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First report of fatal fungemia due *Fusarium oxysporum* in a patient with COVID-19 in Ecuador

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ABSTRACT

Filamentous fungal infections are an important cause of systemic infections in immunocompromised patients. *Fusarium* genus members potentially cause disseminated infections, especially in patients with catheters, due to the ability to adhere to these devices. We describe a case of fatal fungemia due to *Fusarium oxysporum* in a patient with COVID-19 in Ecuador. The genus identification was carried out with conventional techniques and species identification by molecular and phylogenetic techniques through sequencing of the ITS region.

1. Introduction

Filamentous fungal infections are an important cause of systemic infections in immunocompromised patients [1]. The incidence of co-morbid invasive fungal infections increased during the coronavirus disease (COVID-19) pandemic. This is due to its effect on the immune system, and treatments (corticosteroids and other medications) which may weaken or alter the immune response against fungi. SARS-CoV-2 has been associated with aspergillosis, invasive candidiasis, and mucormycosis [2,3]. However, human infections by *Fusarium* spp. are rare. *Fusarium* spp. are widely distributed in nature, and its conidia are dispersed by air. They can produce superficial, localized, invasive, and disseminated infections [4]. The corticosteroid treatment predisposes patients to *Fusarium* infection due to impaired function of macrophages [5].

The *Fusarium* genus comprises around 300 species, grouped into more than 20 species complexes. However, *Fusarium* species that cause disease in humans are grouped into seven species complexes: *Fusarium solani species complex* (FSSC), *Fusarium oxysporum species complex (FOSC), Fusarium fujikuroi species complex (FFSC), Fusarium incarnatum-equiseti (FIESC), Fusarium chlamidosporum species complex (FCSC), Fusarium dimerum species complex (FDSC) and Fusarium sporotrichoides species complex* (FSAMSC) [6]. Certain phytopathogen species such as *Fusarium solani* (approximately 50 % of all infections), followed by *Fusarium oxysporum, Fusarium proliferatum and Fusarium verticillioides*, can cause invasive infections in humans [7].

Infection occurs mainly by airborne transmission, or by direct inoculation via broken skin due to trauma, burns, or catheter insertion [8]. During dissemination, *Fusarium* spp., can adhere to medical devices such as indwelling catheters, artificial heart valves, and joint replacements, and then grow into biofilms on the surface of these materials, which are difficult to cure with antifungal therapy. Invasive fusariosis is associated with a high mortality rate, ranging from 50 % to 80 % [4].

Fusarium genus members have quite similar macroscopic and microscopic characteristics, which makes it difficult to accurately identify species complexes with phenotypic methods, requiring the use of molecular methods for the correct identification of the microorganism [9]. In the present report, we describe the first case of fungemia due *Fusarium oxysporum* associated with SARS-CoV-2 in Ecuador.

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2. Case

A 70-year-old male patient from Quito-Ecuador presents at the outpatient clinic with a productive cough, sore throat, headache, dysphonia, arthralgia, myalgia, rhinorrhea, fever, profuse sweating, dyspnea on medium exertion, listlessness, and fatigue. He received treatment with intravenous (IV) dexamethasone 8 mg and ceftriaxone.

On day 16, due to the persistence of symptoms, he went to the emergency service at a tertiary care hospital. COVID-19 was confirmed by a SARS-CoV-2 positive PCR on a nasopharyngeal swab. On day 17 he was admitted to the Intensive Care Unit with acute respiratory distress syndrome, where he required invasive ventilatory assistance with 3 cycles of pronation. Due to the poor evolution, corticosteroid therapy with dexamethasone 6 mg IV, hydrocortisone 200 mg IV, and antibiotic therapy were implemented again. During the hospital stay, he received broad-spectrum antibiotic therapy with carbapenems, glycopeptides, aminoglycosides, and quinolones.

Eight days after admission to the hospital, on day 22, four blood culture bottles were sent for microbiological identification. On day 24, *Klebsiella pneumoniae* resistant to carbapenems (New Delhi metallo-beta-lactamase (NDM) type) was identified in blood culture, and the new antibiotic scheme was reconsidered.

