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

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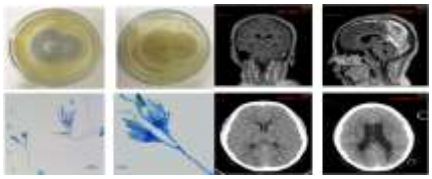
Graphical abstract



Highlights

- The first case of meningoencephalitis associated *Penicillium* spp.
- Case report of brain injury caused by *P. chrysogenum*, in immunocompetent patient
- The need for studies to determine treatment to severe forms of invasive infections

Abstract

Fungal infections of the CNS present a variety of clinical syndromes, such as meningitis; encephalitis; raised intracranial pressure (ICP) with a nonspecific presentation, and, in the last two decades, have increased the incidence of these fungal infections. Fungal meningoencephalitis is frequently associated to *Cryptococcus* but, this report stands out for presenting one species of *Penicillium* genus. Here, we present the first case of meningoencephalitis associated with brain injury caused by *P. chrysogenum*, in an immunocompetent patient admitted to Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil. To identify the fungal species, were performed phenotypic and genotypic methododologies, from the cultive to

sequencing of ITS region and B-tubulin gene. Was identified a rare fungus in CSF cultures, belonging to the genus *Penicillium*. We highlight the importance of the first report of meningoencephalitis in an immunocompetent patient, caused by *P. chrysogenum*, registered in Brazil. We also emphasize the need for further studies in order 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.

Keywords: Fungal infections; meningoencephalitis, *Penicillium*, *Penicillium chrysogenum*, immunocompetent patient

Introduction

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

Fungal meningoencephalitis is frequently associated to *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 *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 (Frisvad and Samson 2004; Sousa et al., 2017).

Here, we present the first case of meningoencephalitis associated with brain injury caused by *P. chrysogenum*, in an immunocompetent patient admitted to Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil. This case demonstrates the importance of an accurate identification of the etiologic agent, since, although the standard of reference for the diagnosis of fungal infections is isolation and identification in

culture, molecular diagnosis has been increasingly used, mainly in order to reduce time, which is crucial in the most severe cases of the disease.

Case report

A 14-year-old Brazilian woman, overweight, was admitted in the hospital on September 22th, 2021, with nonspecific frontal headache, photophobia and vomiting, without fever. No co morbidities, as diabetes, tuberculosis, no primary immunodeficiencies or treatments for any diseases that requires immunomodulation were related. The patient was also tested HIV-negative. We decided to perform a brain CT-scan and a lumbar puncture (LP) to further investigate the meningeal finding of neck stiffness on physical examination. The initial CSF analyzes 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. Acid-fast bacilli (AFB) initially negative. Direct examination of sputum discarded pulmonary tuberculosis. Four days after the admission, the Microbiology Laboratory reported the growing of fungus in CSF cultures and this fungal isolate was sent for taxonomic identification in Laboratory of Taxonomy, Biochemistry and Bioprospecting of Fungi (LTBBF), Fiocruz, RJ.

The fungus was grown in PDA (potato dextrose agar) medium at 30°C. The mycelium, initially white, with a cottony texture, quickly expanded and, after seven days, took on a grayish green color (Figure 1A) with a yellow back (Figure 1B). In direct microscopy, were 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. 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 CSF cultures, belonging to the genus *Penicillium*, but phenotypically incompatible with *P. marneffei*, a species associated with severe infections (Han et al., 2019). It was not possible to make the identification at the species level only by classical taxonomy; therefore, partial sequencing of the region internal transcribed spacer (ITS) and β-tubulin genes (BT2) was performed, using ITS1 and ITS4, according to Lindsley et al., 2001 and Barreira et al., 2022. Briefly were

used 100 ng and 25ng of DNA respectively, 10 pmol of each primer, and 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 by BLAST (Basic Local Alignment Search Tool) with sequences available from NCBI/GenBank, and there was 100% agreement *P. chrysogenum* for ITS and 100% agreement *P. chrysogenum* sequences deposited in Genbank for β-tubulin genes. The ITS and β-tubulin sequences of the LTBBF/HNMD01 strain has been deposited in Genbank under accession numbers OP537069 and ON950424, respectively.

Antifungal therapy was started after identification the positive culture and detection of fungus *Penicillium* spp., with Voriconazole (300mg 12/12 hours) and Amphotericin B (1mg.kg^{-1}/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 ICU. There was an oscillation in the level of consciousness and progressive worsening of the sensorium, holocranial headache of progressive intensity, also evolving with bilateral amaurosis, despite of the use of multiple antimicrobial therapies. Orotracheal intubation was initiated.

Magnetic Resonance Imaging (MRI) was performed after clinical worsening and showed extensive cerebral venous thrombosis (Figure 3 A,B). A new CT scan of the head showed signs of acute supratentorial intracranial hypertension, with increased dimensions of the ventricles (Figure 3C-F), 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 anticonvulsant were already underway.

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

Discussion

P. chrysogenum is considered an opportunistic fungus, rarely described as causal agent of invasive infections, especially in immunocompetent patients, due its difficulty to grow at host temperature. However, it is important to observe that, despite of its low capacity to grow at elevated temperatures 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 are able to cause severe disease is most common in immunocompromised individuals, 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 *Penicillium* non-*marneffei* species is not well established; however, the use of Amphotericin B 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 deregulated 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 (Sheng et al., 2021; Delaloye and Calandra, 2014).

