Prototheca zopfii as a Cause of Pneumonia and Disseminated Infection in Febrile Neutropenia: A Case Report and Literature Review

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Patient: Female, 48-year-old
Final Diagnosis: Disseminated Prototheca zopfii • sepsis
Symptoms: Diarrhea • fatigue • fever • vomiting
Medication: —
Clinical Procedure: —
Specialty: Infectious Diseases • Microbiology and Virology

Objective: Rare disease
Background: Prototheca spp. are common and found in various environments, including animal and human intestines, on the skin and in respiratory tissues, and colonizing fingernails. Few strains pathogenic for humans have been discovered. Here, we describe an infection by the pathogenic fungus species Prototheca zopfii in a patient. The infection was initially classified as a fungus based on colony morphology, fungal staining results, and growth in some fungi culture media (Sabouraud dextrose agar [SDA]). Reports of Prototheca spp. infections are increasing, often with poor outcomes. The use of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) technique for identification has been widely described. Phenotypic identification depends on microscopic examination of the direct wet mount and after subculturing in blood and SDA using different stains that show a typical morphological characteristic of Prototheca spp.

Case Report: A 48-year-old woman was diagnosed with a P. zopfii infection after 22 days of hospitalization in the critical care unit. The patient had profound febrile neutropenia and absolute neutrophil count (ANC) was zero, associated with hypotension and disseminated intravascular coagulation (DIC) 10 days after receiving the first cycle of chemotherapy for metastatic breast adenocarcinoma. Unfortunately, the patient died within 2 days of the initiation of treatment with amphotericin B.

Conclusions: This case report highlights algae infections as a possible opportunistic infection type in patients with profound neutropenia, and we discuss the use of MALDI-TOF MS-based technology in detecting such infections and predicting poor prognosis, especially in patients with the disseminated form with underlying febrile neutropenia.

Keywords: Febrile Neutropenia • Prototheca • protothecosis

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/933694
Background

Prototheca species are achlorophyllous, saprophytic, and yeast-like aerobic algae commonly found in the environment [1-4]. There are many sources of Prototheca spp., such as fresh and saltwater, soil, and mud, but these algae have also been found in animals, including cattle, deer, dogs, cats, and goats, and in certain foods, including potatoes, bananas, and dairy products [1]. These algae seem to play a weak infectious role in immunocompetent individuals. However, infections are being increasingly reported in immunocompromised patients [5,6].

Prototheca spp. causes infections to varying degrees, ranging from mild superficial skin infections and olecranon bursitis to severe disseminated infections in immunocompetent and immunocompromised patients, respectively [1,6]. P. wickerhamii is the cause of most human infections. However, P. zapfii, P. blaschkeae, P. cutis, and P. miyajii can also cause human infections [7-11].

A better understanding of this microorganism is needed because advances in chemotherapy and immune suppression therapy are increasing the burden of this disease. In this report, we describe the first reported case of disseminated P. zapfii infection in the Middle East. This invasive infection manifested as pneumonia in a patient with metastasized breast cancer who presented with febrile neutropenia. The patient was treated with fluconazole and then caspofungin. After receiving the culture result, amphotericin B was started, but, unfortunately, she died within 2 days after the initial dose.

Case Report

We report a case of a 48-year-old single, unemployed woman, originally from Jazan (southern Saudi Arabia). The patient known to have diabetes mellitus type II, hypothyroidism because of total thyroidectomy due to thyroid cancer 10 years ago, and right breast adenocarcinoma (diagnosed and managed in another specialized hospital) with spine metastases complicated by paraplegia. She presented on 8 April 2021, 10 days after receiving the first cycle of chemotherapy (docetaxel) and 5 previous cycles of radiotherapy, with fever reaching 38.6°C, vomiting (grade 2-3), and watery diarrhea (grade 4), associated with fatigue and dizziness (grade 3) evaluated according to NCI CTCAE (National Cancer Institute common terminology criteria for adverse events). She reported having no history of hematemeses, melena, hematochezia, abdominal pain, herbal exposure, recent travel, or contact with domestic animals.

