

## CASE REPORTS

### *Prototheca wickerhamii* Mimicking Yeast: a Cautionary Tale<sup>∇</sup>

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***Prototheca* spp. are environmental algae that may cause serious infection in the immunocompromised patient. Clinical manifestations may mimic other diseases. We present a case of fatal infection in a 78-year-old cardiac transplant recipient and discuss pitfalls in the clinical and laboratory diagnoses.**

#### CASE REPORT

A 78-year-old woman with a history of cardiac transplantation 15 years earlier presented for routine review in the transplantation clinic in June 2010. She reported shortness of breath, cough, and increased sputum production. On examination, she was afebrile with right basal crepitations. Right lower zone infiltrates were noted on her chest but had been observed at a previous visit. The patient's comorbidities included hypothyroidism, bronchiectasis, type 2 diabetes mellitus, atrial flutter, chronic renal impairment, and chronic back pain. Her current immunosuppressive agents included 10 mg of cyclosporine twice daily, 500 mg of mycophenolate mofetil twice daily, and 7.5 mg of prednisolone daily. Other medications included inhaled fluticasone-salmeterol, insulin, ferrous sulfate, and omeprazole.

The patient was admitted to the hospital for intravenous (IV) antibiotic therapy (ticarcillin-clavulanate), chest physiotherapy, and IV immunoglobulin. Initially, there was some improvement in the respiratory symptoms, and the patient remained afebrile. Pedal edema and pleural effusions developed and were initially attributed to cardiac failure. Her antibiotic therapy was changed to cefepime due to concern about the possible exacerbation of fluid overload caused by the sodium component of ticarcillin-clavulanate. An echocardiogram, however, demonstrated normal systolic function, and there was no evidence of rejection on myocardial biopsy specimens.

Subsequently the patient reported pain in her fingers, which she had first noted 1 month prior to admission to the hospital. Her uric acid level was mildly elevated at 0.51 mmol/liter (0.20 to 0.40). Finger swelling was noted at the first metacarpophalangeal (MCP) joint of the right hand. A consulting rheumatologist examined her on day 10 of hospital admission and

recommended increasing the prednisolone dose to 15 mg daily and commencing low-dose colchicine. The colchicine was subsequently ceased after 2 doses.

Three days later, the patient became acutely confused. A single elevated temperature of 37.8°C was recorded. Computed tomography (CT) scanning of the patient's head was unremarkable, and the C-reactive protein (CRP) level was 20 mg/liter. Blood and urine cultures were ordered, and the antimicrobial therapy was changed from cefepime to ciprofloxacin and metronidazole. At this point, the patient developed new skin lesions on her fourth and fifth fingers and bipedal and arm edema (Fig. 1C). On examination, her jugular venous pressure was not raised and she had a normal albumin level (39 g/liter). She was examined by both a dermatologist and rheumatologist, who suspected gout or vasculitis and recommended increasing the prednisolone and restarting colchicine. The latter, however, was not done.

Two sequential blood cultures from this time (days 14 and 15 of admission) were reported to be growing identical yeast-like organisms at 48 h after collection. The microbiology laboratory reported the isolate as "non-*albicans* *Candida* spp.," with further identification to follow. The laboratory was unable to further identify the isolate, and at this stage, the treating team was told (verbally) that it was likely to be an uncommon, possibly environmental yeast. Antifungal susceptibilities were performed by broth microdilution using the Sensititre Yeast-One Test Panel (Trek Diagnostic Systems). The MICs for fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole, caspofungin, 5-fluorocytosine, and amphotericin B were 128, 1, 1, 0.5, 1, 16, 64, and 0.25 mg/liter, respectively. Initially, the patient was not given specific treatment for these isolates by the primary care team, and a subsequent blood culture yielded no organisms.

