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Invasive infection by *Fusarium verticillioides* in a pediatric leukemia patient and literature review on a series of fusariosis cases

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Abstract

Introduction. *The emergence of fungi causing infections to humans has increased as a serious public health problem, representing an important cause of morbidity and mortality among hospitalized patients. Fusarium verticillioides, regarded as an emerging pathogen, is capable of causing invasive infections to immunocompromised patients, especially those carrying hematologic malignancies.*

Gap statement. *There are few reports on invasive and disseminated fusariosis in children and the severity of this infection in patients with weakened immune system.*

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Aim. *The aim of the current study was to present a case of invasive fusariosis caused by F. verticillioides in a child with T-cell acute lymphocytic leukemia.*

Results. *Successful treatment was obtained with amphotericin B and fluconazole.*

Conclusion. *Considering that the number of invasive cases by filamentous fungi has increased, their identification is extremely important for an accurate diagnosis and a more effective treatment.*

Keywords: Fungi; invasive infection; nosocomial infection; hematologic malignancy; immunocompromised patients

Repositories: Sequence data was deposited into GenBank under accession numbers MZ014397.

INTRODUCTION

Filamentous fungi of the genus *Fusarium* Link, 1809 (Ascomycota, Sordariomycetes, Hypocreales), are ubiquitous saprophytes that are found in the soil, air and water, showing a wide geographic distribution [1, 2]. These microorganisms can cause superficial infections to humans, including onychomycosis, keratitis and cutaneous lesions on traumatized skin, as well as invasive and disseminated infections, especially to immunosuppressed patients in hospitals [1-4].

The major risk factors for the development of an invasive infection by *Fusarium* fungi are prolonged neutropenia, concomitant catheters and T-cell immunodeficiency. The mortality rate of this type of infection ranges from 50% to 70% among adults receiving chemotherapy or carrying hematologic malignancies [2-4] and reaches up to 80% among pediatric patients [5].

The genus *Fusarium* has global distribution and is divided into phylogenetic species complexes. Approximately ten complexes are estimated to be related to human pathogens, such as *F. solani*, *F. oxysporum*, *F. fujikuroi*, *F. incarnatumequiseti*, *F. clamydosporum*, *F. dimerum*, *F. sambucinum*, *F. concolor* and *F. lateritium*. Considering these complexes, members of *F. solani* are most common and virulent (comprising around 40%-60% infections), followed by *F. oxysporum* (~ 20%), *F. fujikuroi* and *F. moniliforme* (~ 10%) [6].

One of the problems related to these infections is their rare or late diagnosis, leading to a lethal course [7]. In addition, little is known about the most appropriate therapeutic treatment. Fusariosis can be refractory to antifungal treatment in patients with prolonged neutropenia, especially in those with hematologic and oncologic diseases [4].

Considering the scarce reports of invasive *Fusarium* infection in children, the aim of the present study was to describe a case of invasive fusariosis caused by *F. verticillioides* in a pediatric patient with T-cell acute lymphocytic leukemia (T-ALL).

CASE

An 11-year-old male patient diagnosed with T-cell acute lymphocytic leukemia (T-ALL), started his treatment in October 2017, CSF1 (lumbar puncture of cerebrospinal fluid was not traumatic and there were no cancer cells - blasts), showing an early second relapse. After 12 chemotherapy cycles (October 2017 to December 2018), the patient presented prolonged neutropenia and was re-hospitalized on January 03rd, 2019, due to the recurrent disease, to start a new treatment regimen. On January 08th, 2019, the patient evolved with febrile neutropenia, starting empiric broad-spectrum antimicrobial treatment with piperacillin-tazobactam. Blood samples were collected for blood culture on January 08th and 12th, 2019, when the patient had good general state and clinical examination revealed no changes. Growth of white fungal mycelia was detected on blood cultures and identified as *Fusarium* spp.. Treatment with amphotericin B lipid complex, 3mg/kg/day, was introduced on January 15th and 16th, 2019, and the central catheter was removed. Blood samples were collected from the catheter and sent to the laboratory for identification. The catheter tip culture was positive, and the colonies had macromorphological aspects compatible with those of the fungal etiological agent from the blood samples, which was identified as *Fusarium*. The patient had normal funduscopic examination and negative serial galactomannan test, evolving clinically well and afebrile, without alterations shown by complementary investigation: normal echocardiogram and tomography of the thorax and abdomen. From January 17th to 29th, 2019, the patient was medicated with fluconazole on an outpatient basis. Control cultures were negative on the days subsequent to the treatment. Showing good clinical evolution, the patient was discharged on January 23rd, 2019. After discharge, he remained clinically stable, following a chemotherapy protocol. In March 2019, the patient underwent bone marrow transplantation

and, in August 2019, he died of new recurrent leukemia, associated with graft versus host disease (GVHD) and cytomegalovirus infection.

