et al. 2020). Moreover, it is already recommended for diagnosing CAPA (White et al. 2021). However, bronchoscopy for the collection of BAL samples from COVID-19 patients poses a significant risk to healthcare workers as it produces aerosols (Nasir et al. 2020). Therefore, this procedure was avoided in our study, and a serum sample was preferred instead. In our study, serum GM positivity was detected in a total of 11 patients diagnosed with IPA.

The reason for the high mortality associated with IA in critical non-neutropenic COVID-19 patients has also been attributed to difficulties in timely diagnosis due to nonspecific clinical manifestations and lack of definitive diagnostic criteria (Palacios and Moffarah 2021). In our study, 25 (71.4%) of 35 patients evaluated according to IPA algorithms died.

Obtaining mycological evidence of airway invasive aspergillosis in patients with COVID-19 is complicated by the reduced use of diagnostic bronchoscopy necessary to protect healthcare workers from aerosol exposure and the low sensitivity of circulating GM detection in serum. Also, the detection of *Aspergillus* in upper respiratory tract specimens such as sputum or TA generally does not distinguish between aspergillus colonization and invasive disease. TA and sputum samples are usually positive in critically ill COVID-19 patients but may represent upper airway colonization (Caggiano et al. 2022; Rouzé et al. 2022).

Conclusions

The development of new diagnostic tests, assessment of host immune responses, mycological screening (biomarkers and mycological diagnosis) of patients infected with COVID-19, and regular air quality checks of the ICU may perhaps lead to faster diagnosis and immediate initiation of antifungal therapy to manage the poor prognosis of IPA and reduce the risk of mortality. Considering all these may lead to the development of new prevention strategies for secondary infections.

Author contributions Ali Ozturk: Conceptualization, Methodology, Resources, and Validation; Ali Ozturk and Merve Erdogan: Performing the experiments; Ali Ozturk and Taylan Bozok: Analyzing the data, preparing the figure and writing the manuscript; Ali Ozturk, Taylan Bozok, and Bashar MS. Ibrahim: Editing the manuscript and reviewing the literature.; All authors: read and approve the final manuscript.

Data availability All data generated during this study are publicly available from the GenBank database at http://www.ncbi.nlm.nih.gov/blast.

Declarations

Conflict of interest The authors declare no competing interests.

References

Al-Tawfiq JA, Alhumaid S, Alshukairi AN et al (2021) COVID-19 and mucormycosis superinfection: the perfect storm. Infection 49(5):833–853. https://doi.org/10.1007/s15010-021-01670-1

Antinor S, Galimberti L, Milazzo L, Ridolfo AL (2020) Bacterial and fungal infections among patients with SARS-CoV-2 pneumonia. Infez Med 28(1):29–36

Arastehfar A, Carvalho A, van de Veerdonk FL et al (2020) COVID-19 associated pulmonary aspergillosis (CAPA)-from immunology to treatment. J Fungi (Basel) 6(2):91. https://doi.org/10.3390/jof6020091

Arıcı N, Özgültekin A, Kansak N et al (2022) Secondary infections in COVID-19 patients hospitalized in a tertiary hospital. OTJHS 7(1):99–105. https://doi.org/10.26453/otjhs.1031970

Bilgi EA, Kiraz N (2019) Comparison of conventional methods, MALDI-TOF MS and sequence analysis methods for identification of *Aspergillus* species isolated from clinical samples. Dicle Med J 46:543–551. https://doi.org/10.5798/dicletip.620589

Caggiano G, Apollonio F, Consiglio M et al (2022) Tendency in pulmonary aspergillosis investigation during the COVID-19 era: what is changing? Int J Environ Res Public Health 19(12):7079. https://doi.org/10.3390/IJERPH19127079

Caiazzo L, Temperoni C, Canovari B et al (2022) Secondary infections in critically ill patients with COVID-19: a retrospective study. Antibiotics (Basel) 11(11):1598. https://doi.org/10.3390/ANTIBIOTICS11111598

Castro-Fuentes CA, del Reyes-Montes M, R, Frías-De-León MG, et al (2022) *Aspergillus*-SARS-CoV-2 coinfection: what is known? Pathogens 11(11):1227. https://doi.org/10.3390/PATHOGENS11111227

Chiumello D, Modafferi L, Fratti I (2022) Risk factors and mortality in elderly ARDS COVID-19 compared to patients without COVID-19. J Clin Med 11(17):5180. https://doi.org/10.3390/JCM11175180

Chong WH, Neu KP (2021) Incidence, diagnosis and outcomes of COVID-19-associated pulmonary aspergillosis (CAPA): a systematic review. J Hosp Infect 113:115–129. https://doi.org/10.1016/j.jhin.2021.04.012

