Case Report

Probable case of *Cephalotheca foveolata* bloodstream infection

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Introduction: *Cephalotheca foveolata* is a saprobic fungus recently described among clinical isolates.

Case presentation: A 59-year-old man presented with fevers, upper back pain and shortness of breath after gastric tube removal following an oesophagectomy, oesophagojejunostomy and gastrostomy for management of oesophageal adenocarcinoma. He had a suspected oesophageal leak and polymicrobial empyema. Blood cultures grew only *C. foveolata*. He improved clinically on empiric antifungal and broad-spectrum antibacterial therapy.

Conclusion: We report a probable case of *C. foveolata* bloodstream infection. This is a newly recognized pathogen requiring molecular diagnostic methods to make an accurate diagnosis.

Keywords: *Cephalotheca foveolata*; saprobic fungus; bloodstream infection.

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Introduction

Unusual fungal pathogens are increasingly reported to cause disease in humans. Identification of rare and novel pathogens can be challenging, and often requires molecular diagnostic methods. The difficulty in identifying these pathogens may result in underdiagnosis. Saprobic fungi in the genus *Cephalotheca* have rarely been reported to cause human disease.

*Cephalotheca* spp. are found in the soil and on other environmental matter. These fungi exhibit both teleomorph (sexual) and anamorph (asexual) stages in nature and the laboratory. The anamorphs are similar in morphology to the *Phialamonium* and *Acremonium* spp., which are known to cause invasive human disease (Gavin et al., 2002; Kan et al., 2004; King et al., 1993). In 2006, the first case of subcutaneous hyalophyomycosis due to *Cephalotheca foveolata* was reported in a 67-year-old immunocompetent farmer with erythematous plaques of 10 years duration on his right foot (Suh et al., 2006). An additional six clinical isolates have been reported, recovered from eyes, lymph nodes, cardiac tissue and bronchial lavage fluid in a series of emerging fungal pathogens (Perdomo et al., 2011). The clinical context to ascertain whether these isolates were suspected to be the causal agent of infection and the immune status of patients were not available in these additional cases. Although it was not possible to confirm that this organism caused infection in these cases, the increasing frequency of isolation of this fungus from clinical samples and its ability to grow at 37°C suggest that it may have pathogenic potential.

In this report, we describe the first case, to the best of our knowledge, of a probable *C. foveolata* bloodstream infection. This report adds to existing evidence that *C. foveolata* may be a newly appreciated human pathogen.

Case report

A 59-year-old man with a history of morbid obesity for which he underwent a distant Roux-en-Y gastric bypass, non-alcoholic cirrhosis and newly diagnosed oesophageal adenocarcinoma underwent distal oesophagectomy, oesophagojejunostomy, gastrostomy, and thoracic and abdominal lymph node dissection with adjuvant cisplatin/paclitaxel and radiation therapy. He had a routine gastric tube placed for enteral nutrition. After its removal 1 month later, he began to experience increasing upper back pain, cough and low-grade fever.

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Abbreviations: ITS, internal transcribed spacer; MEC, minimum effective concentration.

The GenBank/EMBL/DDBJ accession number for the ITS and D1/D2 sequences of *Cephalotheca foveolata* determined in this study are KJ573100 and KJ573101, respectively.

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Investigations

The patient presented to an outside hospital where a computed tomography scan of his chest showed a large right hydropneumothorax with a possible oesophageal perforation. Initial laboratory findings were significant for a leukocyte count of $34.6 \times 10^9 \text{ l}^{-1}$ with a leukocyte differential cell count of 94.8% neutrophils. His alkaline phosphatase was $167 \text{ U l}^{-1}$ and total bilirubin $37.6 \mu\text{mol l}^{-1}$, with direct bilirubin $32.5 \mu\text{mol l}^{-1}$. His transaminases, renal function and electrolytes were unremarkable. A chest tube was placed with a creamy tan output. A Gram stain of this output showed Gram-negative rods and budding yeast with pseudohyphae. No cultures of pleural fluid were done. He was intubated and transferred to our hospital, where his chest tube was removed and five new drains were placed in mediastinal collections from which cultures grew *Candida albicans*, *Candida krusei*, *Klebsiella pneumonia*, a *Lactobacillus* sp. and alpha-haemolytic *Streptococcus*. Subsequently, one bottle from one set of blood cultures drawn prior to antibiotic administration at the outside hospital grew a fungus at 4 days with fungal elements noted. Other blood cultures drawn were negative, and none was subcultured. A transthoracic echocardiogram did not identify valvular vegetations. An ophthalmological examination did not identify evidence of endophthalmitis. He had a history of pedal fissures more than 5 years ago requiring moisturizing emollients but otherwise had minimal risk factors for environmental exposure including minimal outdoor activities and no distant travel. He tested negative for human immunodeficiency virus type 1 and 2 antibodies several months earlier during his initial oesophageal adenocarcinoma work-up.