At this time, urine culture grew vancomycin-resistant *Enterococcus*, and the patient was treated with nitrofurantoin. She remained, however, intermittently confused but afebrile, with persistent edema of her extremities and ongoing nausea and vomiting. Cefazolin was commenced for suspected cellulitis of the extremities. On day 27 of admission, the treating team commenced 200 mg of itraconazole twice daily. On days 36 and

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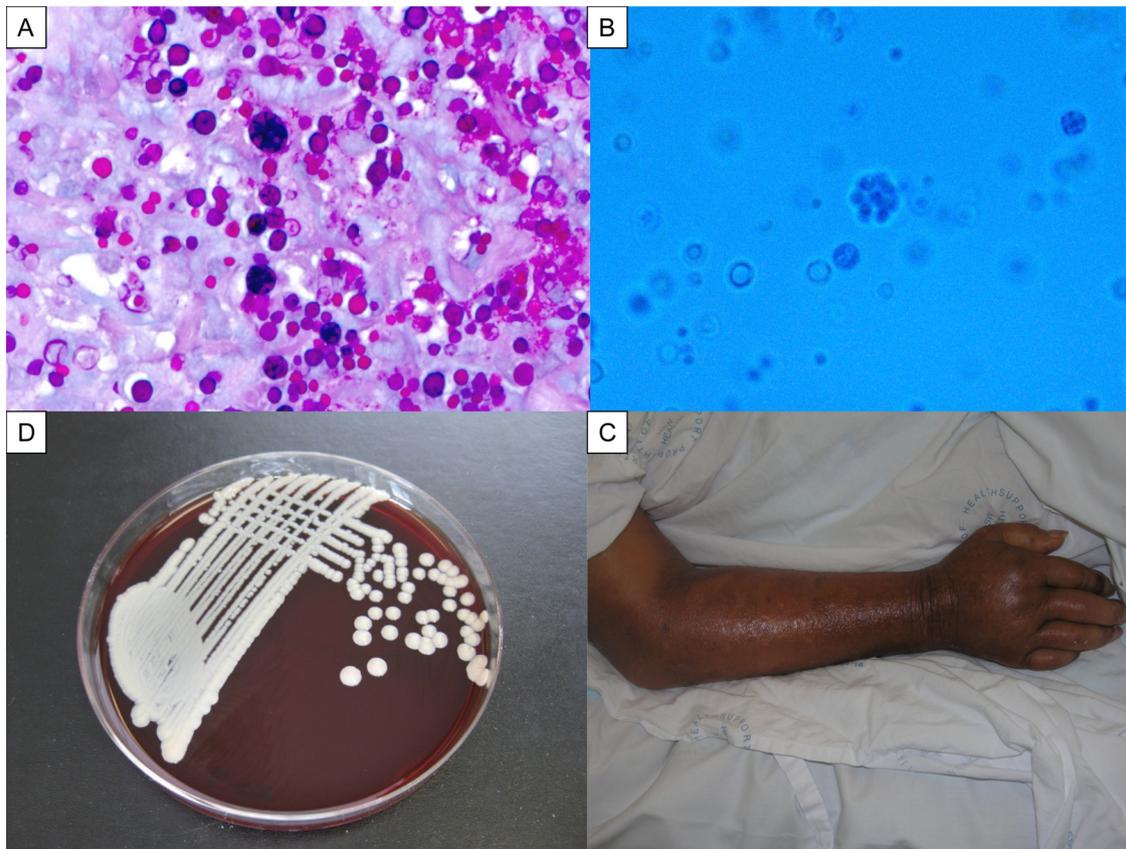


FIG. 1. (A) Histopathological section of tissue sample obtained from the patient’s skin and stained with PAS. Magnification,  $\times 400$ . (B) Wet mount of colony, stained with lactophenol cotton blue, showing organisms of different sizes and morula forms. Magnification,  $\times 400$ . (C) Patient’s arm showing edema and skin induration. (D) Isolate on horse blood Columbia agar, after incubation at  $35^{\circ}\text{C}$  for 96 h, showing smooth, creamy colonies.

37, two further sequential blood cultures yielded identical yeast-like organisms in the aerobic bottles only. Ongoing swelling of the extremities and abdominal pain were noted.