MATERIAL AND METHODS

The addressed case of invasive fusariosis occurred during 2019 in a pediatric patient admitted to the Oncology Ward of the Children's Hospital Darcy Vargas, located in the city of São Paulo, SP, Brazil. The fungal strain was isolated from the blood of this patient; positive culture was detected through automated BACTEC® system (Becton-Dickinson, USA). To confirm the infection, at least two samplings were conducted on different days.

After isolation in culture medium, the strain was identified according to its macroscopic and microscopic features (giant colony and slide culture) [8]. The species was confirmed by molecularly studying the strain through amplification and sequencing of the TEF gene region with primers EF1 and EF2 [9]. The obtained nucleotide sequence was deposited in the GenBank database. Similar sequences were compared with the sequences available at *Fusarium* MLST database (http://www.wi.knaw.nl/fusarium/). The final EF sequences were used in the phylogenetic study of the strain through multiple sequence alignment with ClustalW, and the phylogenetic tree was built according to the Methods of Maximum Likelihood and Kimura 2-parameters [10]; analyses were performed with Mega X software [11].

Antifungal sensitivity test was carried out for the strain according to the E-test method (AB BIODISK, Solna, Sweden), based on the manufacturer's recommendation. The isolates *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC6258 were included as quality control. Minimum inhibitory concentration (MIC) was determined for the antifungals fluconazole, itraconazole, voriconazole, amphotericin B and caspofungin.

RESULTS

The strain isolated from the patient's blood was identified by standard morphological criteria, according to macroscopic and microscopic features, as belonging to the species *F. proliferatum* ((Matsushima) Nirenberg) (Figures 1 and 2).

In the molecular study, after TEF gene region sequencing, the strain was identified as *F. verticillioides,* showing 100% identity to with the reference strains in Blastn. PCR amplification, sequencing and comparison of IGS region with sequences at GenBank allowed the creation of a dendrogram

for this region (Figure 3). The studied isolate (FV01) was allocated to the group of *F. verticillioides* reference strains. For this clade, bootstrap support was high: 96.

As regards the *in vitro* sensitivity test, high MIC values were found for fluconazole (>256µg/mL), itraconazole (>32 µg/mL) and caspofungin (>32 µg/mL), while lower values were obtained for amphotericin B (4 µg/mL) and voriconazole (4 µg/mL). MIC values were not interpreted since there are no clinical breakpoints formally proposed for fungi of the genus *Fusarium*.

Table 1 presents a review of cases of *Fusarium* infections in pediatric patients over the last 20 years.

DISCUSSION

The frequency and the diversity of invasive fungal infections have changed recently. Emergence of less common but medically important fungi has increased, and the risk of infections by these microorganisms became higher with the inclusion of medical conditions like cancer, hematologic diseases, immunosuppressive therapy, prolonged neutropenia and T-cell immunodeficiency [4]. Clinical manifestations are significantly different depending on the host immune status, the portal of entry for the pathogenic fungus, and the inoculum size [12].

Thus, fungi of the genus *Fusarium* spp. have emerged as important pathogens of nosocomial infections. These microorganisms can be transmitted via inhalation, direct inoculation or contaminated food intake [13, 14]. The water distribution systems in hospitals have been identified as potential reservoirs of *Fusarium* species and are consequently regarded as responsible for infections in such environments [15, 16]. For the studied patient, the possible portal of entry was the central venous catheter, since laboratorial analyses after its removal indicated presence of a fungus showing macromorphological aspects compatible with the fungal etiological agent isolated from the blood samples. No skin, nail or airways injury was revealed by the daily comprehensive physical examination of this patient. The importance of asepsis must be highlighted for the hands of healthcare workers, catheters and other devices that may carry the fungus; similarly, proper maintenance of the hospital air conditioning system is essential.

Invasive fusariosis is considered an opportunistic infection of severe consequences for immunocompromised or weakened patients, especially those carrying hematologic and angioinvasive neoplasias and other malignancies [2,

4, 17]. For these individuals, *Fusarium* corresponds to the second most frequent cause of invasive filamentous fungal infections [18].

