First bloodstream infection due to *Prototheca zopfii* var. *hydrocarbonea* in an immunocompromised patient

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ABSTRACT

Here we describe a bloodstream infection due to *P. zopfii* var. *hydrocarbonea* in a patient with acute lymphoblastic leukemia. Identification was performed by DNA sequencing of the D1/D2 domain of 26S ribosomal DNA and by MALDI-TOF MS technique. Antifungal susceptibility tests against amphotericin B, fluconazole, itraconazole, and voriconazole showed the following MIC values, respectively: 0.25 mg/L, 128 mg/L, 0.064 mg/L, and 0.125 mg/L. The patient received amphotericin B treatment with a successful outcome.

1. Introduction

*Prototheca* spp. are yeast-like chlorophyllous, saprophytic, aerobic algae commonly found in nature [1]. Their typical sources are fresh and salt water, soil, mud and tree sap; but these algae have also been isolated from animals such as cattle, dogs, deer, cats, and goats, as well as from certain foods, like potato skins, cows’ milk, butter and bananas [1–4]. Humans are undoubtedly often exposed to these algae appear to have a low ability to infect the human host. Despite this situation, an increasing number of protothecosis cases have been reported recently. This is probably due to an increase in the number of immunocompromised patients detected and an improvement in the diagnosis of protothecosis [7,15]. Therefore, an interest in this disease has emerged among microbiologists and the term “medical phycology” has been adopted to refer to the study of infections by *Prototheca* and *Chlorella* species in human and lower animals [7]. Moreover, in 2014 a new ISHAM (International Society for Human and Animal Mycology) working group, “Medical Phycology: Protothecosis and Chlorelloisis working Group”, was created.

*Prototheca* species cause cutaneous infections, olecranon bursitis and disseminated infections in both immunocompetent and immunocompromised patients [1,6]. Most infections are likely to be caused by traumatic inoculation into subcutaneous tissues. Localized cutaneous infections and olecranon bursitis are more commonly developed in immunocompetent patients, whereas dissemination and visceral involvement mainly affect immunocompromised patients and had the worst prognosis [1,6,7].

The genus *Prototheca* include the following species: *P. wickerhamii*, *P. zopfii*, *P. blashkeae*, *P. stagnora*, *P. ultnea*, *P. cutis*, *P. miyajii* and *P. tumuncola* [5]. *P. wickerhamii* causes the majority of human protothecosis. However, *P. zopfii*, *P. blashkeae*, *P. cutis* and *P. miyajii* can also cause human infections [8–11].

In this report, we describe the first bloodstream infection due to *Prototheca zopfii* var. *hydrocarbonea* in a patient with acute lymphoblastic leukemia. The patient was treated with amphotericin B with a successful outcome.

2. Case

A 19-year-old man was hospitalized with a one-week history (day −7) of adynamia, asthenia and pancytopenia (hematocrit 17%, white blood cell count (WBC) 1100/μL, lymphocytes 75%, granulocytes 22%, platelets 123,000/μL). He had received a transfusion of red blood cells. Signs of phlebitis had been identified and empiric piperacillin/tazobactam (18 g/d) had been prescribed. An ultrasonography scan had revealed a 30 × 20 mm collection of solid material under the skin plane of the intergluteal region.

At the time of admission (day 0) the patient was febrile (39 °C) and neutropenic. On day +4, he was diagnosed with acute lymphoblastic leukemia (ALL) and received a therapeutic drug regimen that included immunosuppressants and corticosteroids. On day +13, the collection in the intergluteal region was drained and sent for culture, which was...
negative. He received empiric antibiotic therapy (imipenem 2 g/d and vancomycin 2g/d). On day +26, he developed neutropenia, phlebitis and perianal painless erythema without desquamation. Perianal and phlebitis swab cultures were negative. Transcatheter blood, catheter and peripheral blood samples were collected. *Staphylococcus epidermidis* grew in the transcatheter blood and catheter cultures. The catheter was removed and a 14 days course of vancomycin (2 g/d) was prescribed. Yeast-like colonies were obtained from the peripheral blood culture, which showed morula-like structures compatible with *Prototheca* spp. on a lactophenol cotton blue (LPCB) wet mount preparation. Antifungal treatment of amphotericin B 5 mg/kg/d (lipid formulation) was prescribed for 10 days. Post-treatment blood cultures were negative.