On day 30, after 14 days of hospitalization, a fungus grew in two BacT/ALERT FAN aerobic blood culture bottles (bioMerieux, France). The Gram stain performed on the positive blood cultures showed Gram-negative septated hyphae, Gram-positive macroconidia, and microconidia (Fig. 2). Based on the characteristics observed in Gram stain, the microbiological differential diagnosis was established between *Fusarium* spp., and *Scedosporium* spp. Immediately, treatment with amphotericin B deoxycholate (0.7 mg/kg/day) was administered once a day, and voriconazole 200 mg every 12 hours was started for persistent septic shock. On day 37 the genus of the microorganism was confirmed based on the macroscopic and microscopic characteristics of the colony (Fig. 2). The macroscopic characteristics showed on the obverse a downy, flat, white colony with a central orange coloration that spreads throughout the colony. On the reverse, an orange-cream coloration is observed in the culture (Fig. 2). Additionally, the microscopic structures observed were septate hyaline hyphae, long conidiophores with monophialides, abundant crescent-shaped, hyaline and septate macroconidia, and abundant ovoid microconidia. Based on the characteristics observed the microorganism was identified as *Fusarium* spp. Due to the regulations established in the country during the public health emergency caused by the pandemic, no additional microbiological studies of medical devices or in other hospitalization areas were carried out [10].

Subsequently, molecular techniques using the ITS region (ribosomal intergenic spacer) were used to identify the microorganisms at the genus and species level. Genomic DNA was extracted from fungal strains using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The ITS region was amplified using specific primers ITS1 and ITS4 [11]. We obtained a PCR product of 517 bp. The Blast search percent identity was 100 % to *Fusarium oxysporum* using the GenBank NCBI and the International Society of Human and Animal Mycology (ISHAM) databases. ISHAM is a curated ITS database that stores sequences mainly from clinically relevant human and animal fungi [12,13]. For the construction of the phylogenetic tree based on the ITS gene, an alignment matrix resulting from 490 base pairs or positional characters per 52 sequences/taxa was constructed using ClustalW on MacVector, Inc. ver 18.6.0. We used gaps creation and extension penalties of 30.0 and 15.0 searching for high homology alignment. The phylogenetic tree was constructed by Maximum Parsimony as a base tree and Maximum Likelihood with the K2+G model using PAUP 4.0a (build 169). In Ecuador, there are only *Fusarium* spp. sequences from environmental samples. Under this context, we used sequences of *Fusarium* species specifically from clinical isolates of different countries and from both GenBank and ISHAM databases, to perform the phylogenetic analysis (Fig. 4). The lack of clinical sequences in our country makes it challenging to know the incidence of this type of fungemia in the territory.

The resulting cladogram is shown in Fig. 3. Two sister clades can be observed: the most basal clade formed by the subclades of the species of the *Fusarium oxysporum* and *Fusarium proliferatum* complex (*Fusarium fujikuroi* species complex), and a second clade formed by members of the *Fusarium solani* and *Fusarium falciforme* species complex. The monophyletic clade formed with the *F. oxysporum* species including the patient sequence (Accession number. OR251340), showed a bootstrap support percentage of 99 % and a divergence percentage within the *F. oxysporum* subclade between 0 and 1.8 %. The sequence of the patient from Ecuador was identical to the sequences from Japan and Brazil (0 % divergence) and the most distant was the sequence from India with 1.8 %. The interspecific divergence versus *F. proliferatum*, the sister group was 7.2 %, and with the *F. solani* clade between 17 % and 20 %. The intraspecific divergence in *F. proliferatum* varied between 0 % and 2 %, similar to that of *F. oxysporum*, corroborating the intra value for a *Fusarium* species. The intra-divergence in the *F. solani* clade was between 1.8 and 7 %, suggesting as in the tree topology a complex of different species. On day 42, the patient died, 26 days after being admitted to the Intensive Care Unit. The patient did not present skin lesions before the hospital stay or during the disease. The timeline of the patient is shown in Fig. 1.

