

Case Report



The Balancing Universe: The Story of Human Invasion and The Primitive yet Evolutionary Prototheca

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Abstract

Prototheca is an achlorophyllic environmental algae and is an underrecognized pathogen. It may cause infections in both immunocompetent and immunocompromised patients but usually misidentified as yeast. The identification of this microorganism requires advanced methods like MALDITOF MS (Matrix assisted Laser Desorption Ionization Time of Flight) and Molecular techniques. Hereby, we report a case of Cutaneous infection by Prototheca wickerhamii in a 49 years old post kidney transplant patient which resembled morphologically as Cryptococcus on histopathological examination but turned out to be Prototheca wickerhamii on fungal culture using MALDITOF MS method for identification. Patient responded to treatment with Amphotericin B.

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Introduction

Protothecosis is a rare infection caused by Prototheca which is an achlorophyllic environmental algae. There are six species of Prototheca: Prototheca wickerhamii, Prototheca zopfii, Prototheca blaschkeae, Prototheca cutis, Prototheca ulema, and Prototheca stagnora.[1] Prototheca wickerhamii is most commonly associated with human infections. Infection usually occurs by a penetrating injury or traumatic inoculation. It may cause infection in both immunocompetent and immunocompromised patients. In immunocompetent patients the infection is localized and mild. Olecranon bursitis and localized cutaneous infections have been reported in immunocompetent individuals.[2] However, in immunocompromised patients, dissemination of infection may occur

into blood, peritoneum, meninges, gastrointestinal tract and liver. It is a colourless microalgae which is spherical, unicellular Gram-positive, 3-30 μ in diameter and may be misidentified as *Candida*. *Prototheca* spp. belong to the family *Chlorellaceae* of the order *Chlorellales*, in the class *Trebouxiophyceae*. [3] There are very few reports of infections caused by *Prototheca*. Worldwide about 200 cases of *Prototheca* have been reported and systemic infections have been reported in 9% of these cases. [4] It is an emerging pathogen with expanding host range and pathogenicity causing both localized and disseminated infections. With increasing awareness and use of advanced diagnostic techniques, this microorganism is being reported across the world. Hereby, we report a case of Cutaneous infection by *Prototheca wickerhamii* in a 49 years old post kidney transplant patient which was identified by MALDITOF MS (Matrix assisted Laser Desorption Ionization Time of Flight).

Case Report

49 years old female presented at our tertiary health care centre with pustular lesions over left hand with swelling, bilateral pedal edema and facial puffiness, weakness, exertional dyspnea and low-grade fever in December 2020. Patient gave history of weight loss in last 3-4 months. Patient was a known case of kidney transplant recipient in 2011 with allograft dysfunction. Besides other drugs, patient was on double immunosuppressants (Prednisolone 10 mg OD and Tacrolimus 0.5 mg OD) and antifungal (Fluconazole) 100 mg OD at the time of admission. Apart from medications, patient was on protein, vitamin and iron nutritional supplements. Patient did not give any history of trauma or injury.

On examination, Patient was afebrile with vital parameters within normal limits. At the time of admission, her Haemoglobin was 6.2 gm/dl, Total leucocyte count was 7900/mm³, urea was 70 mg/dl and creatinine were 2.68 mg/dl. There was no history of cough and haemoptysis, no swelling in neck/axilla. HRCT chest showed small focus of lower lung parenchymal fibrosis in right apical region. There was evidence of small rounded lesion with central cavitation and speculated margins in posterior segment of right upper lobe, bilateral basal region subsegmental atelectasis. BK virus RTPCR (Real Time Polymerase Chain Reaction) in urine sample was positive with more than 1 lakh copies/ml. Patient gave history of pustular lesion on lateral side of right ankle two years back for which incision and drainage was done.

After one year, patient developed pustular lesion on dorsum of left hand with intermittent pus discharge. On examination, it was 5x4 cm with opening over dorsum of left hand. There was minimal redness and swelling and no fluctuation. Around 1 ml of serous discharge from the wound was aspirated and sent for culture. Bacterial culture showed no growth However, fungal culture showed growth of Yeast cells which were not identified by automated systems (Vitek-2 and MALDITOF Vitek MS, Biomerieux, France).

Wedge biopsy was taken from the lesion on dorsum of hand and sent for histopathological examination. Histopathological examination showed acute on chronic inflammation with ulceration and many variable sized, budding encapsulated invasive yeast forms both intra and extracellularly with morphology closest to *Cryptococcus neoformans*. The culture showed growth of yeast cells (Figure 1 and 2) which were urease negative and India Ink examination did not demonstrate presence of capsule (Figure-3).