Besides acute leukemia and T-cell immunodeficiency, prolonged and deep neutropenia is one of the major risk factors for invasive fusariosis [4]. Under such conditions, fusariosis occurs as a severe and disseminated disease [19], refractory to therapy [20], which has become the most frequent and challenging form of this infection, accounting for around 70% of all cases [7]. In a multicentric study, Pérez-Nadales et al. [21] described 58 cases of invasive fusariosis in 18 Spanish hospitals over 15 years. During this period, there was an increase in incidence from 0.40 to 0.79 cases/100,000 admissions which, for neutropenic patients, was 0.32 to 0.57 cases/100.000 admissions. In Brazil, Nucci et al. [22] eported an increment in invasive fusariosis prevalence from 0.6% to 3.7% in ten years for immunocompromised patients. According to those authors, the increases may be related to the larger at-risk population, the environmental exposure to *Fusarium* conidia, and the more frequent use of antifungal prophylaxis, or the combination of all factors [21].

Recently, the raise in cases of invasive fusariosis in immunocompromised pediatric patients has been alarming, especially because they have survived longer [4]. Most reports have included the species *F. solani* and *F. oxysporum* [4, 23-37].

In the current study, the neutropenic T-ALL pediatric patient presented disseminated fusariosis caused by *F. verticillioides*. The strain isolated from the patient's blood was phenotypically identified as *F. proliferatum* ((Matsushima) Nirenberg); after sequencing, it was identified as *F. verticillioides* ((Saccardo) Nirenberg). According to Indian researchers [38], careful morphological characterization is required for the species *F. verticillioides* and *F. proliferatum* since they present chains and false heads, and the major difference between them is formation of monophialids in *F. verticillioides* and formation of monophialids and poliphialids in *F. proliferatum*. According to Barros [39], the fungus *F. verticillioides* have clavate unicellular microconidia of rounded or truncated base and fusiform macroconidia that are thin and septate, straight or slightly curved, presenting elongated and frequently curved apical cell. Phylogenetic analysis indicated that, considering the clade to which it was allocated, the studied isolate was genetically closer to the species *F. verticillioides* than to the strains of *F. proliferatum*, showing high bootstrap value.

Fusarium verticillioides is more commonly associated with corn grains and their byproducts in European countries like France, Spain and Italy. It is also found in other crops such as banana, beetroot, sugarcane and

oat. This fungus is considered a human opportunistic pathogen that causes sporadic cases of fusariosis, especially in immunocompromised patients. There are few cases reported in the literature relating this species to infections in children [20, 31-33, 40, 41].

Although *Fusarium* spp. are not particularly aggressive to humans, their infections, especially systemic manifestations, have a poor prognosis. Early detection and identification of fusariosis in immunocompromised patients, resolution of neutropenia and removal of any primary infection foci allow timely intervention, specific therapy and improved outcome [42]. However, no clear consensus exists over the management of disseminated invasive infections by *Fusarium* in these patients [43, 44]. *Fusarium* specimens seem to be resistant to most currently available antifungal drugs. Considering this scenario, the antifungal agents regarded as an option for combating this infection include natamycin, amphotericin B, voriconazole and posaconazole [40]. Nevertheless, depending on the clinical case, the drugs of choice are amphotericin B and voriconazole [44, 45]. The use of echinocandins as first-line empiric therapy is discouraged due to the intrinsic resistance of *Fusarium* species to these antifungals [12].

Clinical evidence and studies related to antifungal activities on hematologic malignancies have emphasized a synergic association between two or more antifungals for a wider coverage in the treatment of disseminated fusariosis [43, 44, 46-48]. Monotherapy for immunocompromised patients is not always adequate, but there is limited evidence in the literature about the use of combined antifungal therapy in pediatric patients. For this population, specific antifungal therapy is still based on clinical experiences in adults [49].

For the pediatric patient of the present study, the strain of *F. verticillioides* was sensitive to liposomal amphotericin B and fluconazole, evolving to a successful treatment, according to the clinical records [49, 50]. However, on *in vitro* experiment, the strain was highly susceptible to amphotericin B and voriconazole and more resistant to itraconazole, caspofungin and fluconazole. Considering the few reports on isolation of *F. verticillioides* from immunocompromised pediatric patients, successful treatment has been obtained with the administration of amphotericin B [31], liposomal amphotericin B [32] and liposomal amphotericin B plus voriconazole [33]. On the other hand, other studies on infection by this species in adult patients have demonstrated failure in therapy with amphotericin B and caspofungin [41], amphotericin B [51] and liposomal amphotericin B plus voriconazole [20].

