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BRIEF REPORT

Prototheca Infection: A Descriptive Study

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Prototheca is a microalgae known to cause infections in humans, with protothecosis most commonly presenting as olecranon bursitis or localized soft tissue infection. Disseminated disease can be seen in immunocompromised patients. In this retrospective single-institution case series, we describe our experience with 7 patients with *Prototheca* infections.

Keywords. *Prototheca*; protothecosis.

Protothecosis is a rare infectious disease caused by nonphotosynthetic algae belonging to the genus *Prototheca*, which is closely related to the green algae genus *Chlorella* [1–4]. Originally classified as fungi given their yeast-like morphology, *Prototheca* were later reclassified as algae based on shared reproductive properties with *Chlorella* [4]. *Prototheca* spp. are achlorophyllous obligate heterotrophs present in the environment (rivers, ponds, mud, sewage), with some of them identified as opportunistic pathogens in vertebrates [1, 2, 4]. There are several species known to cause infection in mammals, with *Prototheca wickerhamii* being the most common in humans, followed by *P. zopfii,* and more rarely *P. cutis* and *P. miyajii* [1, 3–6]. In humans, protothecosis presents as 3 syndromes: skin or soft tissue infection, olecranon bursitis, or disseminated infection [1, 2, 4]. Soft tissue infections and olecranon bursitis are typically seen in immunocompetent patients, with infection often caused by traumatic inoculation [2, 4]. In immunocompromised hosts, disseminated infections have been reported with high mortality [1, 7]. The pathogenicity of *Prototheca* spp. may in part be due to their ability to create biofilms, yet there is still much about their pathogenesis that is not known [1]. Moreover, given its rarity in clinical practice, protothecosis is an often unknown or underrecognized disease, resulting in misdiagnosis or delayed diagnosis [1, 7]. Treatment typically involves surgery along with antifungal therapies, but the optimal agents and treatment course are not known [1]. Two classes of antifungal medications, polyenes and azoles, have been used to treat protothecosis, as they have shown both in vitro and in vivo algicidal activity [8, 9]. In this case series, we sought to evaluate our institution's experience with protothecosis and describe presenting characteristics, treatment, and outcomes.

METHODS

We performed a case series of patients diagnosed with protothecosis at Mayo Clinic sites in Arizona, Florida, and Minnesota from January 1998 through August 2021. Inclusion criteria were culture growth of a *Prototheca* species from a clinical specimen accompanied by signs, symptoms, and/or radiographic abnormalities consistent with protothecosis. Patients without research authorization were excluded per Minnesota state statute.

Patients were identified from our institution's microbiology database by cultures yielding growth of a *Prototheca* species and manually screened for inclusion. Data from included patients were manually abstracted from the electronic medical record. Abstracted data included patient demographics, infection-specific characteristics, treatment characteristics, and outcomes including mortality and infection recurrence. Descriptive statistics were utilized.

Patient Consent

This study was reviewed by our institution's institutional review board and granted an exempt status and waiver for informed consent (IRB#21–007789).

RESULTS

Our search yielded 7 patients with infection caused by *Prototheca* spp. (Table 1). Five of the 7 patients were identified to have infections with *P. wickerhamii*, 1 identified as *P. miyajii*, and 1 patient's isolate was not identified to the species level [6]. Five (71%) patients were male, with an average age of 63 years. Six patients were White, while ethnicity information for 1 patient was unavailable. Four of 7 patients were immunocompetent (Cases 1, 2, 4, and 7). These patients presented with olecranon bursitis or soft tissue infections. Case 5, while he did have evidence of immunosuppression on presentation, also had a localized soft tissue infection. Cases 3 and 6 were immunocompromised. Case 3 developed a localized deep

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Table 1. Patient Characteristics