These yeast look-alike cells remained unidentified by Vitek-2 YST card and MALDITOF MS by yeast identification protocol using prior extraction with formic acid. A mold extraction protocol was experimented with and the organism could then be further identified as *Prototheca wickerhamii* by MALDITOF MS after the extraction of the proteins was done as per molds extraction protocol, briefly as below:

The yeast-like organism was suspended in a 2ml tube containing 900 μ l 70% Ethanol and was mixed well. Then, it was centrifuged at 10000-14000g for 2 minutes. Supernatant was discarded and 40 μ l of 70% formic acid was added and vortexed. Then, 40 μ l of

acetonitrile was added and centrifugation was again done at 10000-14000g for 2 minutes. After that, 1 μ l of supernatant was deposited on MS target slide and allowed to dry. Then 1 μ l CHCA (α -Cyano-4-hydroxy cinnamic acid) matrix was added. Once it got crystallized, the slide was run in MALDITOF MS as per the standard protocol.

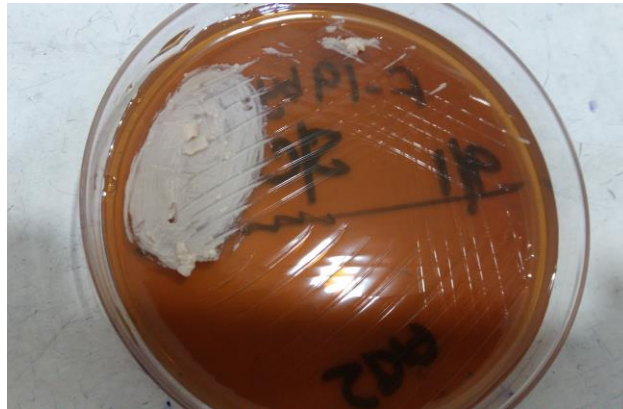


Figure 1: Growth of *Prototheca wickerhamii* on Sabouraud's Dextrose Agar (SDA)-showing dry cream coloured growth

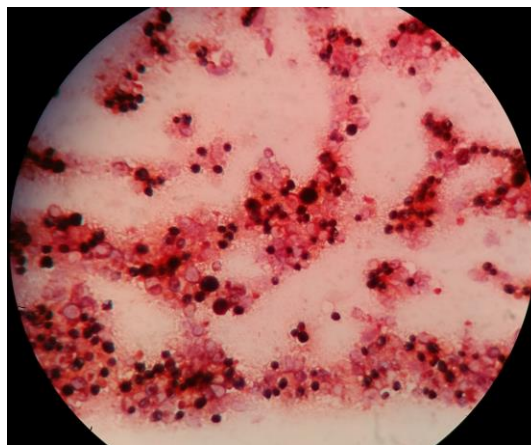


Figure 2: Gram stain examination (1000X)-showing round yeast cells with budding

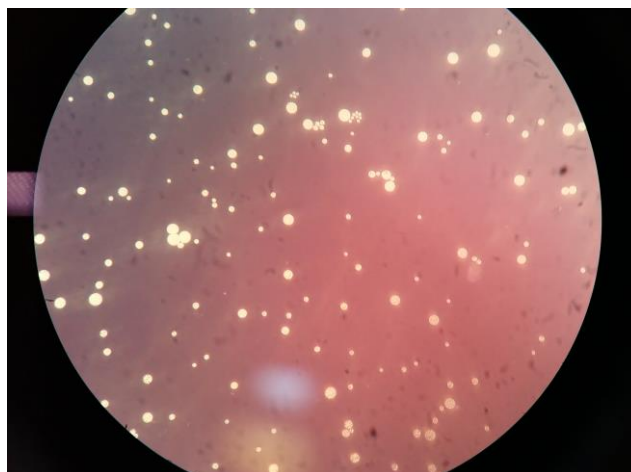


Figure 3: India Ink examination (400X)- showing round yeast cells without capsule

Patient was administered liposomal amphotericin B in the dose of 1.5 mg in 250 ml Dextrose (D5) over 4 hrs intravenously and daily dressing with Fluconazole lotion. Amphotericin B was stopped after 15 days. CT guided right lung nodule biopsy showed fibrinoid exudative and scattered acute on chronic inflammatory cells. No granuloma, malignancy or fungal elements were seen. The patient responded to the therapy and there was no recurrence till one year of follow up.

Discussion

Prototheca spp. are ubiquitous in nature and are found in trees, grass, soil, fresh water and salt water, animals and food items.[5] They are actually algae but are diagnosed like fungi. There are very few reports of *Prototheca* infections in literature. The diagnosis of *Prototheca* infection is a great challenge because of its resemblance with yeast, less awareness about this microorganism and non-identification or difficult identification by routine conventional and automated methods. When not identified, this microorganism may be commonly misinterpreted as *Cryptococcus*, *Candida* or yeast form of dimorphic fungi based on microscopic and macroscopic appearance. *Prototheca* differs from fungi in lack of glucosamine in the cell wall and from bacteria in lack of muramic acid in cells wall. Also, unlike other algae, it does not possess chloroplast and has two layered cell wall instead of three layered. Conventionally, it may be differentiated from yeast on Lactophenol cotton blue stained wet mounts where typical morula forms (mature forms containing endospores) may be seen. It may be identified by both Vitek-2 and MALDITOF Vitek MS. But, in our case, it remained unidentifiable by both the systems using standard protocol giving an opportunity to use a different protocol. The identification could be done by MALDITOF MS by following protocol for mycelial fungi.

