


Successful Treatment of Cutaneous Protothecosis Due to *Prototheca wickerhamii* with Terbinafine

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Abstract: Protothecosis, an infrequent human infection, is caused by achlorophyllic algae belonging to the genus *Prototheca*, particularly *Prototheca wickerhamii*. The skin stands as the most commonly affected organ. This report documents a case involving an 82-year-old male with Protothecosis. Histopathological analysis revealed granulomatous inflammation in the dermis, exhibiting necrotic features and hosting numerous non-budding spherical organisms. These organisms were positively stained using methenamine silver and periodic acid–Schiff stains, confirming identification as *P. wickerhamii* after validation through tissue culture and sequencing procedures. Initially, the patient received oral itraconazole at a dosage of 200 mg daily, accompanied by topical 1% naftifine-0.25% ketoconazole cream for a duration of 4 weeks, resulting in significant improvement. Subsequently, due to gastrointestinal discomfort presumably linked to itraconazole, terbinafine was administered. Over a span of 3 months, the patient received oral terbinafine at a dosage of 250 mg/day alongside the application of topical 1% naftifine-0.25% ketoconazole cream, leading to complete healing of the skin lesion, leaving behind a fibrotic scar.

Keywords: protothecosis, *Prototheca wickerhamii*, terbinafine

Introduction

Protothecosis represents a rare opportunistic human infection caused by the genus *Prototheca*, consisting of achlorophyllic algae widely present in nature.^{1,2} *Prototheca* species, including *P. ciferrii* (previously known as *P. zopfii* genotype 1), *P. bovis* (previously known as *P. zopfii* genotype 2), *P. wickerhamii*, *P. blaschkeae*, *P. cutis*, and *P. miyajii*,³ can cause infections in both humans and animals. While *Prototheca* species are commonly isolated from domestic animals, such as cattle, dogs, and cats, cases have also been reported in goats, horses, and other nondomestic animals, with *P. wickerhamii* being the primary cause of human infections.^{3–5} *Prototheca* algae have been found to be isolated from terrestrial sources, including soil, mud, and stream sediments, in regions characterized by a high organic matter content and moisture level.⁶ The prevailing belief is that *Prototheca* species infect humans either through direct contact with exposed areas or via traumatic inoculation of materials contaminated with the algae.^{1,7–10} This infection typically manifests in three primary forms: intra-cutaneous to subcutaneous nodules and plaques, olecranon bursitis, and systemic (disseminated) disease.¹¹ Notably, the skin emerges as the organ most frequently affected. Cutaneous infections commonly arise in immunocompetent patients, while disseminated infections predominantly impact immunocompromised individuals.^{1,11,12} Clinical management lacks a standardized protocol due to varying treatment efficacies.^{13,14} In this context, we present a case of cutaneous protothecosis effectively cured through a three-month course of terbinafine treatment.

Case Report

An 82-year-old man presented at the hospital with a 4-month history of skin lesions. Preceding these lesions, he experienced a stab wound on the dorsal side of his left forearm from firewood, promptly cleaned and dressed with an

antiseptic iodine solution. Subsequently, he noticed moderate itching and redness in the same area a few days later. Initially diagnosed with eczema, he received symptomatic treatment involving a potent corticosteroid cream containing clobetasol and antihistamine, which failed to control the rash. Instead, the affected area rapidly expanded to the flexor aspect of the left arm and the back of the left hand, accompanied by itching and pain. Concurrently, he received oral cefdinir, compound glycyrrhizin, and loratadine. The patient denied any prior medical history or conditions except hypertension. Dermatological examination revealed diffuse infiltrated erythematous and indurated plaques covering the dorsal side of the left forearm, the back of the left hand, and the flexor aspect of the left arm. Within the affected area, numerous insect bite-like papules, ulcers, papillae, pustules, atrophic scars, and scabs were observed (Figure 1a–d). There was no tenderness upon pressure, and axillary lymph nodes were impalpable. Laboratory tests indicated an elevated white blood cell count of $12.89 \times 10^9/L$ (normal, $3.5\text{--}9.5 \times 10^9/L$), decreased albumin at 34.7g/L (normal, $40\text{--}50\text{g/L}$), elevated urea nitrogen at 19.28mmol/L (normal, $1.7\text{--}8.3\text{mmol/L}$), and elevated total cholesterol levels at 7.57mmol/L (normal, $2.9\text{--}5.68\text{mmol/L}$) and 4.31mmol/L (normal, $<3.36\text{mmol/L}$). Blood sugar, urinalysis, ANA, and HIV tests showed no abnormalities.