On physical examination in the Emergency Department, the patient was pale, hypotensive (blood pressure of 89/59 mmHg) and tachycardic (heart rate of 129 beats per min); body temperature was 38.6°C; respiratory rate was 22 breaths/min and oxygen saturation, and oxygen saturation was 97% on room air. She was conscious, alert, and oriented to time, place, and person. Results of an examination of the chest and abdomen were unremarkable. She had a clean sacral pressure ulcer grade 2 measuring 2.5×1.5 cm in size. After fluid resuscitation, her blood pressure improved.

Laboratory investigations on admission revealed pancytopenia, with a white blood cell (WBC) count of 0.2 k/µl and absolute neutrophil count (ANC) of 0 cell/µl; hemoglobin level was 8.9 g/L and platelet count was 7000/µl. The serum creatinine value was 1.1 mg/dl and blood urea nitrogen was 24 mg/dl. Estimation of electrolytes showed sodium 138 mEq/L, potassium 3.2 mEq/L, and bicarbonate 25 mmol/L. Urine analysis was normal and liver function tests were as follow: protein 5 g/dl, albumin 3.4 g/dl, total bilirubin 1.2 mg/dl, alanine transaminase 49 IU/L, aspartate transaminase 33 IU/L, alkaline phosphatase 179 IU/L, and gamma-glutamyl transferase 447 IU/L. Her lactate level was 1.8 mmol/L, procalcitonin was 138 ng/ml, and C-reactive protein was 36 mg/L. The patient had a clinical picture of DIC with prolonged thromboplastin time PT (47.3 s) and partial thromboplastin time PTT (49 s), high international normalized ratio INR (3.4), fibrinogen level 290 mg/dl and elevated D-dimer (3.0 µg/ml). Peripheral blood film shows normocytic normochromic anemia with mild anisocytosis, mild poikilocytosis, and few schistocytes. A chest X-ray (CXR) on admission (Figure 1) showed a normal lung field, clear bilaterally, costophrenic angle. No evidence of pneumonia. On the day of admission (8 April 2021).

![Figure 1. AP view CXR on the day of admission](image)

Figure 1. AP view CXR on the day of admission shows normal lung fields, clear bilaterally, costophrenic angle. No evidence of pneumonia. On the day of admission (8 April 2021).
Prototheca zopfii as a cause of pneumonia and disseminated infection

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Table 1. Important laboratory findings and antimicrobial management during ICU admission.

| Date   | CRP (mg/dL) (0.1-5) | WBC (k/µl) (4.0-11) | ANC* (cell/µl) (1.5-8) | Procalcitonin (ng/mL) <0.1 | Culture site and findings | Antimicrobial treatment                  |
|--------|---------------------|---------------------|------------------------|-----------------------------|---------------------------|----------------------------------------|
| 9 April | 40.56               | 0.2                 | 0                      | 137.4                       | Pseudomonas aeruginosa (blood) | Vancomycin 1 g i.v. every 12 h, Meropenem 1 g i.v. every 8 h |
| 12 April | 22.13              | 0.1                 | 0                      | 27.37                       | No growth (blood)           | Fluconazole started (800 mg i.v. loading dose then 400 mg IV every 24 h) |
| 15 April | 28.01              | 0.3                 | 0                      | 3.90                        | No growth (blood)           | No change                             |
| 18 April | 26.20              | 0.2                 | 0                      | 3.40                        | No growth (blood)           | No change                             |
| 21 April | 20.14              | 0.4                 | 0                      | 3.33                        | No growth (blood)           | No change                             |
| 24 April | 28.12              | 0.2                 | 0                      | 3.47                        | No growth (blood)           | No change                             |
| 27 April | 32.97              | 0.8                 | 1                      | 4.70                        | No growth (blood)           | Antifungal treatment shifted to Caspofungin (70 mg i.v. loading dose then 50 mg i.v. every 24 h) |
| 30 April | 35.68              | 1.1                 | 1                      | 6.31                        | P. zopfii (blood, TA)       | No change                             |
| 2 May    | 39.7               | 1.3                 | 1                      | 6.54                        | P. zopfii (TA)              | Caspofungin stopped, Amphotericin B lipid complex 300 mg i.v. every 24 h started |

TA – transtracheal aspirate.* ANC=WBC×(PMN^/100)+(Bands/100)^polymorphonuclear neutrophil.