In conclusion, the present study delineates a case of opportunistic infection by *F. verticillioides* in a pediatric T-ALL patient that was successfully treated with amphotericin B and fluconazole. Early diagnosis and treatment are emphasized as important measures for pediatric patients with deficient immune system, which is a risk factor for opportunistic infections caused by *Fusarium* spp.. This paper also highlights the importance of molecular study for cases involving morphologically similar species.

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ETHICAL STATEMENT
Ethics approval from the Darcy Vargas Children's Hospital Ethics Committee (application number 0321128.2.0000.0075) was obtained. A written informed consent was provided by the patient's parents, acknowledging that patient data could be accessed through electronic or paper means, provided that the data is anonymised to protect their identity.

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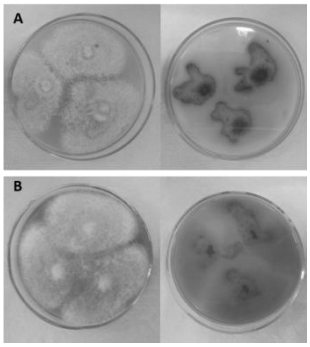

Figure 1 – Macromorphological aspects of the colonies of *F. verticillioides* after 10-day incubation: **A** – Potato dextrose agar, cotton-like colonies presenting white aerial mycelium with violet-like center, and brownish reverse; **B** – Sabouraud dextrose agar, cotton-like colonies presenting white aerial mycelium with violet-like center, and orange-like reverse. (Photo: Leite-Jr, DP)

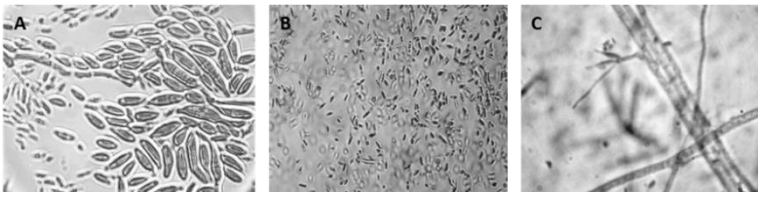

Figure 2 – Micromorphological aspects observed under a 40x optical microscope: **A** – macroconidia; **B** – microconidia; **C** – septate hyphae in cluster. (Photo: Leite-Jr, DP)

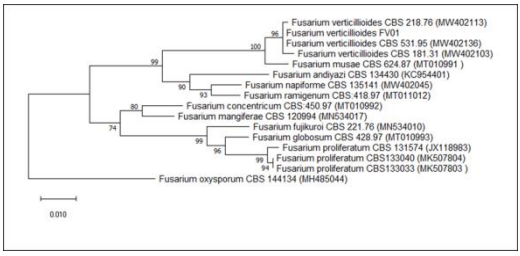

Figure 3 - The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The tree with the highest log likelihood (-1769.29) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4616)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 621 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. Isolated studied designated by FV01.

Table 1. Characteristics of cases involving patients with infection by *Fusarium* and combined antifungal therapies for the treatment of disseminated fusariosis in immunocompromised and immunosuppressed patients from 2000 to 2020.