3. Discussion

The causative diagnosis of fungal infections remains a problem to be solved at the clinical laboratory level. The clinical manifestations of invasive mycoses are not specific and existing diagnostic tools often lack sensitivity. Conventional and molecular techniques can help facilitate and accelerate diagnosis [14]. The traditional techniques based on morphology (phenotypic characters), for the identification of fungal species are important to understand the evolution of morphological characters. However, morphological approaches may not always work well for species or lower-level classifications [15]. The development of molecular methods for the detection and identification of fungi present in clinical samples is growing at a very fast pace, either through nucleic acid amplification techniques or through specific probes or nucleic acid sequencing [14]. In our report, molecular and phylogenetic techniques were used to identify the microorganism at the genus and species level, using the ITS region, a standard marker for the identification of most fungal species and widely used for evolutionary studies and phylogenetic identification [16].

We found 3 comparable case reports in the literature (Table 1). The identification of the species was not achieved in one case [17]. Proteomic technique mass spectrometry was carried out in the other 2 cases to identify *Fusarium proliferatum* and *Fusarium verticillioides* for the first and second case, respectively [18,19]. Both molecular and proteomic techniques use structural information of microorganisms to be able to identify them at the genus and species level. These techniques are a valuable contribution in routine practice laboratories to the identification of pathogens. In addition, the data obtained strengthen the monitoring and epidemiological control of pathogenic fungal species in humans.

Approximately 70 % of the cases of invasive disease that occur in hematology patients are caused by FSSC and FOSC, but there are geographic differences in species distribution [20], being FSSC and FOSC the largest complexes involved in human infections [7]. We observed a close relationship between *Fusarium proliferatum* and *Fusarium oxysporum* sequences suggesting that they share genotypic characteristics, similar to results found using the TEF1 and RPB2 genes, which, like the ITS genomic region, serve to identify species of this fungus, and report this same close phylogenetic relationship between the FOSC and FFSC complexes [21].

The WHO categorized the genus *Fusarium* within the group of high-priority pathogens due to inherent resistance to most antifungals. Causative pathogens are rarely confirmed microbiologically, due to a