The patient was a student who had finished high school, did not work, and lived in an urban area. He did not report any leisure activity or travel prior to hospitalization. Patient outcome was successful and he remained hospitalized to continue his ALL treatment until successful hospital discharge.

The yeast-like isolate was sent to the Department of Mycology of the National Reference Laboratory of the National Institute for Infectious Diseases “Dr. Carlos G. Malbrán” for further studies. On Sabouraud dextrose agar (SDA) after 7 days at 28 °C, the colonies showed the following characteristics: white color, irregular margin, rough surface, butty consistency and 10 mm of diameter. The isolate grew well at 37 °C and 40 °C on SDA. On Malt extract broth after 7 days at 28 °C, subglobose cells and subglobose to reniform sporangiospores were observed (Fig. 1). Glucose, galactose, sucrose, maltose, and trehalose fermentation tests were negative. The glucose assimilation test was positive, but the isolate was not able to assimilate any other of the following compounds: galactose, l-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, d-xyllose, l-arabino- se, d-ribose, D-mannitol, inositol, D-rhamnose, citrate, and nitrate. The urease test was negative. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was performed in a Microflex LT mass spectrometer (Bruker Daltonics, Bremen, Germany) by using both in-situ with formic acid 100% and in-tube with ethanol/formic acid/acetonitrile protein extractions and the Bruker Daltonics database MBT-5627 plus an in-house database named “LevDMic” version 1 [12]. In both cases, the result was a not reliable identification. DNA sequencing of the D1/D2 domain of the large subunit (LSU) rDNA showed 100% identity to the strain *P. zoophilii* var. *hydrocarbonacea* RND16 isolated from hot spring [13]. Consequently, the isolate was identified as *P. zoophilii* var. *hydrocarbonacea* and deposited in the culture collection of the Department of Mycology as DMic 154962 (GenBank accession number: MH352133). The phylogenetic analysis is shown in Fig. 2. After molecular identification, the main spectra (MSP) of the isolate DMic 154962 was included in the in-house database “LevDMic”, following manufacturer instructions. After that, the same isolate was successfully identified (with its own MSP) by using both in-situ with formic acid 100% and in-tube with ethanol/formic acid/acetonitrile protein extractions (scores values 2.399 and 2.786, respectively). Antifungal susceptibility tests were carried out by determining the minimum inhibitory concentration (MIC) according to the E. Def 7.3.1 reference document from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14]. The MIC values were 0.25 mg/L for amphotericin B, 128 mg/L for fluconazole, 0.064 mg/L for itraconazole and 0.125 mg/L for voriconazole.

3. Discussion

*Prototheca wickerhamii* is the most frequently species associated with human protothecosis [8]. On the other hand, *P. zoophilii* has been associated more frequently with bovine mastitis [7] and less commonly recovered from human infections. A literature review on human protothecosis, based on 160 total cases, showed that *P. wickerhamii* was responsible for 115 cases, whereas *P. zoophilii* was responsible for only 11 cases; the other 34 cases were not specified. Protothecosis has a worldwide distribution, equal sex distribution, and cases occur at all ages after the neonatal period but more frequently in older ages, with an average age of 53 years. *Prototheca* isolation was found not only in the surface of the skin but also in blood or inner organs. Host defense should be considered as a determining factor for disease progression, beyond direct tissue inoculation. The effects of immunocompromise and corticosteroid therapy produce a marked increase in the incidence of *Prototheca* infections; 67% of patients with one or both risk factors were diagnosed with protothecosis, whereas the incidence for those who were neither immunocompromised nor receiving corticosteroid therapy was 33% [8].

Normally, *Prototheca* infections tend to improve and be cured after antifungal treatment, but small skin lesions or olecranon bursitis can also be treated by surgical excision. These last two types of infections are more frequent than others, but it should be considered that a wide variety of infections have been reported. Systemic infections with a positive culture from bloodstream are less frequent; they represent only 6 of 160 cases described in this review. Intravenous amphotericin B alone was one of the common and effective treatments for *Prototheca* infections, with 77% success rate. When the treatment result was observed according to species, the success rate was 54% for *P. zoophilii* [8].

In the case presented here, the patient was a young man, whose age was far from the mean in Prototheca infections. We found no source of algaeamia in our patient. He had an immunocompromising condition and a history of immunosuppressant drug treatment, which are recognized risk factors to protothecosis. Despite the negative culture, soft tissue compromise might have been involved in the source of the *Prototheca* infection.