Histopathological analysis depicted granulomatous inflammation with necrosis in the dermis, revealing abundant non-budding spherical structures observed under 100x magnification (Figure 2a). The application of Periodic acid-Schiff staining demonstrated thick spore cell walls with characteristic internal septations. Highlighted in Figure 2b were multiple sporangia containing endospores, displaying a morula appearance. Conversely, acid-fast staining yielded negative results (Figure 2c), while hexamine silver staining showcased positivity (Figure 2d).

Culturing the biopsy specimen on Sabouraud dextrose agar (SDA) at 30°C resulted in smooth, creamy white, yeast-like colonies after 48 hours (Figure 3a). Further microscopic examination stained with lactophenol cotton blue and fluorochrome revealed characteristic endospores scattered or enclosed in a sporangium (Figure 3b and c). All of the reagents were purchased from Sigma-Aldrich. Molecular biology identification was performed using PCR with fungal-specific universal primers. The TIANquick Yeast Genomic DNA Extraction Kit (W9317) was utilized to extract DNA from the saved strain. PCR was conducted using fungal-specific universal primers, namely NS3: 5'-GCAA GTCTGGCCAGCAGCC-3'; and NS8: 5'-TCCGC AGGTTACCTACGGA-3'. The amplification parameters were as follows: an initial denaturation at 94°C for 4 minutes, followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 49°C for 45 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The amplified DNA sequence showed a 92.63% sequence homology with *Prototheca wickerhamii* (GenBank Accession No. MG832940.1), as confirmed by MALDI-TOF analysis. In vitro susceptibility testing yielded minimum inhibitory concentration (MIC) values for various antifungals: amphotericin B (AMB) 0.5mg/L , itraconazole (ITR) 1.0mg/L , fluconazole (FCA) $>128\text{mg/L}$, voriconazole (VOR) 0.25mg/L , 5-flucytosine (5-FC) $>64\text{mg/L}$, caspofungin (CAS) $>8\text{mg/L}$, anidulafungin (ANI) $>8\text{mg/L}$, and posaconazole (POS) 1.0mg/L . Confirmation of antifungal susceptibility was achieved using the microdilution method according to Clinical and Laboratory Standards Institute (CLSI) standards, indicating MIC values of ITR 0.5mg/L , AMB 0.125mg/L , and terbinafine $>16\text{mg/L}$. Based on prior experiences, the patient received oral itraconazole at 200mg/day combined with 1% naftifine-0.25% ketoconazole cream for external application. After 4 weeks of treatment, the lesions significantly improved, with the erythematous lesions and patches halting their extension. While papillomatous hyperplasia, atrophic scars, and scabs remained, the pustules, insect-bite-like erosions, and ulcers healed. However, due to gastrointestinal discomfort from itraconazole, the treatment shifted to terbinafine at 250mg/day along with topical 1% naftifine-0.25% ketoconazole cream, considering the patient's age and compromised kidney function. No further side effects were reported thereafter. Over 3 months of treatment, the skin lesions continued to improve, resulting in wound healing and leaving behind only fibrotic scars (Figure 1e and f). Subsequent follow-ups of one and a half years showed no evidence of relapse.

Discussion

Protothecosis, an infrequently reported infection, stems from the *Prototheca* genus, achlorophyllous algae derived from common green algae like *Chlorella*. The pathogenesis remains largely elusive.^{1,2} These algae thrive in diverse environmental reservoirs such as water, grass, trees, animals, food items, and even household waste. Human protothecosis primarily results from *Prototheca wickerhamii*, causing localized infections in immunocompetent individuals, yet



Figure 1 Clinical photos of the patients. (a–d) Diffuse infiltrated erythematous plaques observed on the dorsal side of the left forearm and back of the left hand. These plaques are accompanied by multiple insect-bite-like erosions, ulcers, papules, pustules, atrophic scars, and scabs within the affected area. (e and f) Following a 3-month treatment of oral terbinafine at a dosage of 250 mg/day combined with topical 1% naftifine-0.25% ketoconazole cream, an improvement in skin lesions was observed. Fibrotic scars emerged on the flexor aspect of the left arm as the skin lesions evolved.

