Medical Mycology Case Reports 40 (2023) 1–4

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Human protothecosis: A case report in northeastern Brazil

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ARTICLE INFO

Handling Editor: Dr Adilia Warris

Keywords:
Protothecosis
Skin diseases
Soft tissues infectious

ABSTRACT

We describe a case of extensive human cutaneous protothecosis in the northeastern of Brazil. The diagnosis was made through histopathological examination of skin samples and culturing on Sabouraud dextrose agar, with identification through mass spectrometry. Treatment with itraconazole failed and was switched to liposomal amphotericin followed by voriconazole with clinical cure. Prothotecosis should be a diagnostic hypothesis when there are skin lesions after trauma, specially in situations of atypical evolution.

1. Introduction

Prototheca spp. belong to the family *Chlorellaceae* and are distinguished from other algae through their lack of chloroplasts. It has been speculated that the lack of chloroplasts promoted the emergence of their pathogenic role [1,2]. The agent is ubiquitous and is found in contaminated soil and water, animals, and food. Many published cases have been described in patients with a comorbidity or immunosuppressive condition such as hematological or solid-tissue malignancy, solid organ transplant, HIV infection, steroid use, or diabetes mellitus. Thus, protothecosis, the disease caused by *Prototheca* spp., is an opportunistic infection [1–5].

The genus *Prototheca* was first described by Wilhelm Krueger in 1984, as a pathogen belonging to the kingdom Fungi. It was later reclassified as algae because of its spores, which are produced internally in a manner identical to how this occurs in the green alga *Chlorella*. *Prototheca* spp. can be found in other environments such as soil, salt water, sewage, animal waste and some foods. It was isolated as a colonizing agent of the skin, nails, and the digestive and respiratory tracts of humans. Human infection can occur through inoculation of the agent after traumatic injuries in the presence of contaminated soil and water [1–3]. In humans, the main risk group includes farmers, gardeners, fishermen, crustaceans, and aquarium handlers. On the other hand, the disease is also observed in animals such as cattle, dogs, cats, roe deer, goats, horses, atlantic salmon, carp, fruit bat, and a corn snake [3]. There have been rare reports of inoculation during surgical and/or orthopedic procedures [2–4].

The disease can be local or disseminated, acute or chronic, and it has been classified into three main clinical forms: cutaneous, articular, or systemic. Cutaneous and subcutaneous diseases are the main clinical manifestations. Visceral infections involving the gut, spleen, eyes, liver, meninges, lung and blood are uncommon, but life-threatening [2,3,5]. The initial cutaneous manifestations are erythematous papular lesions, plaques, nodules, vesiculobullous lesions, ulceration or hypopigmented or atrophic lesions [2,3,6]. The five main species that have been isolated are *P. zopfii*, *P. wickerhamii*, *P. ulmea*, *P. stagnora* and *P. blaschkeae* [1–3]. *Prototheca wickerhamii* and *P. zopfii* are the species that have been described in infections in humans and animals [1–3,6,7].

The differential diagnosis of human infections caused by *Prototheca* spp. includes bacterial infections, fungal infections (phaeohyphomycosis), herpes simplex virus infections, eczema and pyoderma gangrenosum [2,3,6,7].

Thus, the aim of the present study was to report the first case of human cutaneous protothecosis identified in the city of São Luís, Maranhão, northeastern Brazil, an endemic area for occurrence of fungal and bacterial diseases.

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https://doi.org/10.1016/j.mmcr.2023.02.003
Received 6 December 2022; Received in revised form 13 February 2023; Accepted 13 February 2023
Available online 14 February 2023

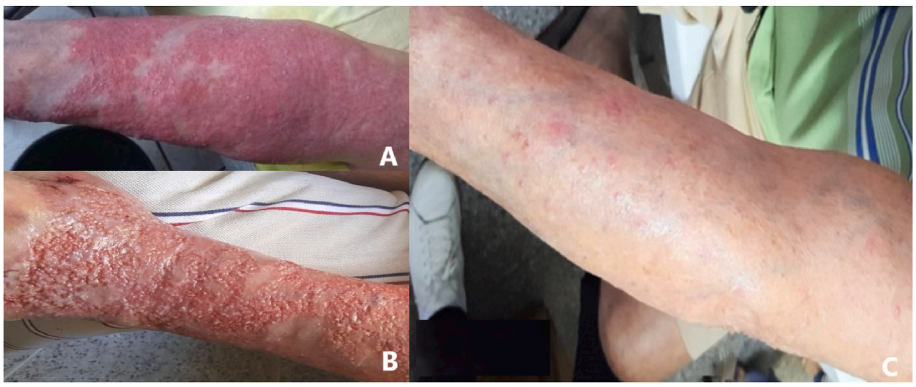

Fig. 1. A – Hyperemic infiltrative lesion with burning pain, presented at the first consultation (D0). B – Lesion with worsening of its characteristics, presenting microulcerations, worsening of pain and mild secretions, with secondary infection, under treatment with itraconazole (400 mg/day) (D+30). C – Upper limb, 18 months after the end of treatment with voriconazole (200 mg, every 12 hours for 6 months) (D+615).