*Prototheca zoophilii* var. *hydrocarbonacea* was first described in 2002 based on a strain isolated from a hot spring in Japan [13]. This thermotolerant strain was found to produce an appreciable amount of ethanol and CO2 from glucose under anoxic conditions at 25 °C and 40 °C [13]. As a human pathogen, it has only been reported twice worldwide; the first time in 2007 and the second time in 2018, both of them as a causal agent of meningitis in China [5,16]. The ability of this variety to grow well at temperatures as high as 40 °C and the fact that it has been related to systemic infections reinforces its role as a human pathogen. This is the first report of this variety causing a bloodstream human infection in an immunocompromised host under corticosteroid treatment.

The identification of *Prototheca* species requires DNA-based methods, such as ribosomal DNA sequencing, not usually available in

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**Fig. 1.** Lactophenol cotton blue wet mount preparation showing globose to subglobose cells and sporangium with 2-6 subglobose to reniform sporangiospores.
Fig. 2. Phylogenetic tree based on the D1/D2 domain of the LSU rDNA. The tree was constructed using the Neighbor Joining method and Tamura-3 model. Numbers at nodes are percentage bootstrap from 1000 replicates. The Scale Bar represents the genetic distance. GenBank accession numbers are shown in parentheses.

clinical laboratories. MALDI-TOF MS is a useful technique that allows clinical laboratories to identify microorganisms at species level, including species that in the past could only be correctly identified by DNA-based methods. In our case, the isolate was not identified by using the manufacturer database, since it had no reference spectra of P. zopfi. However, the isolate was identified after the introduction of its own MSP in an in-house database. Other authors have already shown that this technique is useful for rapid identification of Prototheca species [17–19].

Regarding in-vitro susceptibility tests, our findings are in agreement with other reports highlighting the highest activity of amphotericin B, itraconazole and voriconazole (MIC values ≤ 0.25 mg/L).

Several antifungal agents have been used for the treatment of protothecosis. A study based on 174 cases reported since the first protothecosis in 1964 to April 2017 showed different success rates depending on the antifungal treatment (58%–72%). However, none was superior to the others in terms of statistical significance [7]. Nonetheless, amphotericin B was the most effective treatment (deoxycholate or lipid-associated preparations), followed by itraconazole and fluconazole. Amphotericin B treatment was used more frequently in serious infections than other antifungal agents. In addition, data on antimicrobial susceptibility from 21 cases showed that the organisms were in-vitro inhibited by amphotericin B [20]. Protothecosis in immunocompromised patients is rarely described and optimal antifungal treatment is controversial, probably due to difficulties related to the interpretation of in-vitro susceptibility tests and lack of clinical breakthroughs. Moreover, there is not enough evidence to suggest that P. zopfi treatment might be different from P. wickerhamii treatment [8].

In summary, we report a case of disseminated protothecosis due to P. zopfi var. hydrocarbonea in a patient with acute lymphoblastic leukemia, re-marking this variety as a human pathogen. The patient received amphotericin B treatment with a successful outcome.