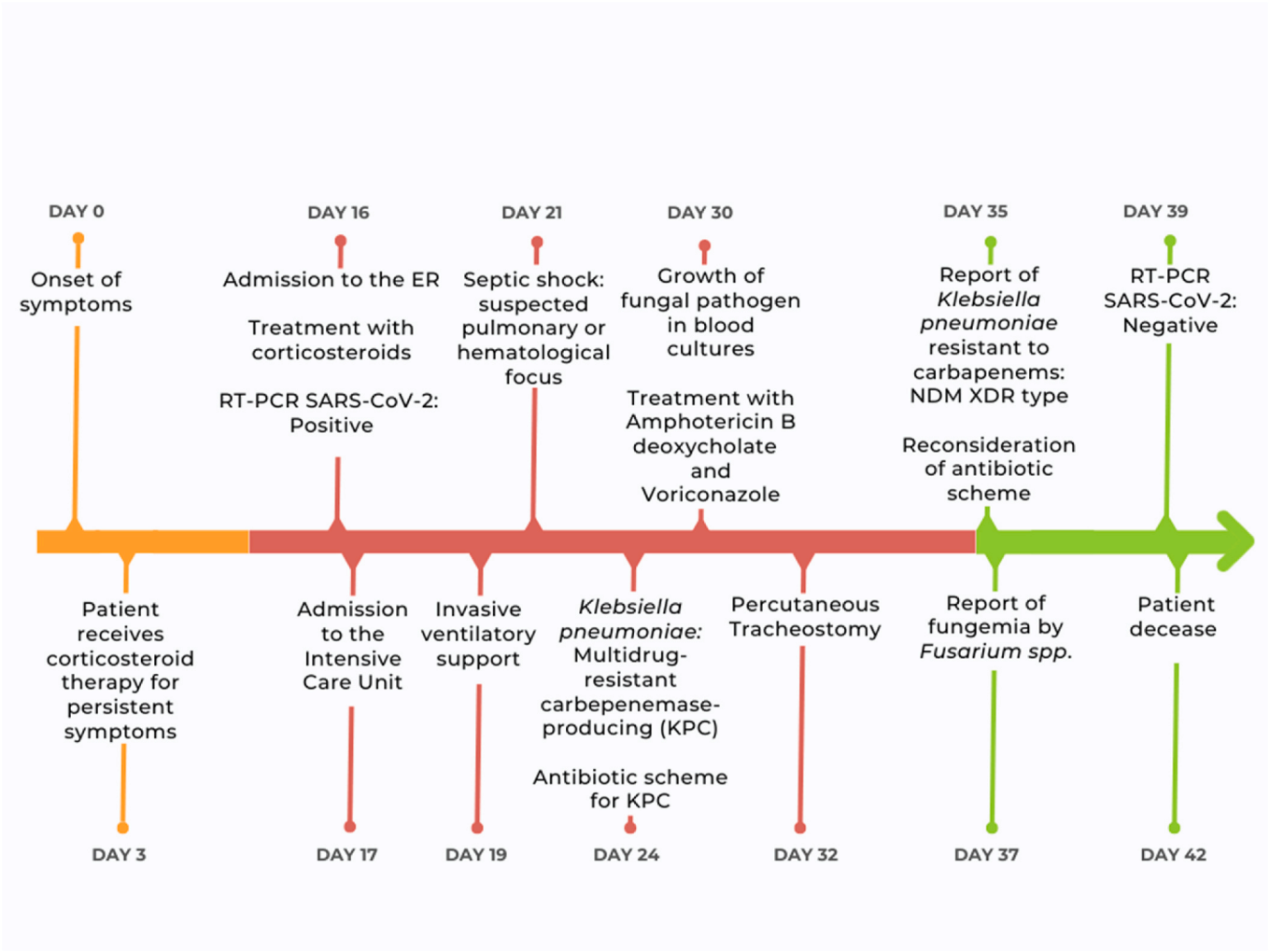

Fig. 1. Timeline of the patient during the course of the disease.

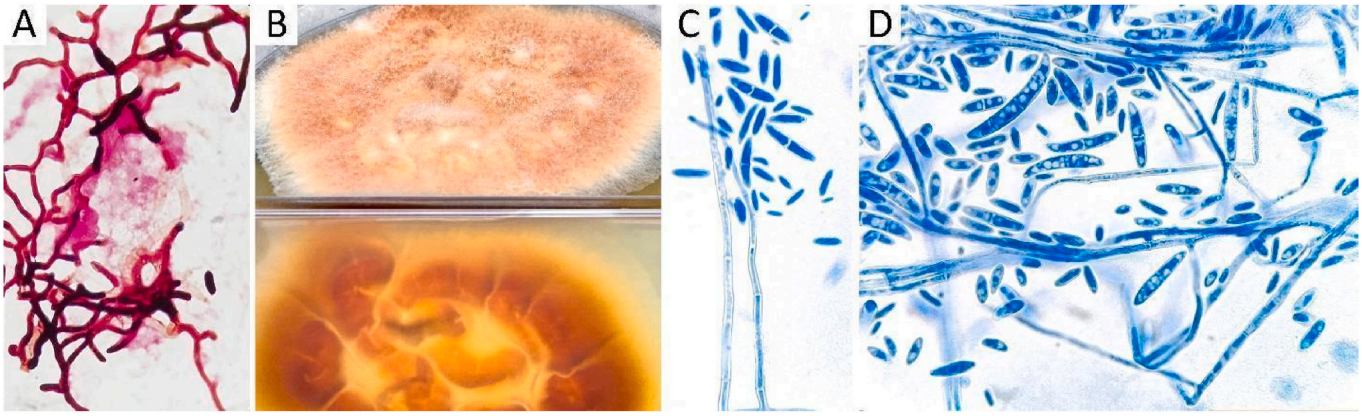

Fig. 2. Morphological characterization of *Fusarium oxysporum* isolated from bloodstream infection. **A.** Gram stain of positive blood culture bottle showing Gram-negative fungal hyphae and Gram-positive macroconidia. **B.** Macroscopic colonies of *Fusarium oxysporum* on Sabouraud dextrose agar medium. **C.** Hyaline septate hyphae and slightly curved microconidia (40X). **D.** Microscopic colony morphology of *Fusarium oxysporum* (100X) with lactophenol blue stain: abundant, elongated, cylindrical, slightly curved, fusiform septate macroconidia. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

lack of qualified personnel and low-quality or non-existent surveillance data in most settings [22].

