Title: *Prototheca wickerhamii* mimicking yeast; a cautionary tale.

Running Title: *Prototheca wickerhamii* mimicking yeast

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Abstract
Prototheca spp. are environmental algae which 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 diagnosis.

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 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 cyclosporin 10mg twice daily, mycophenolate mofetil 500 mg twice daily and prednisolone 7.5 mg daily. Other medications included inhaled fluticasone/salmeterol, insulin, ferrous sulfate and omeprazole.

The patient was admitted to hospital for intravenous antibiotic therapy (ticarcillin/clavulanate), chest physiotherapy and IV immunoglobulin. Initially there was some improvement in respiratory symptoms and the patient remained afebrile. Pedal oedema 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 biopsies.

Subsequently the patient reported pain in her fingers, which she had first noted 1 month prior to this admission. Her uric acid level was mildly elevated at 0.51 mmol/L (0.20-0.40). Finger swelling was noted at the 1st
metacarpophalangeal (MCP) joint of the right hand. A consulting rheumatologist reviewed her on day 10 of hospital admission and recommended increasing the prednisolone dose to 15mg 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 degrees was recorded. Computed tomography (CT) scanning of the patient’s head was unremarkable and the C-reactive protein (CRP) was 20. 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 4th and 5th fingers, bipedal and arm oedema (figure 1c). On examination, her jugular venous pressure was not raised and she had a normal albumin (39g/L). She was reviewed 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 hours post-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, it was verbally reported to the treating team as likely to be uncommon, possibly environmental yeast. Antifungal susceptibilities were performed by broth microdilution using the Sensititre® YeastOne™ Test Panel (Trek Diagnostic Systems, USA). Minimum inhibitory concentrations (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/L, 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 oedema 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 itraconazole 200mg twice daily. On day 36
and 37, two further sequential blood cultures yielded identical yeast-like organisms in the aerobic bottles only. Ongoing swelling of the extremities and abdominal pain was noted.

Histopathological examination of the skin biopsies 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. Haematoxylin 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 (figure 1a) and allowed visualisation 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 initial reporting, the significance of the morula forms were not appreciated, and the biopsy was reported as a probable fungal infection with recommendations for microbiological correlation. From the skin biopsy samples, an isolate identical to the previous blood culture isolates were grown on culture.

The consulting infectious diseases physician then diagnosed disseminated fungal infection at this point and recommended ceasing itraconazole and commencing liposomal amphotericin 1mg/kg/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

Conventional Methods

After 5 days’ incubation at 35º 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-coloured colonies grew on Horse Blood Columbia and Sabouraud’s Dextrose Agar (figure 1d). The appearance was similar to Candida parapsilosis on ChromAgar at 48 hours and further testing with the API 32C (BioMérieux, Marcy L’Etoile, Paris, France) gave “not valid” identification, with the first organism listed as Candida catenulata (Sequence Number 1201010001, Identification 21.3%). 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 descriptions of *Candida catenulata* phenotypically, and was reported provisionally as “yeast, not Candida albicans”. After the final identification of *Prototheca wickerhamii* was made post-mortem by molecular methods described below, the organism was then re-examined with a wet mount, stained with lactophenol cotton blue, and morula forms were noted (figure 1b).

The organism was then tested with API 20C AUX (BioMérieux, Marcy L’Etoile, Paris, France), which had been obtained for this purpose and sent to another laboratory for identification with Vitek 2 (BioMérieux, Marcy L’Etoile, Paris, France). In both cases, “excellent identification” for *Prototheca wickerhamii* was obtained (API 20C AUX Sequence Number 6040040, Identification 99.9%; Vitek 2 Sequence Number 450210000205110, Identification 99%).

**Molecular Methods**

DNA was extracted using the EZ1 automated extraction system (Qiagen, Australia) after 48 hours 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 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) as per 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](http://www.ncbi.nlm.nih.gov)). No products were observed with the ITS1/ITS2 and ITS1 and ITS4 primer pairs. However the primers NL1 and NL4 produced a product (GenBank Accession Number JF812646). Sequence analysis of the amplicons showed 100% homology with *Prototheca wickerhamii* 26S rRNA gene (GenBank: AB244738.1)

**Discussion**

*Prototheca* species are generally considered to be achlorophyllous algae which are ubiquitous in nature but rarely described in human infection. These spherical unicellular organisms range from 3 to 30 µm in diameter.\(^2\)
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.\(^2\) These infections often present as bullous or ulcerative lesions\(^2\) but may resemble cellulitis or oedema\(^5\), as in our case, and the diagnosis may be unsuspected initially. The incubation period is unknown, but thought to be weeks to months, with spontaneous resolution uncommon.\(^2\) Disseminated infection chiefly occurs in the severely immunocompromised patient and has been reported rarely in individuals following solid organ transplantation, and also in those with malignancy or AIDS.\(^2\) In the immunosuppressed, opportunistic infection with *Prototheca* species may be associated with bacterial, viral, or fungal co-infection, complicating both diagnosis and treatment.\(^6\) 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 treatment of choice and some cures have been achieved with the use of this agent.\(^2\)\(^3\)

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, Paris, France) as well as the database of Vitek 2 (BioMérieux, Marcy L’Etoile, Paris, France) but the API 32C (BioMérieux, Marcy L’Etoile, Paris, France) 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.\(^2\)
In our case, several features contributed to the delayed identification of this infection: firstly, the rarity of this organism in human infection (our laboratory, in a tertiary 380-bed hospital which caters for a specialized cardiac and pulmonary transplant as well as bone marrow transplant service, has not identified a case of human protothecosis in the last 15 years); secondly, the resemblance of this isolate to yeast on routine media, microbiological and histopathological staining; thirdly, the lack of inclusion of this organism in the database of the API 32C, used by our laboratory for identifying yeasts; fourthly, 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 non-infectious 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 correct diagnosis and treatment is provided.

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References


Figure 1: (clockwise from top left) a: histopathological section of tissue obtained from skin, magnification x400, stained with PAS; b: wet mount of colony, stained with lactophenol cotton blue, magnification x400, showing organisms of different sizes and morula forms; c: patient’s arm showing oedema and skin induration; d: isolate on horse-blood Columbia agar, after incubation at 35 degrees for 96 hours, showing smooth, creamy colonies.