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Case Report

First report of fungal meningoencephalitis by *Penicillium chrysogenum* in Brazil

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ABSTRACT

Introduction: Fungal infections of the central nervous system present a variety of clinical syndromes, such as meningitis, encephalitis, raised intracranial pressure with a nonspecific presentation, and, in the last two decades, have increased the incidence of these fungal infections. Fungal meningoencephalitis is frequently associated with *Cryptococcus*, but this report stands out for presenting one species of *Penicillium* genus.

Objectives: Here, we present the first case of meningoencephalitis associated with brain injury caused by *Penicillium chrysogenum*, in a patient who is immunocompetent and was admitted to Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil.

Methods: To identify the fungal species, we performed phenotypic and genotypic methodologies, from the culture to the sequencing of internal transcribed spacer region, and β-tubulin gene, a rare fungus in cerebrospinal fluid cultures, belonging to the genus *Penicillium*, was identified.

Conclusion: We highlight the importance of the first report of meningoencephalitis caused by *P. chrysogenum* in a patient who is immunocompetent, registered in Brazil. We also emphasize the need for further studies to determine an effective treatment with the least possible side effects for patients infected by fungi that are rarely related to the most severe forms of invasive infections.

Introduction

Fungal infections of the central nervous system present a variety of clinical syndromes, such as meningitis, encephalitis, raised intracranial pressure with a nonspecific presentation, and, in the last two decades, have increased the incidence of these infections (Góralska et al., 2018). They are characterized by recurrent headaches, changes in mental faculties, focal neurological deficits, and deterioration of level of consciousness (Góralska et al., 2018). It is important to highlight that the expansion of immunosuppression conditions, such as HIV/AIDS, among others, constitutes a host condition that can influence the severity of a disease (Khanna *et al.*, 2016); however, fungi have also emerged as causal agents of infection in immunocompetent hosts (Sharma and Jakati, 2020).

Fungal meningoencephalitis is frequently associated with *Cryptococcus* sp. and more than a century ago, this fungus has been described as the cause of this serious infectious condition (Stott *et al.*, 2021). Nevertheless, this report stands out for presenting one species of the *Penicillium* genus, *Penicillium chrysogenum,* a fungus with a wide global distribution found in different habitats, including environments with extreme temperatures, such as the Antarctic soil (de Sousa *et al.*, 2017; Frisvad and Samson, 2004).

Here, we present the first case of meningoencephalitis associated with brain injury caused by *P. chrysogenum*, in a patient who

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is immunocompetent and was admitted to Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil. This case demonstrates the importance of an accurate identification of the etiologic agent because although the standard of reference for the diagnosis of fungal infections is isolation and identification in culture, molecular diagnosis has been increasingly used, mainly to reduce time, which is crucial in the most severe cases of the disease.

Case report

A female Brazilian patient aged 14 years, overweight, was admitted to the hospital on September 22, 2021, with nonspecific frontal headache, photophobia, and vomiting, without fever. No comorbidities, such as diabetes and tuberculosis, no primary immunodeficiencies, or treatments for any diseases that requires immunomodulation were related. The patient also tested HIV-negative. We decided to perform a brain computed tomographic scan and a lumbar puncture to further investigate the meningeal finding of neck stiffness on physical examination. The initial cerebrospinal fluid (CSF) analysis revealed increased cellularity (203 cells, 98% mononuclear), elevated protein levels (137 mg/dl), reduced glucose levels (35 mg/dl), and negative direct microscopy for fungi or bacteria. The test for acid-fast bacilli was initially negative. Direct examination of sputum ruled out pulmonary tuberculosis. Four days after the admission, the microbiology laboratory reported the growth of fungus in the CSF cultures, and this fungal isolate was sent for taxonomic identification in the Laboratory of Taxonomy, Biochemistry and Bioprospecting of Fungi (LTBBF), Fiocruz, Rio de Janeiro.