Diagnosis

Mycology

Gram staining of initial positive blood cultures showed fungal elements. Further subculture on various media showed pseudohyphae but were insufficient for purposes of identification by colony and microscopic morphology. Therefore, the isolate was referred to the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center at San Antonio, TX, USA (isolate UTHSCSA DI 14-21), for identification by combined phenotypic characterization and DNA sequencing. Colonies on potato flakes agar (PFA) and V8 agar, prepared in house, were yellow–brown, velvety and effuse, produced a yellowing diffusing pigment after 3 days’ incubation at 25 °C (Fig. 1a, b). On Sabouraud dextrose agar (SDA) (Remel), colonies were yellowish grey and produced a brown to reddish diffusing pigment. Growth on carnation leaf agar (CLA) prepared in house restricted filamentous growth but enhanced the development of cleistothecia (Fig. 1d–f). Microscopically, a *Phialemonium*-like anamorph was present on PFA, V8 agar and SDA, exhibiting cylindrical conidia borne from reduced phialides, some of which lacked basal septa (Fig. 1g, h). Black, cleistothecial ascomata and reddish-brown ascospores were produced on both PFA and CLA after 25 days’ incubation at 25 °C. Examination of sexual structures by scanning electron microscopy revealed ciliated cleistothecia that appeared to be five to six cell layers thick (Fig. 2a, b) containing numerous ascospores (Fig. 2a–c). The isolate has been deposited in the University of
Alberta Microfungus Collection under accession number UAMH 11820.

Molecular characterization

For DNA sequencing, template DNA was prepared by subculturing the isolate onto PFA and incubating at 30 °C for 24 h. Hyphal elements were scraped from the agar surface, suspended in CPL-100 Buffer (VWR International), lysed in a bead beater instrument and isolated manually by chloroform extraction. Extracted DNA was used for PCR amplification of the internal transcribed spacer (ITS) and D1/D2 regions of the 28S rRNA subunit as described previously with slight modifications (Romanelli et al., 2010). PCR products were then sequenced using the ITS1 and ITS4 primers, as well as the NL1 and NL4 primers, at the UTHSCSA Molecular Diagnostics Laboratory (White, 1990). Sequences were assembled and analysed using DNASTAR SeqMan Pro (DNASTAR) and queried in GenBank using the BLASTN algorithm at the NCBI site (http://www.ncbi.nlm.nih.gov). Sequences were also compared with those available in the CBS-KNAW Fungal Biodiversity Centre database (http://www.cbs.knaw.nl). The ITS sequence of this isolate showed 99.7% nucleotide identity to *C. foveolata* (GenBank accession no. AB278171.1; base pair match 564/566) and a 99.8% nucleotide identity match to a previous *C. foveolata* isolate identified at the Fungus Testing Laboratory (GenBank accession no. HE599376; base pair match 487/488), and the D1/D2 sequence also showed 100% identity to *C. foveolata* (GenBank accession no. AB178269.1; base pair match 605/605).

Susceptibility testing

Antifungal susceptibility testing was performed by the Fungus Testing Laboratory according to Clinical and Laboratory Standards Institute guidelines for filamentous fungi (CLSI, 2008). For amphotericin B, itraconazole, posaconazole and voriconazole, the MIC was defined as the lowest concentration of each agent that resulted in 100% inhibition of growth after 48 h of incubation at 35 °C. For caspofungin, the minimum effective concentration (MEC) was defined as the lowest concentration of drug that resulted in abnormal growth characterized by short, stubby, abnormally branched hyphae after 24 h of incubation.