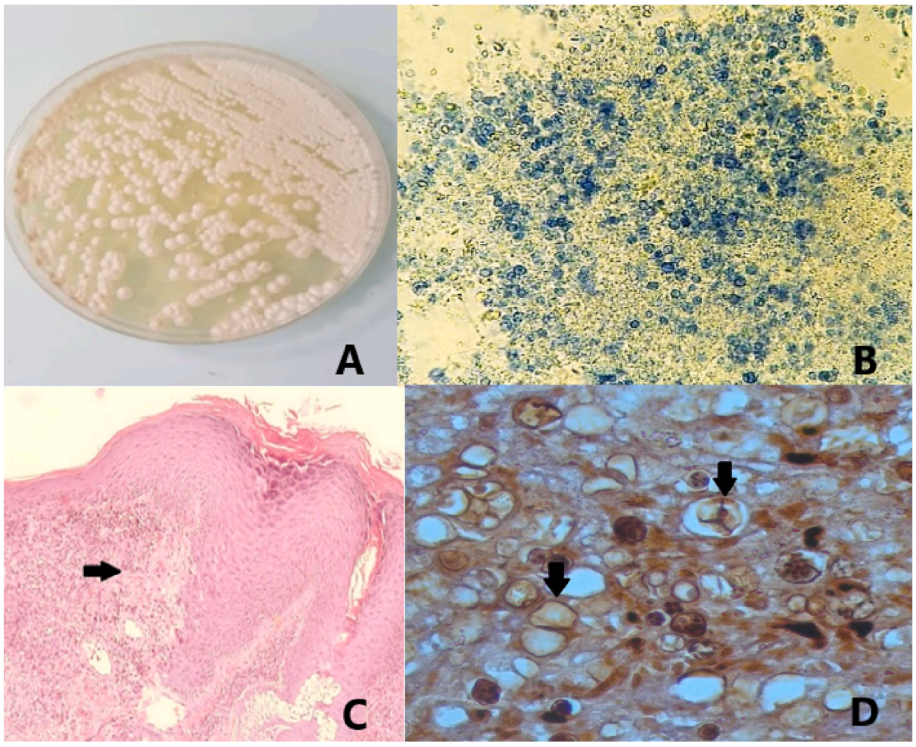

Fig. 2. A - *Prototheca* colony seeded on Sabouraud dextrose agar medium. B - Direct investigation under light microscopy, using KOH and permanent black Parker ink. C - Protothecosis. HE 20x. Skin section showing large thick-walled *Prototheca*, containing spores, intensely silvered. Note multiple Langerhans giant cells. Elsewhere, the epidermis is involved, showing hyperplasia. D - Tissue section, 40x, showing ovoid or spherical *Prototheca* with conspicuous walls that became intensely stained using the usual stains for fungi, i.e., Gömöri's methenamine and periodic acid-Schiff (PAS).

2. Case

A 75-year-old male, born in São José de Ribamar on the island of São Luís, state of Maranhão, Brazil, presented with a history of painful erythematous plaque, with itching in the left arm for 6 months. During the physical examination, an infiltrative plaque spreading to the entire length of the left upper limb was observed (D0) (Fig. 1A). The patient reported that one week before the onset of the condition, despite protection for his upper limb, he had been exposed to sewage material while repairing a clogged clay pipe in a house in which these clay pipes formed part of its sewage network. He noticed that he had suffered a small traumatic injury on his arm, which during the healing process evolved with an itchy hyperemic lesion measuring 2 × 2 cm, with progressive worsening.

After medical evaluation at a dermatological center, dermatophytosis was diagnosed based only on clinical characteristics. Treatment with terbinafine hydrochloride (250 mg once a day) was started. Because of the progressive worsening of the lesion, a skin biopsy was performed, with histopathological analysis. This histopathological examination showed lymphocytes, macrophages, neutrophils and, especially, giant cells, with epidermal hyperplasia. Grocott staining showed the presence of many spherical sporangia containing multiple endospores, either inside macrophages or free in the exudate, consistent with protothecosis (Fig. 2C and D).

Treatment with itraconazole, 400 mg/day, was started. However, after 16 days of use of this antifungal drug, the lesion was found to have spread throughout the patient's left upper limb (D+30) (Fig. 1B). A new skin biopsy was performed and direct mycological examination and cultures for aerobic bacteria, fungi and mycobacteria were performed on a skin fragment. There was growth of *Prototheca wickerhamii* on Sabouraud glucose agar, which was identified by means of the MALDI-TOF® BRUKER automated mass spectrometry system (Fig. 2A and B). This identification was confirmed by sequencing the ITS region (GenBank number MZ409514.1).

The isolate was revived by subculture on Sabouraud 4% dextrose agar (SDA, Difco Laboratories, Detroit, MI, USA) and incubated for

48–72 h at 37 °C. *In vitro* susceptibility testing of *P. wickerhamii* was performed, and Etest® strips (Biomerieux, Brazil) were used for MIC determination, showing MICs of 0.19, 0.5, 2.0, 256 μg/ml for amphotericin, itraconazole, voriconazole, and fluconazole, respectively.