Blood culture at presentation was positive for Pseudomonas aeruginosa, for which she was treated for 14 days with meropenem, and eradication was proved by repeated negative cultures, which showed no growth (Table 1). Beta-D-glucan and galactomannan antigen tests were not available.

Methicillin-resistant Staphylococcus aureus (MRSA) screening was positive for nasal and throat samples. Because neutrophils failed to recover by the fifth day of admission despite antibiotics and colony-stimulating growth factor (Filgrastim 300 μg i.v. daily for 21 days) treatments, the patient was started on fluconazole (800 mg loading then 400 mg daily for 16 days) as a preemptive antifungal management for febrile neutropenia.

On 13 April, the patient developed upper gastrointestinal (GI) bleeding followed by lower GI bleeding with fresh blood per rectum and was evaluated by General Surgery. She underwent proctoscopy, which showed normal mucosa with the presence of fresh blood. She received multiple blood products. The Gastroenterology team was consulted, and because of hemodynamic instability, gastroscopy and colonoscopy were delayed by a few days, and when performed, the delayed gastroscopy was unremarkable; however, the colonoscopy showed severe, inflamed, ulcerative lesions throughout the whole colon. Biopsies revealed inflamed fibromuscular stroma, ulcerative surface, and reactive atypia without malignancy, and no evidence of P. zopfii in histopathology. Cytomegalovirus (CMV) colitis was suspected but not confirmed histologically or by PCR.

On 19 April, total parenteral nutrition (TPN) was initiated through the right brachial by a peripherally-inserted central catheter (PICC) line. On 27 April, the antifungal coverage shifted to caspofungin (70 mg i.v. loading dose then 50 mg i.v. every 24 h).

On 30 April, she had respiratory deterioration, requiring intubation and mechanical ventilation (Figure 2). The anteroposterior (AP) view CXR showed patchy infiltration involving the upper left radiological zone, and multiple patches over the entire right lung field, as well as middle and lower left radiological zone, obliterating the left costophrenic angle (Figure 3). On post-intubation day 2, we detected severe bilateral pneumonia involving the middle and lower zone of the left lung and right upper zone. Tracheal aspirate and blood culture on 30 April were positive for P. zopfii (Table 1). Despite the initiation of amphotericin B on the day these results became available, unfortunately, she died the following day.

Discussion

Disseminated protothecosis is a fatal opportunistic infection that can arise in immunodeficient populations [12]. The pathogenesis of Prototheca is poorly understood; it mainly occurs in immunocompromised hosts and rarely in patients with intact immune systems. The genus Prototheca includes 8 species: P. wickerhamii, P. zopfii, P. stagnora, P. blaschkeae,
P. ulmea, P. cutis, P. tumumicola, and P. miyajii [5]. There are 3 clinical types of protothecosis: cutaneous, the most common; olecranon bursitis; and systemic protothecosis, which can also affect the skin [13,14]. Unlike the previous series on patients infected with P. Wickerhamii, mortality from disseminated P. zopfii infection was much higher [12]. Milk and dairy products contaminated with P. zopfii primarily cause skin diseases, and can be a source of infection in humans and milking cows, then are disseminated in immune-compromised hosts [15]. In our patient, the contaminated food may have been the source of infection, and the colon ulcerations were a possible portal of entry.

Disseminated protothecosis may be clinically unsuspected and underdiagnosed; thus, patients may receive various treatments for extended periods with no satisfactory results [1]. The definitive diagnosis of infection often depends on the morphological identification of organisms in the preparation of the wet culture slide and/or direct identification in tissue samples [1-3].

Recently, differences in the protein expression profiles of environmental strains (genotype 1) and pathogenic strains (genotype 2) have been reported, suggesting that it is related to the level of P. zopfii pathogenicity [5]. For suspected cases, a combination of microbiological and histopathological examination is recommended [1]. Identification of Prototheca species requires multiple detection methods, including DNA-based methods, such as ribosomal DNA sequencing; however, this test was not available in our laboratory and is not part of most clinical trials.