Histopathological examination of the skin biopsy specimens was reported to show a normal epidermis overlying a florid infiltrate of yeast-like organisms within the dermis and subcutis, accompanied by necrosis and an inflammatory infiltrate, including macrophages and neutrophils. The organisms were focally refractile with a 10-fold variation in size. Hematoxylin and eosin (H&E) stain demonstrated variable eosinophilic staining, but each organism had homogenous staining of its unicellular structure. Periodic acid-Schiff with diastase (DiPAS) stained the organisms bright magenta (Fig. 1A) and allowed visualization of scattered morula forms. These morula forms consisted of numerous small rounded organisms encapsulated in larger rounded structures and were also demonstrated on astral blue-PAS combination, silver, astral blue, and mucicarmine stains. No hyphae were identified. At the time of the initial report, the significance of the morula forms were not appreciated, and the biopsy specimen was reported as a probable fungal infection with recommendations for microbiological correlation. An isolate from the skin biopsy samples that was identical to the previous blood culture isolates was grown on culture.

The consulting infectious diseases physician then diagnosed

disseminated fungal infection at this point and recommended ceasing itraconazole and commencing liposomal amphotericin treatment at 1 mg/kg of body weight/day. The patient, however, soon became increasingly drowsy with decreased urine output and increased respiratory effort and died on day 43 of admission. The cause of death was presumed to be overwhelming fungal infection, and an autopsy was not performed.

**Microbiological investigation. (i) Conventional methods.** After 5 days of incubation at  $35^{\circ}\text{C}$ , the Bactec FX automated blood culture machine signaled positive cultures. Large Gram-positive spherical cells of varied sizes resembling yeast were seen on Gram stain. The following day, cream-colored colonies grew on horse blood Columbia and Sabouraud’s dextrose agar (Fig. 1D). The appearance was similar to the appearance of *Candida parapsilosis* on ChromAgar after 48 h, and further testing with the API 32C (bioMérieux, Marcy L’Etoile, France) gave “not valid” identification, with the first organism listed as *Candida catenulata* (sequence number 1201010001; 21.3% probability of correct identification). Unstained wet mount demonstrated atypical yeast-like organisms. The typical endospores of *Prototheca wickerhamii* were not noted initially. A repeat culture produced identical colonies. The isolate did not match phenotypic descriptions of *Candida catenulata* and was reported provisionally as “yeast, not *Candida albicans*”. After the final identification of *Prototheca wickerhamii* was made

postmortem by molecular methods described below, the organism was then reexamined with a wet mount, stained with lactophenol cotton blue, and morula forms were noted (Fig. 1B). The organism was then tested with API 20C AUX (bioMérieux), which had been obtained for this purpose and sent to another laboratory for identification with Vitek 2 (bioMérieux). In both cases, "excellent identification" for *Prototheca wickerhamii* was obtained (API 20C AUX sequence number 6040040 and 99.9% identification; Vitek 2 sequence number 450210000205110 and 99% identification).

**(ii) Molecular methods.** DNA was extracted using the EZ1 automated extraction system (Qiagen, Australia) after 48 h of growth on horse blood agar (Oxoid, Australia). PCR was performed as described previously (1) using the following primers: ITS1 and ITS2 to amplify the internal transcribed spacer 1 (ITS1) region, ITS1 and ITS4 to amplify both ITS1 and ITS2 regions simultaneously as a single product, NL1 and NL4 to amplify the D1/D2 variable domain of the 26S ribosomal DNA gene. To exclude inhibition, all samples were spiked with an equal volume of genomic DNA from controls and run in parallel with unspiked samples. All PCR products were purified using a QIAquick PCR purification kit (Qiagen) per the manufacturer's recommendations and were sequenced in both forward and reverse directions at the Australian Genome Research Facility Ltd. Database searching was performed with BLAST 2.2.23 on the National Center for Biotechnology Information's World Wide Web site (<http://www.ncbi.nlm.nih.gov>). No products were observed with the ITS1 and ITS2 primer pair and ITS1 and ITS4 primer pair. However, the NL1 and NL4 primers produced a product (GenBank accession no. JF812646). Sequence analysis of the amplicons showed 100% homology with *Prototheca wickerhamii* 26S rRNA gene (GenBank accession no. AB244738.1).