Conclusions

In conclusion, we highlight the importance of the first report of meningoencephalitis in an immunocompetent patient, caused by *P. chrysogenum*, registered in Brazil. We also emphasize the need for further studies in order 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.

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Ethical Approval Statement: This work was approved by the Research Ethics Committee (CEP) Fiocruz, CAAE:28063114.2.0000.5262. The patient has signed the consent form for publication.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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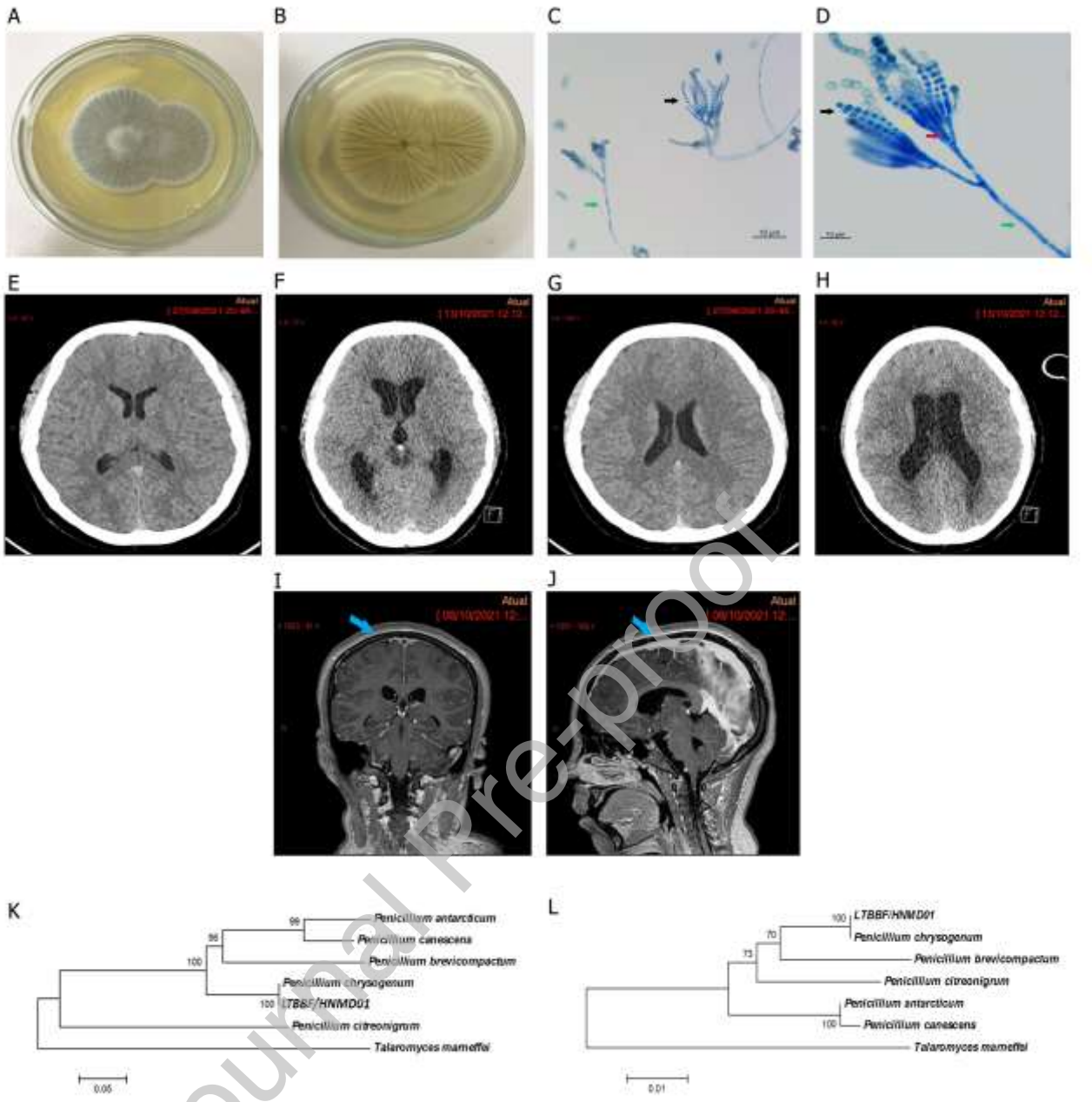

Figure 1: Phenotypic and Genotypic characteristics of *P. chrysogenum* and clinical signs of the patient. **A-D)** *P. chrysogenum* after seven days of culture in PDA medium at 30°C. **A)** Obverse of the colony grayish green coloration and cotton texture; **B)** Reverse in yellow tones; **C-D)** Microculture in PDA 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 two weeks after the initial imaging. **I,J)** MRI 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 Neighbour-Joining, maximum likelihood and maximum parsimony analysis using partial nucleotide sequences of the B-tubulin (K) and ITS (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 7 taxa being six type strains belonging to the main *Penicillium* sp., which sequences were obtained from GenBank.