Case	Sex[a]	Ethnicity[b]	Age[c]	Exposure	Syndrome	Species	Surgery	Antimicrobial Therapy	Treatment Length[d]	Treatment Delay[e]	Time to Last Follow-up[f]	Coinfection	Comorbidities	Immuno-suppression
1	M	W	45	Trauma	Olecranon bursitis	*P. miyajji*	Yes	Voriconazole 200 mg 3 times daily → twice daily	44	3	2	*Bacillus* spp. felt to be contaminant	None	None
2	M	W	49	Trauma	Olecranon bursitis	*P. wickerhamii*	Yes	Fluconazole 400 mg daily	14	4	5	Methicillin-resistant *staphylococcus aureus* (MRSA)	60 pack-year smoking history	None
3	F	W	63	Prior surgery	Abscess and osteomyelitis of hip	*P. wickerhamii*	Yes	Fluconazole 200 mg daily → itraconazole 200 mg twice daily	98	0	19	Group B *streptococci*, *Bacteroides fragilis*, MRSA, *Veillonella* spp. grew from hip	Bullous pemphigoid, alcohol-related liver disease, chronic kidney disease	Yes
4	M	W	67	Trauma	Olecranon bursitis	*P. wickerhamii*	Yes	Itraconazole 200 mg twice daily	18	6	264	None	Coronary artery disease, former smoker, hyperlipidemia	None
5	M	W	58	Trauma	Tenosynovitis (wrist)	Not identified to species level	Yes	Fluconazole 800 mg daily	561	1	1[g]	None	HIV/AIDS	Yes
6	M	Unknown	91	Indwelling lines, intubation	Septic shock	*P. wickerhamii*	N/A	IV amphotericin B 450 mg → 650 mg daily	24	0	1	Many (candidemia, multidrug-resistant *E. coli* bacteremia, *Stenotrophomonas* pneumonia)	Abdominal aortic pseudoaneurysm, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, chronic myelomonocytic leukemia, severe aortic stenosis	Yes
7	F	W	65	Uncertain (possibly gardening)	Tenosynovitis (fingers)	*P. wickerhamii*	Yes	Fluconazole 200 mg daily→ itraconazole 200 mg daily	52	6	257	None	Hashimoto's thyroiditis, hypertension	None

[a]M = male, F = female.

[b]W = White.

[c]Age at time of diagnosis.

[d]In days.

[e]In months.

[f]In months.

[g]Last follow-up in our clinic. Seen by local provider at around 46 months.

periprosthetic joint infection with *Prototheca* spp. and multiple bacterial coinfections. Case 3 had successful treatment of protothecosis but unfortunately continued to have complications from chronic joint infection and died over a year and a half later from complications of bacterial sepsis. Case 6 had mixed septic and cardiogenic shock in the setting of acute respiratory distress syndrome due to large volume aspiration. He required life support with extracorporeal membrane oxygenation (ECMO). One month into his admission in the intensive care unit, he developed a bloodstream infection with *P. wickerhamii*, with the source suspected as either central line or pulmonary. Although *Prototheca* cleared from his bloodstream, the patient died several weeks later when family elected to transition him to comfort measures in the setting of prolonged critical illness.

Diagnosis of *Prototheca* infection was determined by blood (Case 6) and/or body fluid or tissue culture (all cases). In 1 example, blood from Case 6 was cultured on mold-inhibitory agar plates and grew multiple yeast-like colonies (Figure 1A). The characteristic "spoke-and-wheel" sporangia were then seen on wet slide prep with lactophenol cotton blue stain (Figure 1B), as well as wet mount with calcofluor white stain (Figure 1C). Ultimately, 16S rRNA gene sequencing was used to identify the organism to the species level as *P. wickerhamii*. In other cases, histopathology was also used to diagnose protothecosis. In 4 patients, tissue sent for histopathologic examination showed necrotizing granulomatous inflammation with organisms morphologically consistent with *Prototheca*. Case 3, who had a polymicrobial infection including *Prototheca*, showed only acute inflammation on synovial biopsy without organisms seen microscopically.

The 6 patients with skin, soft tissue, or joint infections had surgery for debridement and irrigation of the infected site. These patients were all treated with azole therapy, most commonly fluconazole, although voriconazole and itraconazole were also used. These patients recovered from their infections without reported evidence of recurrence. No patients died from their *Prototheca* infections. The average length of treatment in days for all patients was 115 days, with the shortest time course being 14 days and the longest 561 days. Patient 5, who had the longest course of therapy with 561 days of fluconazole, initially presented with subacute right hemiparesis and right wrist pain. He was diagnosed with wrist tenosynovitis, and tissue cultures grew *Prototheca*. With regards to his new weakness, there was concern for progressive multifocal leukoencephalopathy, but disseminated *Prototheca* infection was considered. While there was no definitive evidence of disseminated infection, he continued fluconazole for this possibility and was lost to follow-up after 561 days of therapy. If Case 5 is excluded, the average duration of treatment in days for our cases was 42.