The fungus was grown in potato dextrose agar medium at 30°C. The mycelium, which was initially white, with a cottony texture, quickly expanded and, after 7 days, took on a grayish green color (Figure 1a) with a yellow back (Figure 1b). In direct microscopy, we observed globose conidia arranged in chains from the ends of the phialides, which, in turn, emerged from metulae oriented in a brush or penicillus-shaped arrangement. The vegetative hyphae were septate. According to these phenotypic characteristics, the fungus was identified as a species of the genus *Penicillium* (Figure 1c/d).

The LTBBF reported the growth of a rare fungus in the CSF cultures belonging to the genus *Penicillium* but phenotypically incompatible with *Penicillium marneffei*, a species associated with severe infections (Han *et al.*, 2019). It was not possible to make the identification of the species only by classical taxonomy; therefore, partial sequencing of the region internal transcribed spacer (ITS) and β-tubulin genes was performed using ITS1 and ITS4, according to Barreira *et al.* (2022) and Lindsley *et al.* (2001). Briefly, we used 100 ng and 25 ng of DNA, respectively, and 10 pmol of each primer; in the reaction, the annealing temperature was 58°C and 60°C, respectively. Automated sequencing was done using the sequencing platform at Fundação Oswaldo Cruz - PDTIS/FIOCRUZ, Brazil. The sequences were edited using the CodonCodeAligner 9.0.2 software and compared using basic local alignment search tool, with sequences available from the NCBI/GenBank, and there was 100% agreement *P. chrysogenum* for ITS and 100% agreement *P. chrysogenum* sequences deposited in the GenBank for β-tubulin genes. The ITS and β-tubulin sequences of the LTBBF/HNMD01 strain has been deposited in the GenBank under accession numbers OP537069 and ON950424, respectively.

Antifungal therapy was started after the identification of the positive culture and detection of the fungus *Penicillium* spp., with voriconazole (300 mg 12 every 12 hours) and amphotericin B (1 mg/kg once per day). Antibiotics were also included (ceftriaxone and vancomycin, the latter suspended after a negative CSF culture for common germs).

The patient was promptly transferred to the intensive care unit. There was an oscillation in the level of consciousness and progressive worsening of the sensorium and holocranial headache of progressive intensity, also evolving with bilateral amaurosis, despite the use of multiple antimicrobial therapies. Orotracheal intubation was initiated.

Magnetic resonance imaging was performed after clinical worsening and showed extensive cerebral venous thrombosis (Figure 1e,f). A new computed tomographic scan of the head showed signs of acute supratentorial intracranial hypertension with increased dimensions of the ventricles (Figure 1g-j) and the possibility of the presence of transependymal exudate/diffuse cerebral edema. Emergency neurosurgical treatment was indicated: interposition of a right frontal external ventricle shunt, with hypertensive output of “rock water”. After a multidisciplinary meeting, thrombectomy by neurointervention was chosen, with partial success. Then, full anticoagulation was started. Dexamethasone and an anticonvulsant were already underway.

The patient died after 29 days of hospitalization, with mixed shock (septic and neurogenic due to severe acute intracranial hypertension syndrome) refractory to clinical support measures.

Discussion

P. chrysogenum is considered an opportunistic fungus, rarely described as the causal agent of invasive infections, especially in patients who are immunocompetent, due to its difficulty growing at the host's temperature. However, it is important to observe that despite its low capacity to grow at elevated temperatures, which limits their ability to infect and establish in mammals, fungi can be trained to evolve thermotolerance and gradual adaptation to increasing temperature caused by climate changes (Nnadi and Carter, 2021).

These organisms are ubiquitous in nature and can be found in soil, decaying matter, sewage plantations, and construction sites (Chuah *et al.*, 2020). They can cause severe disease, which is most common in individuals who are immunocompromised, whose risk factors include comorbidities with HIV/AIDS or tuberculosis, treatments for disease that requires immunomodulation, such as stem cell transplantation, or specific genetic defects resulting in primary immunodeficiency (Burgess *et al.*, 2022). Our patient was not related to these sites or performed any activity that presented risk of infection; therefore, how this infection occurred, especially in a patient without immune compromise, remains unknown to us.