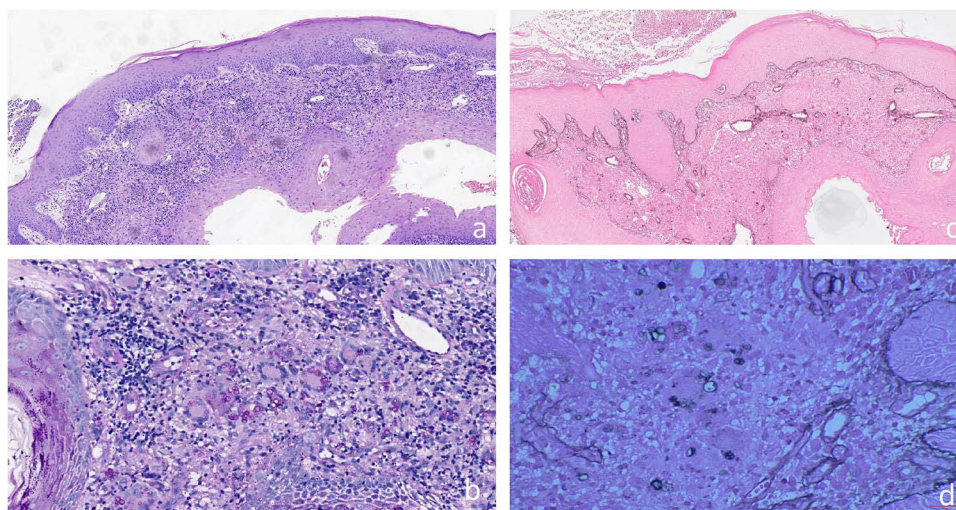


Figure 2 Histological Features of the Patient. (a) Pseudoepitheliomatous hyperplasia and granulomatous inflammation accompanied by localized necrosis and abundant inflammatory infiltration (H&E, magnification $\times 50$). (b) Numerous morula-like formations highlighted through Periodic Acid-Schiff staining (magnification $\times 200$). (c) Acid-fast stain: negative (magnification $\times 50$). (d) hexamine silver stains: positive (magnification $\times 200$).

dissemination is more prevalent in immunocompromised cases.¹⁵ This report details a case of cutaneous protothecosis induced by *Prototheca wickerhamii* in a non-immunocompromised male, notably lacking exposure to stagnant water or animal waste, with no significant medical history except for hypertension. His otherwise healthy status preceding lesion onset was disrupted by a stab injury on his left forearm, a route also posing risk factors for deep fungal diseases like sporotrichosis and chromoblastomycosis. Clinical symptom aggravation may be linked to a potent corticosteroid cream containing clobetasol.

Protothecosis presents with a heterogeneous, nonspecific array of skin lesions, documented as papules, pustules, plaques, nodules, ulcers, crusts, erosions, and various eczematous, herpetiform, granulomatous, and verrucous lesions.^{16,17} Our patient displayed insect bite-like erosions, ulcers, papillae, atrophic scars, and scabs diffusely covering an indurated erythematous area. Given the nonspecific clinical morphology, histopathological examination, aided by Periodic Acid Schiff and Grocott's modification of Gomori methenamine silver staining, remains crucial for diagnosis. Characteristic morulae structure and endosporulation, better highlighted by these stains, were evident in our case. Skin biopsies typically reveal pan-dermal granulomatous inflammatory infiltrates, occasionally accompanied by necrosis and pseudocarcinomatous epidermal hyperplasia.¹⁸ Organism cultivation and identification further support diagnosis.

Establishing a standardized treatment regimen for protothecosis remains elusive, with treatment varying on a case-by-case basis.¹⁴ Amphotericin B appears most effective against *Prototheca* spp., while azole antifungals, particularly itraconazole, are preferred for localized infections.^{19–21} Previous studies have indicated that AMB and most azoles exhibit activity against clinical isolates of *Prototheca* and primarily exert an algicidal effect. However, the activities of individual drugs vary significantly between different *Prototheca* species and among strains of the same species.²² Moreover, several studies have demonstrated the beneficial effects of blue light on pathogenic species of *Prototheca*.²³ Additionally, the study by Jagielski et al demonstrated that silver nanoparticles (AgNPs) and 3-Bromopyruvate also exhibited positive effects on pathogenic species of the *Prototheca* genus.^{24,25} In this case, our patient initially received oral itraconazole (200 mg/day) based on in vitro MIC results but had to discontinue due to side effects, despite significant lesion improvement. Subsequently, a 3-month terbinafine treatment successfully eradicated the infection. While in vitro susceptibility tests suggested *Prototheca wickerhamii*'s resistance to terbinafine, contradicting clinical efficacy, our patient exhibited gradual wound healing and resolved itching and pain with terbinafine (250 mg/day). The discordance between in vitro susceptibility and clinical outcomes challenges current understanding, with no established guidelines for testing this genus.²⁶ Antibacterial agents like amikacin, tetracycline, and gentamicin have shown some efficacy in vitro.^{1,26} However, diverse susceptibility profiles in *Prototheca* species limit establishing direct correlations between in vitro activity and clinical responses.²⁷ As of March 2021, a total of 269 human *Prototheca* infections have been reported since the first case in

