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Case Report

Cutaneous Infection with *Prototheca wickerhamii* Treated Successfully with Voriconazole

Hsun Chang [a], Yu-Hung Wu [b,c], Chia-Chin Chiang [d], Yu-Ting Tina Wang [d], Kam-Hang Leong [d], Shin-Yi Tsai [b,d,e,f,g] *

[a] *Division of Infectious Diseases, Department of Internal Medicine, MacKay Memorial Hospital, Taipei, Taiwan,* [b] *Department of Medicine, MacKay Medical College, New Taipei City, Taiwan,* [c] *Department of Dermatology, MacKay Memorial Hospital, Taipei, Taiwan,* [d] *Department of Laboratory Medicine, MacKay Memorial Hospital, Taipei, Taiwan,* [e] *Institute of Long-Term Care, MacKay Medical College, New Taipei City, Taiwan,* [f] *Institute of Biomedical Sciences, MacKay Medical College, New Taipei City, Taiwan,* [g] *Department of Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, 21205, USA*

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SUMMARY

Human protothecosis is a rare disease that predominantly occurs in immunocompromised patients. Here, we present a case of a 73-year-old man with cutaneous protothecosis. The patient had multiple comorbidities and dermal exposure to herbs. Conventional and molecular diagnostic techniques revealed that the causative agent of protothecosis in this patient was *Prototheca wickerhamii*. Currently, no standard treatment is available for protothecosis. The patient achieved clinical improvement through voriconazole treatment.

1. Introduction

Prototheca is a genus consisting of ubiquitous algae, but it also includes species that cause diseases in humans. The clinical manifestations of protothecosis are often nonspecific and varied. Nevertheless, the conventional diagnostic techniques available for *Prototheca* species identification are time-consuming and require experienced laboratory personnel. Here, we present a case in which both conventional and molecular diagnostic techniques were used for diagnosis.

2. Case report

A 73-year-old man with a history of hypertension, chronic hepatitis C infection, peptic ulcer disease, arrhythmia, benign prostatic hyperplasia, and obstructive chronic bronchitis presented to the dermatological outpatient department of our hospital with progressive bilateral forearm erythema, pruritus, pain, and a burning sensation with skin erosion for 4 weeks. Upon examination, the predominant cutaneous symptoms were vesiculobullous and ulcerative lesions with purulent exudates (Figure 1A and B). The patient was not taking any immunosuppressants. Furthermore, he denied having received an insect bite, experiencing trauma, drinking water from a contaminated source, or having contact with chemical irritants. He stayed at home most of the time and did not have any travel history. However, his daughter stated that he had applied an unknown herb over his bilateral forearms just before this episode.

* Corresponding author. Department of Laboratory Medicine, MacKay Memorial Hospital, Taipei City, Taiwan; Department of Medicine, Institute of Biomedical Sciences, Institute of Long-Term Care, MacKay Medical College, New Taipei City, Taiwan; Department of Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA.
E-mail address: stsai22@jhu.edu; tsaisy@mmc.edu.tw (S.-Y. Tsai)

At the dermatological outpatient department, skin biopsy was performed, which revealed folliculitis with acute and chronic dermatitis, tissue necrosis, and a granulomatous reaction. Periodic acid-Schiff-stain-positive round organisms were found in the dermis, ranging from 8 to 30 μm in diameter. Furthermore, sporangia with endospores in a morula-like pattern were noted (Figure 2A). On the blood agar culture plate, yeast-like colonies were noted within 72 hours (Figure 2B). Large Gram-positive spherical cells of various sizes resembling yeast were seen with Gram staining at 1000× magnification (Figure 2C). Examination of a wet mount with lactophenol cotton blue staining showed morula forms at 400× magnification (Figure 2D). The organisms were weakly positive for Alcian blue stains (pH 2.5) and negative for melanin and acid-fast stains. Two subsequent cultures revealed the presence of *Prototheca wickerhamii*. In

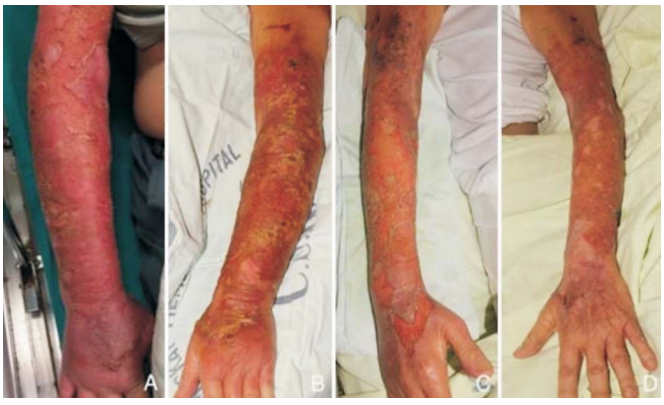

Figure 1. Bilateral forearm at presentation. A and B: Right and left arm with skin lesions consisted of vesiculobullous and ulcerative skin erosion with severe blisters and purulent exudates. C and D: Improvement with tissue regrowth and scar formation on right and left arm.