Susceptibility results were available for 3 patients. The ranges of minimum inhibitory concentrations (MICs) were as follows: fluconazole 8–64 mcg/mL, isavuconazole 0.125 mcg/mL, itraconazole 0.5–2 mcg/mL, voriconazole 0.5 µg/mL, posaconazole 0.5 µg/mL, 5-fluorocytosine 64 mcg/mL, amphotericin B 0.125–0.6 µg/mL, and caspofungin 8 mcg/mL. Despite having a high MIC, fluconazole was successfully used to treat localized infections in Cases 5 and 7, with Case 7 transitioning to itraconazole due to side effects. Patient 6, who had evidence of disseminated protothecosis, was treated with amphotericin B, which had the lowest MIC of the antifungals tested.

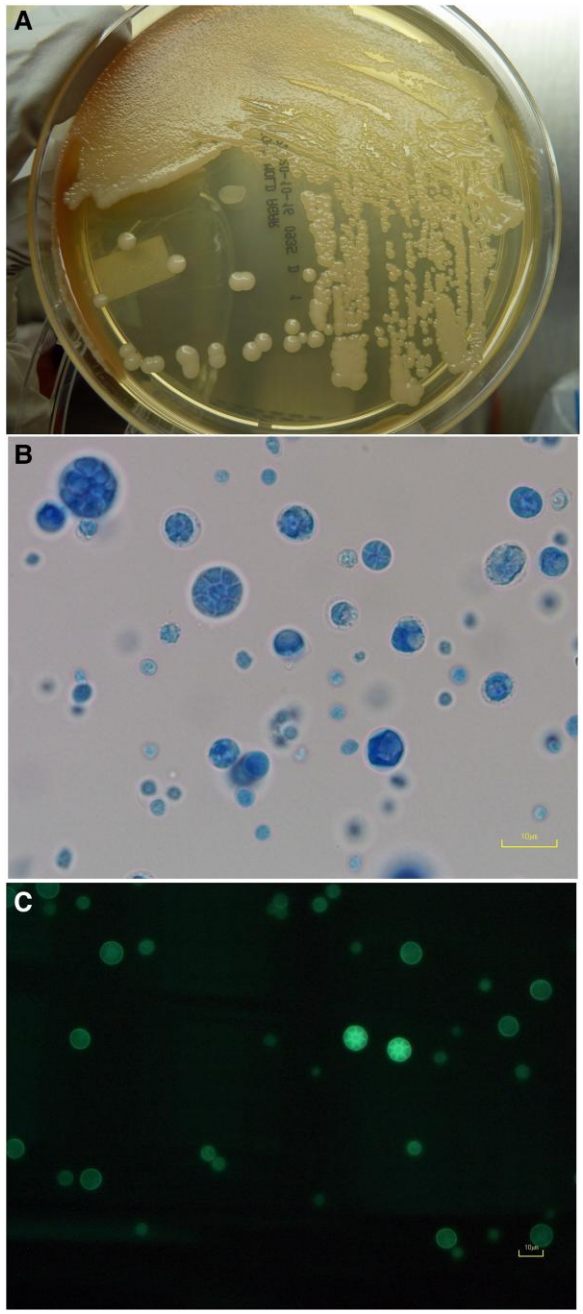

Figure 1. A, Many yeast-like colonies growing on mold inhibitory agar after 3 days (Case 6). B, Numerous spherical daisy-like or spoke-in-wheel–shaped sporangia consistent with *P. wickerhamii* (lactophenol cotton blue stain, wet slide prep 100×; Case 6). C, Characteristic *P. wickerhamii* sporangia (wet mount, calcofluor white stain, 100×; Case 6).

DISCUSSION

We describe 7 patients with protothecosis who presented with a range of pathology. *Prototheca* infections have been classified as cutaneous lesions, olecranon bursitis, or disseminated infections [2, 4]. Among our patients, we had 3 cases of olecranon bursitis, 2 cutaneous lesions resulting in tenosynovitis, 1 periprosthetic joint infection, and 1 systemic infection.