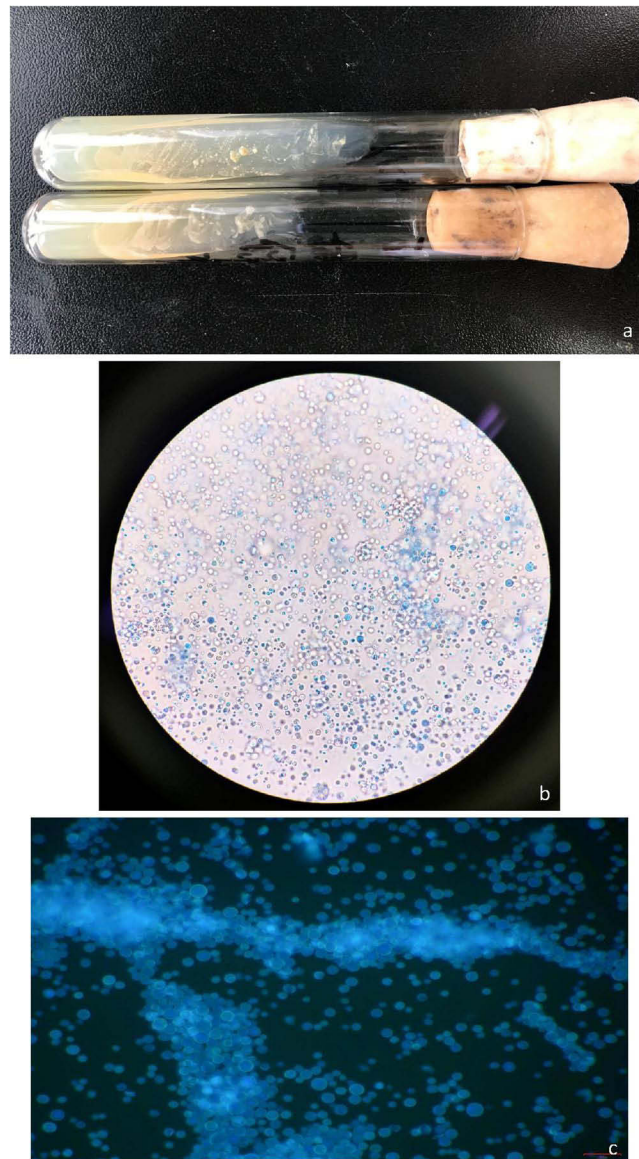


Figure 3 Colony Growth and Characteristics of *Prototheca wickerhamii* under Microscopy. (a) Smooth, creamy, white, and yeast-like colonies observed on SDA culture after 48 hours of incubation at 30°C. The characteristic endospore-containing sporangium of *Prototheca wickerhamii*. (b) Wet preparations stained with lactophenol cotton blue and (c) fluorochrome revealing the distinction from yeast due to the absence of budding.

a barefoot Sierra Leone rice farmer's foot in 1964.^{28–32} Routine in vitro antifungal susceptibility testing for protothecosis is not recommended unless empirical therapy fails.

Conclusions

The correlation between in vitro susceptibility and clinical outcomes, as well as established in vitro antimicrobial breakpoints, remains undefined. While itraconazole and AMB typically serve as the primary treatment options for cutaneous protothecosis, our evidence suggests that terbinafine could serve as a viable alternative in specific situations for treating this condition.

Data Sharing Statement

All data used in this work are publicly available.

Ethical Approval

The ethical committee of the hospital gave the agreement to report this case.

Consent Statement

A formal written consent was obtained for publication the case details and associated images from the patient.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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