The incidence and severity of diseases caused by *Fusarium* species vary according to geographic location. A study conducted in Europe described the complex FFSC as the predominant cause of invasive *Fusarium* infections, similar to a study conducted in Turkey, where FFSC was more frequent, followed by FSSC. A study conducted in France showed that FSSC was the most prevalent complex, followed by FOSC

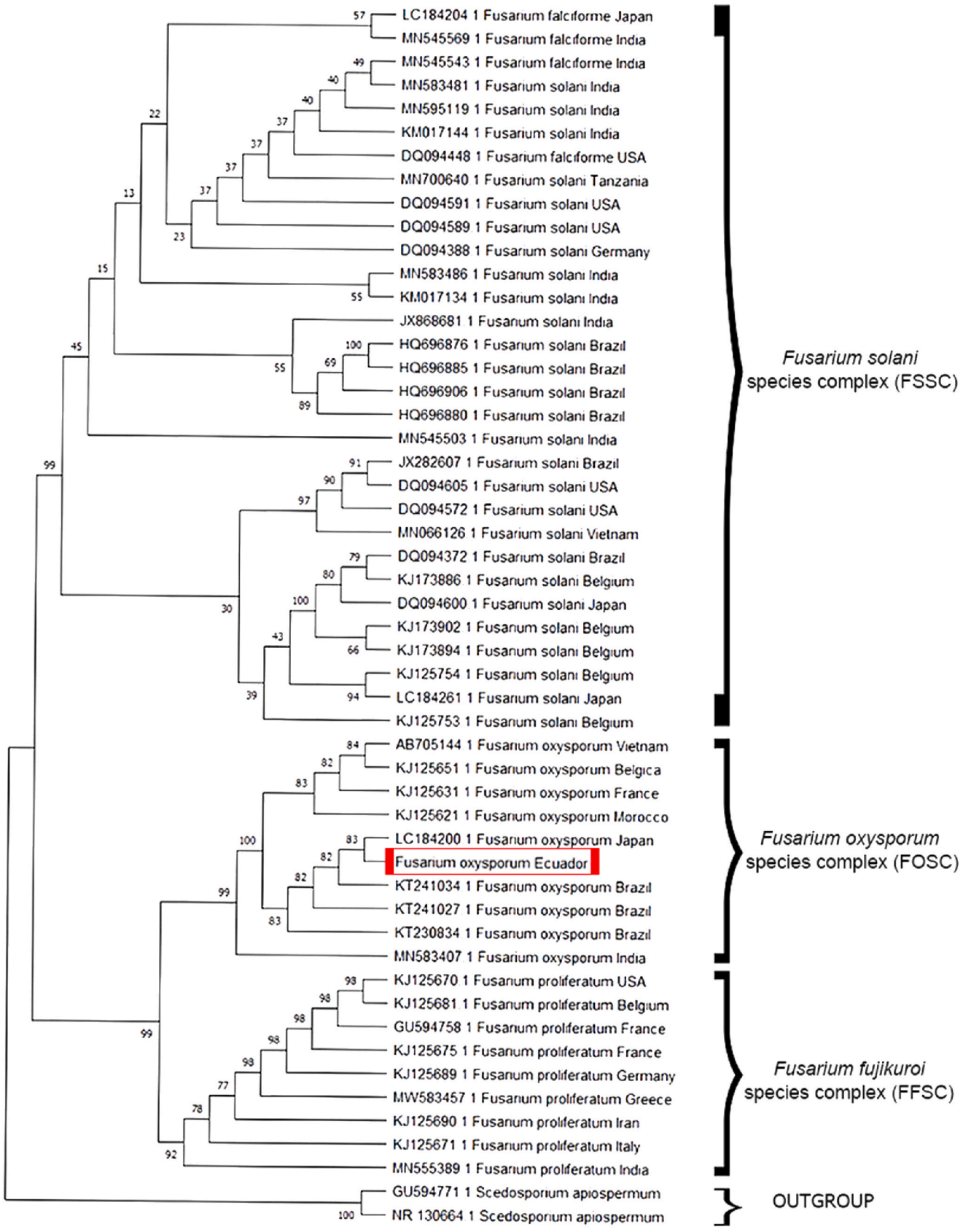

Fig. 3. Maximum Likelihood phylogenetic tree of *Fusarium* species based on complete ITS region. The red square shows the sequence obtained from the patient. Species complexes are indicated in square brackets to the right. At the base of the branches, the value is shown in the percentage of bootstrap as clade support. The clades corresponding to each species complex have 99 % support. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Maximum Likelihood phylogenetic tree of *Fusarium* species based on complete ITS region using sequences from the curated database ISHAM. The red square shows the sequence obtained from the patient. At the base of the branches, the value is shown in the percentage of bootstrap as clade support. The clades corresponding to each species complex have 100 % support. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Case report comparison of fusariosis associated with COVID-19 infections.