Clinical and Laboratory Standards Institute (2008) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; M38-A2. Approved standard-second edition 13–56. Wayne, PA

De Bruyn A, Verellen S, Bruckers L et al (2022) Secondary infection in COVID-19 critically ill patients: a retrospective single-center evaluation. BMC Infect Dis 22(1):207. https://doi.org/10.1186/S12879-022-07192-X

Dellière S, Dudoignon E, Fodil S et al (2020) Risk factors associated with COVID-19-associated pulmonary aspergillosis in ICU patients: a french multicentric retrospective cohort. Clin Microbiol Infect 27(5):790.e1–790.e5. https://doi.org/10.1016/J.CMI.2020.12.005

Er B, Er AG, Gülmez D et al (2022) A screening study for COVID-19-associated pulmonary aspergillosis in critically ill patients during the third wave of the pandemic. Mycoses 65(7):724–732. https://doi.org/10.1111/MYC.13466

Espinel-Ingroff A, Turnidge J (2016) The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds. Rev Iberoam Micol 33(2):63–75. https://doi.org/10.1016/j.riam.2016.04.001

Gouzien L, Cocherie T, Eloy O et al (2021) Invasive aspergillosis associated with COVID-19: a word of caution. Infect Dis Now 51(4):383–386. https://doi.org/10.1016/j.idnow.2020.12.008

Hoenigl M, Seidel D, Sprute R et al (2022) COVID-19-associated fungal infections. Nat Microbiol 7(8):1127–1140. https://doi.org/10.1038/S41564-022-01172-2

Ichai P, Saliba F, Baune P et al (2020) Impact of negative air pressure in ICU rooms on the risk of pulmonary aspergillosis in

COVID-19 patients. Crit Care 24(1):538. https://doi.org/10.1186/s13054-020-03221-w

Koehler P, Bassetti M, Chakrabarti A et al (2021) Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis 21(6):149–162. https://doi.org/10.1016/S1473-3099(20)30847-1

Lai CC, Yu WL (2021) COVID-19 associated with pulmonary aspergillosis: a literature review. J Microbiol Immunol Infect 54(1):46–53. https://doi.org/10.1016/j.jmii.2020.09.004

Marr KA, Platt A, Tornheim JA et al (2021) Aspergillosis complicating severe coronavirus disease. Emerg Infect Dis 27(1):18–25. https://doi.org/10.3201/eid2701.202896

McClenny N (2005) Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture: the traditional approach. Med Mycol 43(1):125–128. https://doi.org/10.1080/13693780500052222

Nasir N, Farooqi J, Mahmood SF, Jabeen K (2020) COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: an observational study from Pakistan. Mycoses 63(8):766–770. https://doi.org/10.1111/myc.13135

Palacios CF, Moffarah AS (2021) Diagnosis of pneumonia due to invasive molds. Diagnostics (Basel) 11(7):1226. https://doi.org/10.3390/diagnostics11071226

Rouzé A, Martin-Loeches I, Nseir S (2022) COVID-19-associated pulmonary aspergillosis: an underdiagnosed or overtreated infection? Curr Opin Crit Care 28(5):470–479. https://doi.org/10.1097/MCC.0000000000000977

Salas B, McCullagh I, Cranfield K et al (2022) COVID-19-associated pulmonary aspergillosis: a year-long retrospective case series. COVID 2(7):976–982. https://doi.org/10.3390/COVID2070073

Sánchez Martín C, Madrid Martínez E, González Pellicer R et al (2022) Invasive pulmonary aspergillosis in patients with acute respiratory syndrome by COVID-19. Rev Esp Anestesiol Reanim 69(1):48–53. https://doi.org/10.1016/J.REDARE.2021.02.007

Song G, Liang G, Liu W (2020) Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from China. Mycopathologia 185(4):599–606. https://doi.org/10.1007/s11046-020-00462-9

Townsend L, Martin-Loeches I (2022) Invasive aspergillosis in the intensive care unit. Diagnostics 12(11):2712. https://doi.org/10.3390/diagnostics12112712

Verweij PE, Rijnders BJA, Brüggemann RJM et al (2020) Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Med 46(8):1524–1535. https://doi.org/10.1007/s00134-020-06091-6

Weber DJ, Peppercorn A, Miller MB et al (2009) Preventing healthcare-associated *Aspergillus* infections: review of recent CDC/HICPAC recommendations. Med Mycol 47(1):199–209. https://doi.org/10.1080/13693780802709073

White PL, Dhillon R, Cordey A et al (2021) A national strategy to diagnose coronavirus disease 2019-associated invasive fungal disease in the intensive care unit. Clin Infect Dis 73(7):1634–1644. https://doi.org/10.1093/cid/ciaa1298

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.