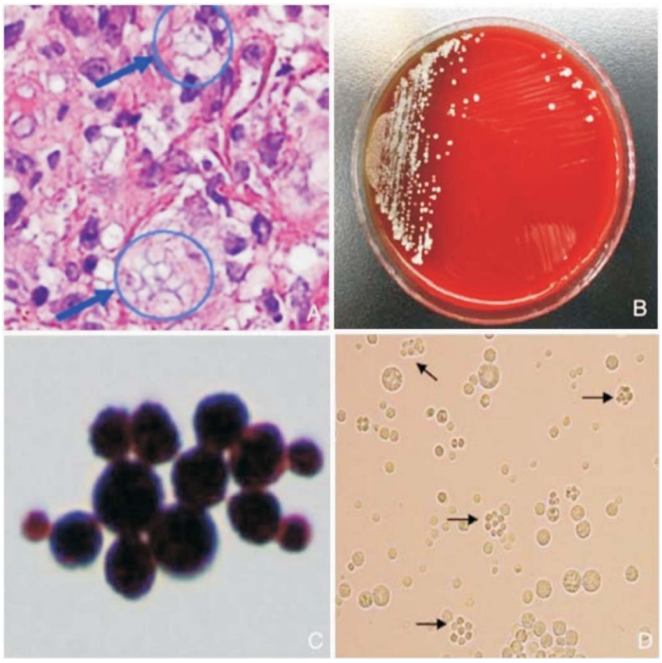

Figure 2. Microbiological investigation in conventional methods. A: Sporangia with endospores in a morula-like pattern are noted in histo-pathology slide. B: Blood agar plate: Yeast-like colonies were noted within 72 hours. C: Large Gram-positive spherical cells of varied sizes resembling yeast were seen on Gram stain at 1000×. D: Examination with a wet mount with lactophenol cotton blue staining at 400×. Morula forms were noted (arrows).

addition to conventional diagnostic techniques, we used the Vitek 2 system and further confirmed the results through nucleotide sequencing of the D1/D2 domain of the 26S/28S rDNA region.

Subsequently, oral tetracycline 250 mg Q6H was administered for a week. However, the erosion worsened. The patient was shifted to the emergency room because of fever flare-ups, reaching 38.3 °C. Laboratory data showed no leukocytosis but bandemia. The basic biochemistry profile revealed bilirubinemia and increased liver enzymes compared with baseline. Under the impression of bilateral forearm cellulitis caused by *P. wickerhamii*, the patient was hospitalized. Voriconazole 200 mg Q12H and adequate wound care with silver sulfadiazine were provided. Although cellulitis symptoms showed improvement, a pneumonia episode complicated with impending respiratory failure developed. He was then transferred to the intensive care unit. Empiric antibiotics with meropenem and teicoplanin were administered. In the following days, sputum culture revealed carbapenem-resistant *Acinetobacter baumannii*, and hence, we switched antibiotics according to the susceptibility profile. The patient was transferred to the general ward after his condition stabilized. Voriconazole was discontinued on the 32nd day because cellulitis had improved (Figure 1C and D). Follow-up at our infectious disease outpatient clinic after discharge revealed that he was free of dermatological symptoms.

3. Discussion

Protothecosis is a rare, opportunistic infectious disease caused by algae belonging to the genus *Prototheca*. *Prototheca* species can be isolated from decomposing plants, water, soil, animal waste, sewage, and even human fingernails, skin, and respiratory and gastrointestinal systems.[1] Through cytochrome b sequence (*cytb*) analysis, the genus *Prototheca* are divided into two main lineages. Some species are mostly associated with bovine diseases, such as *P. ciferrii*, *P. blaschkeae* and *P. bovis*. The others cause human diseases, including *P. wickerhamii*, *P. cutis* and *P. miyajii*.[2] At least 14 species were identified in this genus.[3,4] Humans are infected through exposure to a *Prototheca*-contaminated environment or traumatic inoculation. The incubation period, suspected to range from a few weeks to months, is still unclear because most patients are unaware of when they experienced trauma or contact with *P. wickerhamii*. Furthermore, occupational activities, such as farming, fishing, handling raw seafood, and working in an aquarium, are risk factors for cutaneous protothecosis. During these activities, lesions commonly occur on exposed areas.[5]