Isolated Species/ Complex	Disease	Infection Site	Sex/ Age (years old)	Antifungal/ Therapy/ Treatment	Outcome	Geographical origin	Author/Year of publication (Reference)
F. solani (FSSC)	ALL	Cutaneous lesions	M/17	L-AmB	Died	Brazil	Costa et al. (2000) [29]
F. oxysporum (FOSC)	AA	Lesion on the knee	F/3	L-AmB, VOR	Died	USA	Rodriguez et al. [2003] (30)
F. dimerum (FDSC)	AA	Lesions on the skin and Pulmonary infection	F/19	VOR	Recovered	Europe	Bigley et al. [2004] (52)
F. solani (FSSC) (2 casos)	ALL, AML	Lungs, skin, blood, urine, feces, bones and brain	M/5, 8	AmB	Died	Finland	Kivivuori et al. [2004] (28)
F. verticillioides (FFSC)	CML	Nasal septum abscess	M/9	L-AmB	Recovered	Austria	Dornbush et al. [2005] (31)
F. moniliforme (FFSC)	ALL	Lungs, skin	M/5	AmB + ITR	No	Taiwan	Chi et al. [2007] (32)
F. verticillioides (FFSC)	ALL	Skin and articulations	M/12	L-AmB + VOR	Recovered	Turkey	Tezcan et al. [2009] (33)
F. solani (FSSC)	ALL	Skin	F/5	L-AmB + G-CSF	Recovered	United Kingdom	Cooke et al. [2009] (53)
F. solani (FSSC)	LAD	Gangrenous ecthyma on the knee	F/9	AmB	Recovered	Tunis	Mellouli et al. [2010] (34)
F. oxysporum (FOSC)	AA	Cutaneous and blood Infection	F/1	L-AmB, VOR	Died	Canada	Morris et al. [2012] (35)
F. chlamydosporum (FCSC)	Pyelonephritis	Perinephric abscess	M/12	VOR	Died	India	Sidhu et al. [2013] (36)
F. solani	ALL	Cutaneous lesions and oral mucosa	M, 13543	L-AmB	Died	Spain	Morel et al. [2013] (13)
(*F. solani* (2 cases) *F. oxysporum* (3 cases)	ALL (4), AA (1)	Lungs (4), skin (1), brain (1), liver (1), paranasal sinuses (1)	F and M/1, 3, 8, 9, 15	L-AmB + VOR(2), L-AmB + CAS + VOR, L-AmB (2)	01 recovered and 04 died	Canada	Schwartz et al. [2015] (27)
Fusarium spp. (7 cases), *F. solani* (1 case) and *F. oxysporum* (2 cases)	ALL, AML	Lungs (7), skin (9), liver (6), spleen (4), bone marrow (1), bones (1), kidney (2)	F and M/ 1, 6, 8, 8, 9, 9, 13, 16, 16, 17	AmB(2), VOR + CAS, AmB + VOR	2 recovered and 8 died	Brazil	Litvinov et al. [2015] (24)
F. dimerum	AML	Cutaneous lesions	M, 15	L-AmB, VOR	Died	Spain	García-Ruiz et al. [2015] (25)
Fusarium sp	ALL (9), Shwachman-Diamond Syndrome (1)	Blood (7), deep soft tissue (2), palate (1)	F, 6	VOR + AmB (5), VOR + Echinocandin (3), VOR (2)	5 recovered and 5 died	Germany	Hassler et al. [2017] (54)
F. oxysporum (7 cases)	Solid Tumors (7), ALL	Blood	M,F/ 1, 1, 2, 2, 3, 8 and 9 months	VOR, L-AmB	Recovered	Brazil	Carlesse et al. [2017] (23)
Fusarium spp.	Toxic Shock Syndrome	Skin	F, 5	AmB + VOR	Recovered	Spain	Barranco-Fernández et al. [2017] (55)
Fusarium spp (3 cases) and *F. keratoplasticum* (1 case)	ALL (3) and Wilms' Tumor	Skin (2) and blood (2)	M, F/6, 7, 9, 11	VOR, L-AmB+VOR	03 recovered and 01 died	Brazil	Arnoni et al. [2018] (4)
F. mundagurra	DM and Lung Transplantation	Lungs	M, 13	L-AmB + POS	Recovered	Australia	Al Yazidi et al. [2019] (37)
Fusarium lichenicola	Hyper-IgE Syndrome	Skin	F, 4	AmB, VOR and Luliconazole	Recovered	China	Shi et al. [2020] (56)
F. solani	ALL	Skin and lungs	M, 9	L-AmB, VOR	Recovered	Italy	Biddeci et al.

							[2020] (57)
F. dimerum	B-cell leukemia	Subcutaneous nodules	M, 8	N/C	Recovered	France	Sevestre et al. [2020] (58)
F. petroliphilum	Severe congenital Neutropenia	Skin, blood	M, 7	L-AmB + VOR	Died	Japan	Hoshino et al. [2020] (59)

N/C = not declared; L-AmB= Liposomal Amphotericin B; VOR=Voriconazole; CAS=Caspofungin; FLU=Fluconazole; TBF=Terbinafine; POS=Posaconazole; CET=Ketoconazole; ITR=Itraconazole; DM=diabetes mellitus; ALL= acute lymphocytic leukemia; CLL= chronic lymphocytic leukemia; AML= acute myeloid leukemia; CML= chronic myeloid leukemia; G-CSF= granulocyte colony stimulating factor; AA = aplastic anaemia ; LAD = . Leukocyte adhesion deficiency.