It has been observed that patients with neutropenia are at increased risk of severe infection due to *Prototheca*, with studies suggesting that neutrophils play a key role in host immune defense against the organism [1, 2, 4, 7, 10, 11]. None of our patients, where data was available, had evidence of neutropenia. On the other hand, there have been few reports of patients with HIV/AIDS developing protothecosis, often with localized infections, suggesting that T cells may play less of a role [1, 2, 10, 12, 13]. Case 5, who had HIV/AIDS with a CD4 count of 135, developed a local infection, likely a result of a traumatic inoculation.

Case 6 is an unusual presentation of a systemic *Prototheca* infection, in which he was found to first have growth of *P. wickerhamii* on bronchial washings (first identified 10 days after initial collection). He subsequently developed bloodstream infection with *P. wickerhamii* 13 days later from a long-term central line. ECMO circuit lines, arterial line, and peripheral blood cultures also grew *P. wickerhamii* in the following days. At the time, the patient was receiving anidulafungin and transitioned to amphotericin B. It seems plausible that the patient developed a ventilator-associated *Prototheca* infection or a line-associated infection. Blood cultures were not collected on the same day as bronchial washings, so it is not clear when bloodstream infection first developed. There are other cases of pulmonary *Prototheca* infections reported, both in immunocompromised patients [14, 15]. These cases demonstrate that severe or disseminated infections with *Prototheca* are seen in acutely ill patients with other underlying infections or immunocompromised states.

Diagnosis of protothecosis can be challenging, especially when not clinically suspected. Tissue or blood culture is essential to diagnosis, with both microbiological and histopathological techniques used to identify the organism [2, 4]. *Prototheca* spp. can grow on blood agar plates and fungal media without cycloheximide, appearing as white or cream-colored yeast-like colonies [6]. On wet slide preparations or histopathologic stains, *Prototheca* sporangia appear as spherical organisms measuring 3–30 μm in size, with a prominent wall and theca [2, 6]. They contain many endospores arranged in a symmetric daisy-like pattern, as seen in *P. wickerhamii*, or asymmetrical morula-like structures, as seen in *P. zopfii* [2, 4, 6]. Once isolated from culture, *Prototheca* spp. can be identified with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry or molecular techniques like DNA sequencing of the ribosomal internal transcribed spacer, D2 targets of the large-subunit ribosomal DNA, or 16S ribosomal RNA gene [3, 4, 6]. Identification by sequencing of mitochondrial DNA *ctyb* gene has been proposed as the "new gold standard" in diagnostics of protothecal infections [3]. *Prototheca* spp. stain well with Gridley fungus stain, Grocott's modification of Gomori methenamine silver, or periodic-acid-Schiff stain, but not so well with hematoxylin and eosin–stained smears [2, 4].

There is no standardized therapy for *Prototheca* infections, and treatment choice and duration should be chosen based on infection site and severity and the patient's comorbid conditions. Amphotericin B and azoles, which are antifungal medications with algacidal activity, are usually administered empirically to treat *Prototheca* infections [4, 8, 9]. Among the azoles, in vitro algacidal efficacy based on MIC varies [8, 9]. Susceptibility testing may be helpful to guide treatment choice, when available. However, it is not necessary, and MIC data do not always predict clinical success [2]. Following surgery, 6 of 7 cases presented here were treated successfully with azole therapy. Treatment duration was determined by evidence of healing postoperatively, with both surgical and infectious diseases teams involved in monitoring patients' progress. However, a treatment course as short as 14 days was adequate, and patients with protothecosis may not require prolonged courses of therapy. Case 6, our only patient with systemic infection, was managed with amphotericin B due to the severity of his infection.

CONCLUSIONS

Prototheca spp. are an underrecognized group of algae that can cause infection in both immunocompetent and immunocompromised hosts. Time to diagnosis and treatment can be delayed by misdiagnosis. Many of our cases were initially misdiagnosed and saw a delay in treatment time of an average of 4 months. Multidisciplinary management is important for effective therapy and follow-up of these patients to ensure resolution of infection. In our experience, patients with localized infection had successful treatment outcomes following surgery and antimicrobial therapy. Together with our case series, other institutions' experiences with protothecosis will be helpful in developing a broader understanding of this rare disease.

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