The patient seems to have a chronic infection by this organism which had likely spread taking a disseminated form over a period of almost two years.

Prototheca, which was first thought to be a fungus, is now generally considered to be the apochlorotic equivalent of *Chlorella*. [6] It is achlorophyllous, saprophytic algae with a wide distribution in natural environments and can also be found in many food products, e.g. beef, pork, clams, crabs and dairy milk. [6] Although the exact source of infection cannot be established in this case but it is important to note that certain dietary supplements may have *Chlorella* which resists Pasteurization method used in food industry. [7,8] *Chlorella* is the most cultivated eukaryotic algae as it contains proteins, polysaccharides, lipids, unsaturated fatty acids, carotenoids, immunostimulators, vitamins and minerals. Hence, it is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry. [9] We checked the composition of dietary supplements being used by the patient but the presence of *Chlorella* could not be established.

Infections in human beings have commonly been reported by *Prototheca wickerhamii* and less commonly by *Prototheca zopfii*; which is more reported to cause bovine mastitis and canine systemic infections and also has poor prognosis than *Prototheca wickerhamii* in human infections. [10] Table-1 describes the reported cases of *Prototheca* infection in Indian literature. The different types of infections caused by *Prototheca* include localized cutaneous disease, olecranon bursitis, and disseminated disease which is very rare. Although there are reports of fatal infections but majority of the cases show indolent course. Cutaneous infections are usually associated with trauma to skin, but there are cases where no breach in skin integrity has been identified. [11] As evident in Table-1, infection in immunocompetent individuals is mainly cutaneous and in immunocompromised it's mainly disseminated with mixed outcome to treatment. Treatment of *Protothecosis* is a therapeutic challenge due to inconsistent therapeutic response. Amphotericin B is the mainstay of treatment with or without other antifungal drugs like Itraconazole. There are considerable chances of therapeutic failures in *Prototheca* infection so monitoring of clinical response should be done to review the treatment. [4] In our case, based on review of literature, patient was given Amphotericin B and patient showed response to

treatment with no recurrence till one year.

Table 1

	Number of patients	Risk factor	Clinical specimen	Identification method	Treatment	Outcome
Khan ID et al (2018) ^[12]	12	Malignancy, Neutropenia	Blood	<i>Prototheca wickerhamii</i> Vitek-2	Amphotericin B	Post treatment-Blood cultures were negative for <i>Prototheca</i> in all patients
Rajan A et al (2020) ^[5]	01 (69 Years/ Male)	Immunocompetent, farmer by profession	Skin tissue	Histopathology- Numerous PAS positive bodies forming a symmetrical morula or cartwheel-like structure were seen within the histiocytes	Itraconazole	Improvement till 3 months follow-up
Gandham NR et al (2015) ^[13]	01 (55 years/ Female)	Immunocompetent, House wife	Nail	<i>Prototheca zopfii</i> -Vitek-2	Oral terbinafine 250 mg once a day and local application of Nail lacquer (ciclopirox olamine)	Lost to follow-up
Rao PV et al (2018) ^[14]	01 (36 years/ Male)	Post Liver transplant with right leg cellulitis	Blood culture and Bronchoalveolar lavage	<i>Prototheca zopfii</i> -Vitek-2 and MALDITOF Vitek MS	Amphotericin B and Fluconazole	Expired
Narayanan N et al (2018) ^[15]	01 (46 Years/ Male)	Diabetic, Corneal injury with sparks of fire	Corneal scraping	<i>Prototheca wickerhamii</i> -Vitek-2	intensive Topical 1% voriconazole and 5% natamycin for 1 month followed by penetrating keratoplasty in view of non-improvement	No recurrence of infection post-operatively
McMullan B et al (2011) ^[11]	01 (78 Years/ Female)	Cardiac transplantation on immunosuppression with multiple co-morbidities	Blood and Skin biopsy	<i>Prototheca wickerhamii</i> - Genomic sequencing, API 20C AUX, Vitek-2 (Biomerieux, France)	Liposomal Amphotericin B	Death

Conclusion

Protothecosis is a rare but emerging important infection especially in immunocompromised patients. A possibility of *Prototheca* infection should be kept in mind in case of unidentified yeast-like growth in pus or tissue samples especially from immunocompromised patients. In case of diagnostic challenge, a mold extraction protocol can be used for MALDITOF-MS. Microbiological suspicion and advanced diagnostic techniques contribute to the definitive identification of *Prototheca*. Role of nutritional supplements with *Chlorella pyrenoidosa* needs to be further studied in patients with *Prototheca* infections. Although the treatment of this organism is not well defined but Amphotericin B with or without Itraconazole may be used effectively.

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