Although the sensitivity test showed MIC of 0.5 μg/ml for itraconazole, the medical team chose to change the medication to liposomal amphotericin B (4 mg/kg/day), given the recrudescence of the lesions. After 45 days of treatment with this polyene (D+75), the treatment was changed to voriconazole (400 mg/day) for 6 months, which led to complete healing of the lesions. Monitoring of transaminase levels was performed every 7 days in the first month, followed every 15 days until completing 6 months, with no laboratory and clinical adverse events. After clinical cure had been achieved, no recurrence of the disease was detected over a 18-month follow-up (D+615) (Fig. 1C).

3. Discussion

Because of the rarity of protothecosis, an early clinical diagnosis is not usually achieved. Thus, patients tend to undergo several empirical types of treatment with antibacterial and antifungal agents. Our case report shows that the lesion pattern was initially suggestive of dermatophytosis, with unsuccessful use of terbinafine. In the absence of a clinical response through empirical therapy for diseases that initially do not require a microbiological routine for diagnosis (dermatophytosis), histopathological examination should not be postponed, to avoid rapid dissemination of the disease [2,3,6–8].

Protothecosis is a rare condition, but large number of cases have been described in patients with underlying immunosuppression or several underlying diseases. Systemic steroid use, diabetes mellitus, hematological or solid-tissue malignancy and solid-organ transplantation are the main factors related to increased risk of this disease [2,3,5–12]. Todd et al. reviewed 211 cases of protothecosis published between 1964 and 2017 and showed that 66 (31.2%) of these cases occurred in patients without any chronic condition or immunosuppression [2]. Our patient's case corroborates the information that even in immunocompetent individuals with some incidental disease, protothecosis should be considered in the differential diagnosis, especially when there is no clinical improvement through the antifungal drugs that are used for the most frequent mycoses [2,3,6,9].

In cases of incidental diseases (chromoblastomycosis, phaeohyphomycosis, mycetoma, lacaziosis, protothecosis etc.), a combination of microbiological and histopathological tools is recommended. In tissue, basophilic thick-walled nonbudding spherical bodies of different sizes are seen, especially through Gömöri's methenamine silver or PAS stains. Although the morphology of these bodies was characteristic of *Prototheca* spp., lack of typical endospores may sometimes lead to resemblance between *Prototheca* spp. and nonsporulating tissue cells of *Blastomyces* spp., *Cryptococcus* spp., *Paracoccidioides* spp., muriform cells of chromoblastomycosis or vesicle or yeast-like cells of phaeohyphomycosis agents. Therefore, isolation of the agent in culture media is mandatory for a correct and reliable diagnosis [2,3,7–9]. Our case report showed that a second skin biopsy was necessary for diagnostic confirmation, after the lack of therapeutic response with itraconazole. It is valid to suspect protothecosis in cases with a chronic inflammatory lesion of the skin, without a defined diagnosis, and/or no response to empirical treatment after 2 weeks [2,3].

Classically, identification of *Prototheca* spp. has been based on colony morphology, micromorphology and biochemical activity such as carbohydrate assimilation. However, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry proteomic analysis provides rapid identification of human and animal strains and it has enabled determination of the genotype of *P. zopfii* [1,2,13]. Molecular analysis is a useful tool for phylogenetic and epidemiological studies, and sequencing of ITS, the large subunit D1/D2 region and small subunit of rDNA is sufficient for this characterization [1,13]. In our case report, *Prototheca wickerhamii* was isolated and identified with good accuracy by means of MALDI-TOF, and was confirmed through genetic sequencing of the ITS region.

Despite the lack of specific interpretive criteria for *Prototheca* spp., the most effective drugs are polyenes (amphotericin B and nystatin) [1–3,14]. However, there is no clinical correlation between the MICs obtained through broth microdilution and the clinical response of patients. This was also observed in our case, in which the itraconazole susceptibility test showed a MIC of 0.5 μg/ml, but with a poor response to this antifungal [1,2,14]. In Brazil, itraconazole levels were not available due to the unavailability of this tool in the laboratory routine, representing a problem for monitoring the therapeutic efficacy and drug toxicity. Furthermore, it is important that the EUCAST and CLSI committees should promote standardization of *in vitro* susceptibility testing to assist clinicians in achieving optimal treatment, even if only preliminary epidemiological cutoff values are available [14].

In conclusion, it is important to highlight that protothecosis is mainly an incidental disease. It occurs in a significant number of immunocompetent patients without other risk factors. Isolation of the agent in culture media is an important tool when added to histopathological examination. Moreover, treatment with advanced-generation polyenes and azoles may be more effective.

Declaration of competing interest

The authors declare that is no conflit of interest regarding the publication of this paper.

Acknowledgements

The authors thank FAPEMA (Fundação de Amparo e Pesquisa do Maranhão) and colleagues who referred the patient to our service.

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