The in vitro activities of the antifungal agents tested against this isolate are shown in Table 1. Although MIC data against *C. foveolata* are not available from large surveillance studies, these results were consistent with the limited data available in the Fungus Testing Laboratory antifungal susceptibility database against *C. foveolata* and those reported from previous case reports in the literature (Perdomo et al., 2011; Suh et al., 2006).

Treatment

The patient initially received vancomycin, ceftazidime, metronidazole and fluconazole for empiric treatment of polymicrobial empyema as a complication of a possible oesophageal leak. As described above, mediastinal tubes were placed for drainage. In response to the blood culture

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC/MEC (μg ml⁻¹) for the case isolate</th>
<th>Previous MIC data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>4</td>
<td>4–16</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0.125</td>
<td>1</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>1</td>
<td>1–2</td>
</tr>
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</table>
fungal growth, his antifungal regimen was transitioned from fluconazole to micafungin while awaiting identification. He eventually underwent a right thoracotomy and pulmonary decortication, after which his chest tubes were removed and he was discharged home with a jejunostomy feeding tube in place at 1 month. He was tolerating sips of water with his jejunostomy quickly on broad-spectrum antibacterials and antifungals, and was found to have micafungin-resistant Candida sp. and other oropharyngeal bacterial flora, pathology from this decortication did not identify fungal forms on staining. Decortication material was not cultured. He completed a course of micafungin that extended 2 weeks beyond removal of his chest tube, for a total of approximately 6 weeks.

Outcome and follow-up
His repeat blood cultures were negative. He defervesced quickly on broad-spectrum antibacterials and antifungals, and was tolerating sips of water with his jejunostomy tube in place at 1 month.

Discussion
We have presented a probable case of bloodstream infection with C. foveolata. This organism has been isolated previously from diverse tissues including the eye and cardiac tissue, although the clinical significance of these findings is unclear (Perdomo et al., 2011). This is the first case, to the best of our knowledge, of isolation of this organism from the blood. At the time, the patient had a polymicrobial empyema presumed to be due to an oesophageal leak. We believe it is most likely that this organism entered the blood from skin barrier breakdown in the setting of his multiple surgeries or through disrupted oropharyngeal integrity, thus providing a route of entry through the oesophageal leak. Additionally, his recent chemotherapy and multiple surgeries may have increased his risk of infection by depressing immune system function.

In the limited studies available characterizing the oral and pulmonary fungal microbiome in humans and mice, Cephalotheca spp. have not been identified (Charlson et al., 2012; Ghannoun et al., 2010; Scupham et al., 2006). Studies of skin flora that have caused bloodstream infections have not included Cephalotheca spp. (Paulino et al., 2006). Nevertheless, the pathogenic potential of this organism as part of the ‘rare biosphere’ (Huffnagle & Noverr, 2013) either by ingestion or skin barrier breakdown leading to translocation into the bloodstream is a possibility. The patient did not have any particular exposures such as farming that would expose him to soil pathogens, as was the case in previously described cases of skin and soft-tissue infection with this organism (Suh et al., 2006). Given the limited case reports and environmental surveys of C. foveolata, it is possible that this organism is a contaminant. While we cannot wholly exclude this possibility, this is the first isolate of C. foveolata reported from our group of two quaternary academic centres and five community hospitals. Additionally, only C. foveolata and no other organisms grew from the blood in a relatively short time frame (4 days) in this patient, whilst he had clinical signs of infection including fevers and shortness of breath.

Accurate identification may be difficult, as C. foveolata requires a sufficient length of incubation for development of the sexual state. Cultures may be mistakenly discarded after observance of the anamorphic state. C. foveolata produces characteristic dark, ciliated cleistothecia and hyaline to brown, foveolate (delicately pitted), kidney-shaped ascospores (Sutton, 2008; Yaguchi et al., 2006). The anamorph of C. foveolata is similar in morphology to Phialemonium and Acremonium spp., which are known to cause invasive human disease. Molecular characterization by DNA sequencing is usually required for species identification, as was the case for this patient.

In conclusion, the true mode of acquisition in this patient is uncertain, and the environmental niche and pathogenic potential of C. foveolata is not well understood. We believe this case report contributes to the existing literature supporting the pathogenic potential of this organism. The incidence of infections with C. foveolata is not known and may be elucidated in the future with the use of molecular diagnostic methods.

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