The standard treatment for *P.* non-*marneffei* species is not well established; however, whether the use of amphotericin B is associated or not with azoles has been described by several authors in many cases successfully, regardless of the patient's immune status (Swoboda-Kopec *et al.*, 2003). Unfortunately, in case reports of intracranial infection, corroborating the outcome described in our study, the strategies for treatment failed to prevent death in all cases (Chuah *et al.*, 2020; Lyratzopoulos *et al.*, 2002).

Invasive fungal infections are an increasingly frequent etiology of sepsis in critically ill patients, causing substantial morbidity and mortality (Delaloye and Calandra, 2014). Septic shock is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, and it was the cause of death of the patient. Although the culture results have been negative for fungi and other microorganisms during her hospitalization, it is worth mentioning that some authors observed in *in vivo* studies that, in some cases, the antifungal therapy can cause fungal dysbiosis in the intestinal microbiota and this disruption of commensal fungal populations can influence local and peripheral immune responses and enhance relevant disease states, including sepsis (Delaloye and Calandra, 2014; Sheng *et al.*, 2021).

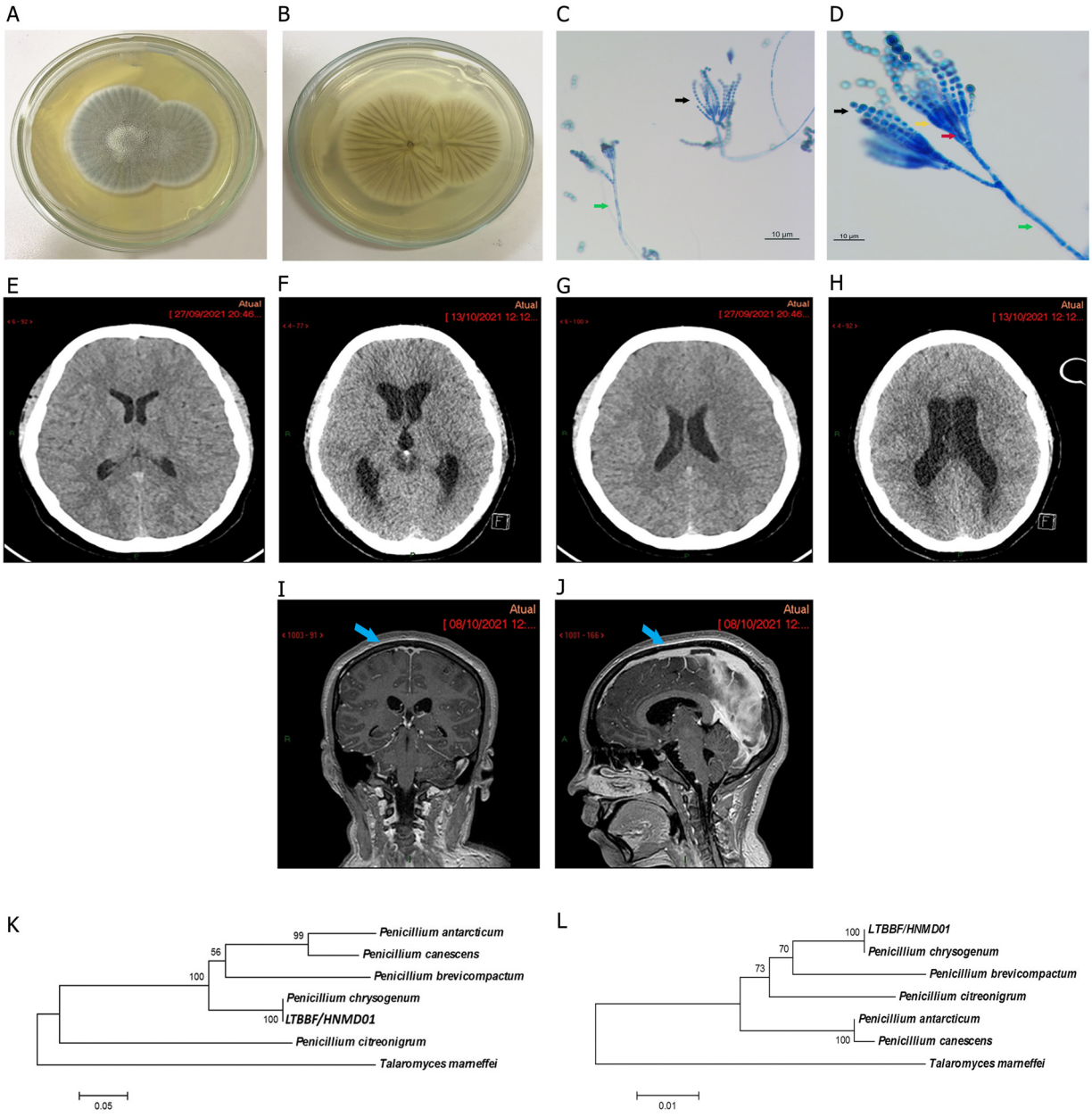

Figure 1. Phenotypic and Genotypic characteristics of *Penicillium chrysogenum* and clinical signs of the patient. (a-d) *P. chrysogenum* after 7 days of culture in potato dextrose agar medium at 30°C. (a) Obverse of the colony grayish green coloration and cotton texture; (b) Reverse in yellow tones; (c-d) Microculture in potato dextrose agar medium and cotton blue staining, highlighting globose conidia in chains (black arrows), from the ends of the phialides (yellow arrow) that emerge from metulae in a brush (red arrow). Metulae branched from conidiophores (green arrows). (c) 400x (d) 1000x. (e-h) CT scan of the patient during hospitalization, showing tetraventricular hydrocephalus, signs of acute supratentorial intracranial hypertension. (e,g) Initial CT imaging; (f,h) Second CT imaging test, performed 2 weeks after the initial imaging. (i,j) Magnetic resonance imaging showing the presence of extensive venous drainage filling failure (blue arrows), absent in the