Cutaneous and subcutaneous infections, or rarely, disseminated infection occur in immunosuppressive and immunocompetent patients, but mostly occur in patients with immunosuppressive disorders. Our patient with chronic hepatitis C virus infection, hypertension, and no other malignancy records or chronic steroid use presented with the cutaneous form of the infection. Because he denied being exposed to most of the possible previously mentioned transmission routes, we suspected his infection was due to the unknown herb dressing. Protothecosis is chronic inflammation with various, unspecific manifestations attributable to delayed diagnosis or misdiagnosis. Our patient presented with cutaneous protothecosis with erythematous patches on a purpuric base and superficial ulcerations, which may easily be misdiagnosed as eczema[6] and thus further treated with a topical steroid. Cutaneous protothecosis can manifest as diffuse erythema, hypopigmentation, nodules, papules, ulcers, verrucous lesions, or even vesiculobullous and purulent exudates. In disseminated disease, internal organs such as the gut, peritoneum, blood, and spleen can be involved.[1]

Skin biopsy can yield hyperkeratosis; focal para-keratosis; pseudo-epithelialization; and granulomatous inflammatory response with infiltrating lymphocytes, neutrophils, eosinophils, and multinucleated giant cells. Necrotic areas are common but not necessary to make the diagnosis. In addition to tissue necrosis and granulomatous reaction, folliculitis was observed in our case. Usually, organisms infiltrate the mid-dermisand papillary dermis, only rarely infiltrating the corneum of the epidermis.[7] Through periodic acid-Schiff, Gridley fungus, Gomori methenamine silver, or Alcian blue stain, the characteristic morula appearance of *P. wickerhamii* can be highlighted.[8] If morula do not present, the results can be misleading, and inexperienced medical personnel might diagnose the organism as a yeast, particularly *Candida* species or adimorphic fungus, such as *Histoplasma capsulatum* var. *duboisii*, *Blastomyces dermatitidis*, or *Paracoccidioides brasiliensis*.[1] However, the ribostamycin inhibition test can be used to differentiate *Prototheca* species from *Candida* species.[9] The culture obtained from our case exhibited smooth, creamy, tiny colonies on the blood agar plate, chocolate agar, and Sabouraud dextrose agar after incubation at 35 °C for 2 days. Moreover, *Prototheca* species present as creamy, white, and yeast-like colonies on MacConkey agars and eosin-methylene blue and Tween 80 medium at 30 °C–37 °C.[7] A wet mount stained with lactophenol cotton blue or calcofluor white can reveal the morula-like structure. In some equivocal results, the absence of chloroplasts under an electron microscope might help to identify *Prototheca* species. A disadvantage in conventional microbiological investigations is that the time-consuming process requires well-trained staff for accurate results. Commercial systems, including the API 20C, Vitek 2, RapID Yeast Plus test, or Vitek MS, can help to identify *Prototheca* species. However, only *P. wickerhamii* was included in the API 20C and Vitek databases.[8] Nucleotide sequencing of the D1/D2 domain of the 26S/28S rDNA region was used to identify *P. wickerhamii* in our case and in several other cases.[10] One study published in 2013 revealed that the sequencing of the ITS region can help to

differentiate *P. wickerhamii* from other *Prototheca* species because *P. wickerhamii* has a relatively large ITS.[11] Jagielski et al. proposed using the mitochondrial *cytb* gene as a robust marker, which became the new classification system of the *Prototheca* genus through molecular taxonomy.[2–4]

The mechanism by which algae infect humans remains unknown, and currently, a standardized treatment protocol for protothecosis is lacking. *P. wickerhamii* is susceptible to polyene and azoles due to the 4% ergosterol in its membrane.[8] Treatment has varied from case to case, depending on the disease severity and immunocompromised status of patients. A review of 160 cases from the literature suggested that itraconazole or fluconazole can be the initial treatment for patients with mild infections, leaving amphotericin-B for serious infections or for infections that have not responded to azole treatment.[12] A 2018 *Medical Mycology* review compared common treatments, including topical, intravenous antifungals, and surgical excision, and suggested that it may be difficult to claim any treatment as having a statistically significant clinical effect due to the rarity of the infection.[13]

In conclusion, human protothecosis is most often observed in immunocompromised patients. However, it can also occur in people without strong evidence of having been exposed. To avoid misdiagnosis, physicians and clinical scientists should be aware of the clinical manifestations and microscopic features of *Prototheca* species.

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Conflict of interest

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

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