	CASE 1 [17]	CASE 2 [18]	CASE 3 [19]	CASE 4 (current case)
Risk factors	Tuberculosis treated w/RIPE therapy 30 years ago	Overweight, type 2 diabetes, hypertension and hypothyroidism	Obesity, no history of SARS-CoV-2 vaccination	Chronic arterial hypertension
Diagnostics results	COVID-19 pneumonia (non-critically ill patient)	Severe COVID-19 pneumonia	Severe COVID-19 pneumonia Catheter-associated bacteremia caused by *Acinetobacter baumannii* *Enterobacter cloacae* NDM-1 bacteremia	Severe COVID-19 pneumonia *Klebsiella pneumoniae* resistant to carbapenems NDM type
Species identification	Identification: *Fusarium* spp. Methodology: Beta-(1,3)-D-Glucan test and positive culture	Identification: *Fusarium proliferatum* Methodology: mass spectrometry	Identification: *Fusarium verticillioides* Methodology: mass spectrometry	Identification*: Fusarium oxysporum* Methodology: molecular techniques
Treatment	Voriconazole	Liposomal amphotericin B	Amphotericin B deoxycholate, voriconazole	Amphotericin B deoxycholate, voriconazole
Outcome	On follow-up 6 weeks later, patient had resolution of his symptoms	Patient extubated and then discharged to a post-ICU area.	Patient died due to respiratory instability, sepsis and shock, despite clearance of fungemia burden during antifungal therapy	Patient died after 26 days in ICU due to multiorgan failure

RIPE: Rifampin, Isoniazid, Pyrazinamide, Ethambutol. **ICU:** Intensive Care Unit. **NDM:** New Delhi metallo-β-lactamase-1.

complex [23]. In Ecuador, there is no precise information about species of the *Fusarium* genus that affect humans. As it is a high-priority pathogen, there should be established diagnostic and identification protocols that enable timely reporting.

The conventional, molecular, and phylogenetic techniques used in this study provide valuable information for the management and surveillance of patients infected with this type of microorganism. The techniques based on the structural composition such as proteins, nucleic acids, and genes of the pathogens, allow the identification of more precise, specific treatments, and relevant data about these infectious diseases.

The enhanced clinical surveillance will depend on the level of knowledge and education about the clinical presentation and risk factors of infections caused by these pathogens. Therefore, access to diagnostic tools is essential for optimal patient care and the generation of surveillance data.

Ethical form

Publication of this case report was approved by the Human Research Ethics Committee (CEISH in Spanish) at Hospital de Especialidades Carlos Andrade Marin, Quito, Ecuador (Code. SGC-EI-FR-011).

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Please state any sources of funding for your research

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CRediT author statement

A.J-Z.: conceptualization, methodology, formal analysis, data curation investigation, original draft preparation, writing, review & editing; G.S: methodology, original draft preparation, writing, review & editing; P.R.: methodology, original draft preparation, writing, review & editing; J.R.R–I.: data curation, validation, methodology; original draft preparation, writing, review & editing J.C.N.: funding acquisition, supervision, formal analysis, initial draft preparation, project administration, final draft preparation, writing, review & editing.

Please state any competing interests

There are none.

Consent

Please declare that you have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

Please state that consent has been obtained from the patient or legal guardian(s)

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case report and accompanying images. A copy of the written consent is available (submission data) for review by the Editor-in-Chief of this journal on request.

Declaration of competing interest

Please declare any financial or personal interests that might be potentially viewed to influence the work presented. Interests could include consultancies, honoraria, patent ownership or other. If there are none state 'there are none'.

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