We typically identify microorganisms at the species level by use of MALDI-TOF MS. In this case, the isolate was identified using the manufacturer database that included a reference
Additionally, the isolate was also identified by characteristic microscopic morphology, which showed on Figures 4-10 a typical morphology with multiple cells, globose to ovoid in shape, varying in size, and from 4 to 6 sporangiospores contained in each cell [1,4-6]. In this case scenario, the antimicrobial management of febrile neutropenia and P. aeruginosa sepsis with a broad-spectrum antibiotic (meropenem) along with adding antifungal agents as part of an empirical treatment of febrile neutropenia (fluconazole then caspofungin) most likely resulted in the selection of a resistant P. zopfii strain as a causative opportunistic agent for an overwhelming fungal infection.

Treatment options are still controversial, and various treatments are being tried. Antifungal agents, such as ketoconazole, itraconazole, fluconazole, conventional amphotericin B, and liposomal amphotericin B, are the most commonly used drugs to date. Among them, amphotericin B showed the best activity.
against Prototheca spp, but there is no strong evidence that P. zopfii treatment should differ from the treatment of P. wick-erhamii [8]. Regarding in vitro susceptibility tests, which were not available in our lab, after review, the references show that several antifungal agents have been used to treat Prototheca spp. One study reported variable success rates depending on the antifungal treatment (58-72%) [7]. Amphotericin B was the most effective treatment if used as deoxycholate or lipid-associated preparations, then itraconazole and fluconazole. Amphotericin B treatment has been used more frequently for severe infections than other antifungal agents [16]. Therefore, we advised starting amphotericin B for our patient. Treatment options also include medical and surgical approaches. Treatment failure is frequently reported (Table 2) [1]. With the increasing use of transplants and chemotherapy in human medicine, the predisposition to protothecosis in immunodeficient individuals or those treated with chemotherapy will continue to be a problem [17].

Ultimately, we reported this case as disseminated protothecosis caused by P. zopfii in a patient with febrile neutropenia due to chemotherapy as a treatment for metastatic breast cancer. She was given amphotericin B but died a few days after receiving the first doses.

### Table 2. Documented disseminated *Prototheca zopfii* cases in the literature review.

| Case No. | Reference | Age/Sex | Underlying medical condition | Treatment | Outcome |
|----------|-----------|---------|------------------------------|-----------|---------|
| 1        | Our case  | 42 years/Female | Febrile neutropenia/breast cancer | Amphotericin B | Death |
| 2        | Zhang Q et al [5] | Chinese | Neck mass/granulomatous lymphadenitis medically free | Amphotericin B | Improving |
| 3        | Takano M et al [14] | Japan | Fever/malaise immune suppressed chemotherapy | Itraconazole | Death |
| 4        | Sari S et al [18] | USA | Bloody diarrhea inherited CARD9 deficiency | Amphotericin B | Respond and improved |
| 5        | Lass-Flörl C et al [19] | Austria | Fever/respiratory distress Acute leukemia after BMT | Voriconazole Amphotericin B | Death |

**Figure 9. Appearance of P. Zopfii wet mount microscopic visualization:** Wet mount microscopic examination, 40×, from transtracheal culture, shows multiple cells, globose to ovoid in shape, varying in size. Budding cells are present and from 4-6 sporangiospores contain in each cell (blue arrow). Image taken on 2 May 2021.

**Figure 10. Appearance in gram stain of P. zopfii from blood agar:** Gram stain of colony from blood agar, seen under 100×, from blood culture, shows gram-positive yeast-like no budding, the sporangiospores not seen by gram stain. Image taken on 2 May 2021.
Conclusions

This case report shows that protothecosis is a serious infection type among immune-compromised patients and is associated with a high mortality rate and limited treatment options. It is increasingly common to combine an immune-suppressing agent with chemotherapy and steroid treatments. This case was diagnosed in our hospital based on laboratory identification, although there was an initial lack of suspicion. Early suspicion of such differential diagnoses would help to rapidly identify the best treatment. Food contaminated with P. Zopfii is often the source of infection and colon ulcerations are a possible portal of entry.

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Declaration of Figures’ Authenticity

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