previous examination, affecting the middle and posterior portion of the superior sagittal sinus, confluence of the sinuses, transverse sinuses, left sigmoid, as well as the superior portion of the ipsilateral internal jugular vein. The appearance is consistent with extensive brain venous thrombosis. (k,l) Phylogenetic tree generated by neighbor-joining, maximum likelihood and maximum parsimony analysis using partial nucleotide sequences of the B-tubulin (k) and internal transcribed spacer (l). Bootstrap values (1000 replicates) were added to respective branches. Each species are indicated at each respective position at the phylogenetic tree. Evolutionary relationships of seven taxa being six type strains belonging to the main *Penicillium* sp., which sequences were obtained from GenBank.
CT, computed tomographic.

Conclusion

In conclusion, we highlight the importance of the first report of meningoencephalitis caused by *P. chrysogenum* in a patient who is immunocompetent, registered in Brazil. We also emphasize the need for further studies to determine an effective treatment with the least possible side effects for patients infected by fungi that are rarely related to the most severe forms of invasive infections.

Declaration of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Rômulo Vieira Mello de Oliveira: Investigation, Methodology. **Danielly Corrêa-Moreira:** Visualization, Investigation, Formal analysis, Writing – review & editing.

Túlio Vieira Mendes: Investigation, Methodology, Writing – review & editing. **Gisela Lara da Costa:** Methodology, Writing – review & editing. **Renata de Magalhães Vieira:** Investigation. **Cynthia Miranda Nascimento Buchele:** Investigation. **Rodrigo Schrage Lins:** Investigation. **Ana Beatriz Teixeira Brandão Camello Ferreira:** Investigation. **Daniela Barbosa Veira:** Investigation. **Rafael Santos de Aragão Pedroso:** Investigation. **Vinícius Tadeu Dias Pereira de Faria:** Investigation. **Manoel Marques Evangelista Oliveira:** Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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Ethical approval

This work was approved by the Research Ethics Committee Fiocruz, CAAE:28063114.2.0000.5262. The patient has signed the consent form for publication.

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