References

[1] C. Lanz-Flied, A. Mayr, Human protothecosis, Clin. Microbiol. Rev. 20 (2007) 230–242, https://doi.org/10.1128/CMR.00032-06.
[2] R.S. Pore, E.A. Barnett, W.C. Barnes, J.D. Walker, Protothecosis ecology, Mycopathologia 81 (1983) 49–62.
[3] R.S. Pore, T.A. Shahan, Prototheca zopfi: natural, transient, occurrence in pigs and rats, Mycopathologia 101 (1988) 85–88.
[4] E.K.A. Camboim, F.J. Garino, A.F.M. Dantas, S.V.D. Simões, M.A. Melo, E.O. Azevedo, R.A. Mata, F. Riet-Correia, Protothecosis by Prototheca wickerhamii in goats, Mycosenes 54 (2011) e196–200, https://doi.org/10.1111/j.1439-0507.2011.01864.x.
[5] N. Hirose, Z. Hu, Y. Kato, Q. Zhang, R. Li, K. Nishimura, M. Masuda, Molecular Characterization of Prototheca strains isolated in China revealed the first cases of protothecosis associated with Prototheca zopfi genotype I, Med. Mycol. 56 (2018) 279–287.
[6] B.C.Q. Leimann, P.C.F. Monteiro, M. Lázéra, E.R.U. Candanoda, B. Wanke, Protothecosis, Med. Mycol. 42 (2004) 95–106.
[7] J.R. Todd, T. Matsumoto, R. Ueno, J. Murugaiyan, A. Britten, J.W. King, Y. Odaka, A. Oberle, C. Weise, U. Roesler, R.S. Pore, Medical mycology 2017, Med. Mycol. 56 (2018) S188–S204.
[8] J.R. Todd, J.W. King, A. Oberle, T. Matsumoto, Y. Odaka, M. Fowler, R.S. Pore, T.A. Shahan, L. Yin, I.D. Sunami, Protothecosis: report of a case with 20-year follow-up, and review of previously published cases, Med. Mycol. 50 (2012) 673–689.
[9] U. Roesler, A. Möller, A. Hensel, D. Baumann, U. Truyen, Diversity within the current algal species Prototheca zopfi: a proposal for two Prototheca zopfi genotypes and description of a novel species, Prototheca blakshoei sp. nov., Int. J. Syst. Evol. Microbiol. 56 (2006) 1419–1425.
[10] K. Satoh, K. Ooe, H. Nagayama, K. Makimura, Prototheca cutis sp. nov., a newly discovered pathogen of protothecosis isolated from inflamed human skin, Int. J. Syst. Evol. Microbiol. 60 (2010) 1236–1240.
[11] M. Masuda, N. Hirose, T. Ishikawa, Y. Ikawa, K. Nishimura, Prototheca miyajii sp. nov., isolated from a patient with systemic protothecosis, Int. J. Syst. Evol. Microbiol. 66 (2016) 1510–1520.
[12] C.G. Taverna, M. Mazza, N.S. Bueno, C. Alvarez, S. Amigot, M. Andreani, N. Azula, R. Barrios, N. Fernández, B. Fox, L. Guelfand, I. Maldonado, O.A. Muriensgo, S. Relloso, M. Vivet, G. Davel, Development and validation of an extended database for yeast identification by MALDI-TOF MS in Argentina, Med. Mycol. (2018), https://doi.org/10.1093/mmy/myy021.
[13] R. Ueno, N. Urano, M. Suzuki, S. Kimura, Isolation, characterization, and fermen-tative pattern of a novel thermotolerant Prototheca zopfi var. hydrocarbonea strain producing ethanol and CO2 from glucose at 40 degrees C, Arch. Microbiol. 177 (2002) 244–250.
[14] M.C. Arendrup, I. Meletiadis, J.W. Mouton, K. Lagrou, P. Hamal, J. Guinea, The Subcommittee on Antifungal (AFST) of the ESCMID European Committe for Antimicrobial Susceptibility Testing (EUCAST), EUCAST DEFINITIVE DOCUMENT EDEF 7.3.1. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts, (2017) http://www.euCAST.org.
[15] H.A. Torres, G.P. Bodey, J.J. Tarrand, D.P. Kontoyiannis, Protothecosis in patients with cancer: case series and literature review, Clin. Microbiol. Infect. 9 (2003) 786–792.
[16] S. Liu, Y. Ran, X. He, D. Zhang, Y. Dai, Identification of a strain Prototheca zopfi var. hydrocarbonea by analyzing the sequence of ribosome RNA gene, Chin J Zoon 23 (2007) 656–660 [In Chinese].
[17] M. von Bergen, A. Eidner, F. Schmidt, J. Murugaiyan, H. Wirth, H. Binder, T. Maier, Acknowledgements

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Conflict of interest

None.

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U. Roesler, Identification of harmless and pathogenic algae of the genus *Prototheca* by MALDI-MS, Proteomics Clin. Appl. 3 (2009) 774–784.

[18] J. Murugaiyan, J. Ahrholdt, V. Kowbel, U. Roesler, Establishment of a matrix-assisted laser desorption ionization time-of-flight mass spectrometry database for rapid identification of infectious achlorophyllous green micro-algae of the genus *Prototheca*, Clin. Microbiol. Infect. 18 (2012) 461–467.

[19] J. Ahrholdt, J. Murugaiyan, R.K. Straubinger, T. Jagielski, U. Roesler, Epidemiological analysis of worldwide bovine, canine and human clinical *Prototheca* isolates by PCR genotyping and MALDI-TOF mass spectrometry proteomic phenotyping, Med. Mycol. 50 (2012) 234–243.

[20] V. Krcmér, Systemic chlorellosis, an emerging infection in humans caused by algae, Int. J. Antimicrob. Agents. 15 (2000) 235–237.