**Discussion.** *Prototheca* species are generally considered to be achlorophyllous algae which are ubiquitous in nature but rarely reported to be involved in human infection. These spherical unicellular organisms range from 3 to 30  $\mu\text{m}$  in diameter (5). They are heterotrophs and reproduce asexually by release of endospores. The two most common species of *Prototheca* in human infection are *Prototheca wickerhamii* and *Prototheca zopfii*.

Three types of human infection predominate: localized cutaneous disease, olecranon bursitis, and, more rarely, disseminated disease. In many cases, infections follow an indolent course, though acute and fatal cases are reported. Cutaneous infections with *Prototheca* have occurred following trauma to skin, with probable local inoculation, but also in cases where no such breach in skin integrity has been identified (2, 5, 6). These infections often present as bullous or ulcerative lesions (5) but may resemble cellulitis or edema (3), as in our case, and the diagnosis may be unsuspected initially. The incubation period is not known but is thought to be weeks to months, with spontaneous resolution uncommon (5). Disseminated infection chiefly occurs in the severely immunocompromised patient and has been reported rarely in individuals after solid organ transplantation and also in individuals with malignancy or AIDS (5). In the immunosuppressed individual, opportu-

nistic infection with *Prototheca* species may be associated with bacterial, viral, or fungal coinfection, complicating both diagnosis and treatment (4). Neutrophils are known to ingest *Prototheca* and are believed to play an important role in immunity (7). Diagnosis requires careful microbiological and/or histopathological confirmation. Once identified, an ideal treatment regimen has not been established: a variety of antibacterial and antifungal agents have been used with inconsistent responses to treatment reported. Amphotericin B is generally recommended as the treatment of choice, and some cures have been achieved by treatment with this agent (5, 6).

The appearance of *Prototheca* is similar to yeast on routine media, and they may be mistaken for *Candida* spp. for this reason. They may be distinguished from yeast on wet mounts with lactophenol cotton blue staining if typical morula forms (mature forms containing endospores) are observed. These organisms grow well aerobically on routine bacteriological and mycological media, and several commercial systems correctly identify *Prototheca*, including API 20C or API 20C AUX (bioMérieux, Marcy L'Etoile, France) as well as the database of Vitek 2 (bioMérieux), but the API 32C (bioMérieux) does not include this organism in its database and, therefore, does not identify it (8). Histopathological examination may also be misleading, as this organism may be mistaken for yeast or dimorphic fungus. Periodic acid-Schiff staining may be best for observing typical morphology in tissue (5).

In our case, several features contributed to the delayed identification of this infection. First, this organism is rarely responsible for human infection (our laboratory is in a tertiary 380-bed hospital, which includes a specialized cardiac and pulmonary transplant service and a bone marrow transplant service, has not identified a case of human protothecosis in the last 15 years). Second, this organism resembles yeast on routine media and microbiological and histopathological staining. Third, this organism was not included in the database of the API 32C, used by our laboratory for identifying yeasts. Fourth, the fact that *Prototheca* species cannot be identified by sequencing of the ITS regions as for fungi. Finally, the fact that the infection was subacute and was mistaken by several consulting physicians as a noninfectious disease such as gout. It is likely that immunosuppressive therapy contributed to the patient's acquisition of cutaneous infection and subsequent progression to algaemia and systemic disease. Despite the lack of correct identification of the organism, however, the patient was eventually treated with liposomal amphotericin B, the antimicrobial of choice, but failed to respond and succumbed to the infection.

Human protothecosis is a rare disease, usually associated with immune compromise. In an era of increasing immune suppression, clinicians may begin to encounter *Prototheca* spp. more commonly than in the past. Given their appearance, similar to yeasts on routine media, but very different implications for prognosis and treatment, both clinicians and laboratorians must be aware of these organisms and work in concert to ensure that correct diagnosis and treatment are provided.

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