Case Report

Central venous catheter infection associated with *Pseudozyma aphidis* in a child with short gut syndrome

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Pseudozyma aphidis is a heterobasidiomycetous yeast related to the smut fungi in the genus *Ustilago*. *Pseudozyma* species are usually isolated from plants and rarely from clinical specimens. We report what is believed to be the first paediatric case of central venous catheter (CVC)-related fungaemia associated with *P. aphidis*. Prompt removal of the CVC in conjunction with anti-fungal therapy resulted in a successful outcome.

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Introduction

The fungal class *Ustilaginomycetes* contains more than 70 genera of moulds that are well-known as parasites of wheat, corn and grasses. The genus *Ustilago* comprises smut fungi, pathogens of corn with sophisticated life cycles including sexual and asexual (yeast) morphologies (Bauer *et al.*, 1997). The best known member of this group is *Ustilago maydis*. *Pseudozyma aphidis* is a heterobasidiomycetous yeast related to the smut fungi in the genus *Ustilago*. Although a few reports of human infections caused by *U. maydis* have been published in the literature (Teo & Tay, 2006; Patel *et al.*, 1995), little is known about the pathogenicity of *Pseudozyma* species. One recent report describes their isolation from three adult clinical specimens (Sugita *et al.*, 2003), but their association with human disease is unclear. To the best of our knowledge, we report the first case of central venous catheter (CVC) infection due to *Pseudozyma* species in a child with short gut syndrome.

Case report

A 7-year-old Hispanic female, who was born with gastroschisis and ileocolonic atresia resulting in short bowel syndrome, presented to the paediatric surgery clinic with a 1 week history of intermittent fevers (up to 39.7 °C) accompanied by chills, malaise and fatigue. Due to her

short gut syndrome, she had been maintained on chronic parenteral nutrition since birth. Her parenteral nutrition was delivered via a gastrostomy tube and consisted of Peptinex, a high nitrogen, low fat, 100 % peptide and free amino acid formula that promotes rapid absorption of nutrients for patients with short gut syndrome. She received the parenteral nutrition through a long-term indwelling central line. Her CVC had been replaced three times since birth due to line infections. She was admitted to the Pediatric Surgery Service, Brenner Children's Hospital, Winston-Salem, NC, USA, and started on intravenous vancomycin (45 mg kg⁻¹ per day, given as 15 mg kg⁻¹ every 8 h) and ceftazidime (50 mg kg⁻¹ per day, given as 15 mg kg⁻¹ every 8 h) for possible CVC-related bacterial infection. Initial blood cultures obtained on admission, and drawn through the CVC, yielded >100 c.f.u. yeast ml⁻¹ and >100 c.f.u. coagulase-negative *Staphylococcus* ml⁻¹, which was resistant to oxacillin (MICs of >2 µg ml⁻¹) and susceptible to clindamycin, vancomycin, and gentamicin with MICs of ≤25, ≤2 and ≤1 µg ml⁻¹, respectively. Subsequent blood cultures obtained on the second and third day of hospitalization were positive for yeast (>100 c.f.u. ml⁻¹) but the bacteraemia had resolved. Based on this finding, intravenous fluconazole (loading dose of 10 mg kg⁻¹ followed 24 h later by a 5 mg kg⁻¹ per day maintenance dose) was started to treat fungaemia due to a possible candida infection. An echocardiogram showed no evidence of intracardiac vegetations or thrombus at the tip of the CVC. High fevers up to 39.3 °C persisted during the first 4 days of hospitalization despite intravenous fluconazole therapy. The CVC was removed on the fifth day of hospitalization, which resulted in prompt clinical

Abbreviation: CVC, central venous catheter.

The GenBank/EMBL/DDBJ accession number for the ITS sequence of *Pseudozyma aphidis* reported in this paper is bankit1010363.

improvement with resolution of the fever and two subsequent blood cultures were negative for yeast. Once the definitive identification and susceptibility data for the yeast were available, fluconazole was discontinued and itraconazole (5 mg kg⁻¹ per day) was added to her medication regimen. She was treated with a 14 day course of intravenous vancomycin, and was discharged home with a 10 day course of oral itraconazole. When last seen at follow-up she was well.

Microbiological studies

Processing of the specimen, culture and procedures for identification of the yeast were performed in the Clinical Mycology Laboratory at Wake Forest University Health Sciences. The blood specimen yielded several colonies on chocolate (Becton Dickson) and Sabouraud dextrose (Gibson Laboratories) agars after 2 days of incubation at 37 °C in ambient air. Mature, moist yeast-like colonies were tan-yellow and wrinkled after 4 days of incubation at 37 °C (Fig. 1). A similar morphology was observed on Sabouraud dextrose plates incubated at 25 °C in ambient air. Wet mount showed fusiform spindle-shaped blastoconidia that were elongate and slightly irregular (Fig. 2). Germ tube production was negative. Identification of the isolate was attempted by using a commercial yeast identification kit (Uni-Yeast Tek plates; Remel). The organism was positive for urea hydrolysis and for assimilation of sucrose, lactose, maltose, raffinose, soluble starch, trehalose and nitrate, and was negative for assimilation of cellobiose. Although the yeast identification system failed to provide an identification of the isolate, the morphological and biochemical features suggested that the organism was yeast of the order *Ustilaginales*. Susceptibility testing was performed by broth microdilution using the YeastOne system (Trek Diagnostic) according to the manufacturer's recommendations. The isolate showed MICs of 4 µg ml⁻¹, 0.125 µg ml⁻¹ and

Fig. 1. Sabouraud dextrose plate demonstrating tan-yellow and wrinkled colonies consistent with *Pseudozyma* species after 4 days of incubation at 37 °C in ambient air.

Fig. 2. Wet mount performed from Sabouraud dextrose agar after 4 days incubation at 37 °C in ambient air showing elongated spindle-shaped blastoconidia consistent with *Pseudozyma* species.

0.25 µg ml⁻¹ to fluconazole, itraconazole, and amphotericin B, respectively.

The isolate was sent to the Myotic Diseases Branch, Centers for Disease Control and Prevention, for species identification. The isolate grew on potato dextrose agar at 30 and 37 °C, and displayed fusiform morphology with polar budding. DNA was isolated and sequenced on an ABI 3700 instrument as previously described (Brandt *et al.*, 2003). A 695 nt sequence was generated using primers ITS-5 and ITS-4, which amplified the intervening transcribed spacer region of the rDNA (White *et al.*, 1990). A BLAST search displayed a 100 % match to all the *P. aphidis* ITS sequences in the GenBank database. The isolate was submitted to a stock collection. The DNA sequence was submitted to GenBank with the accession number bankit1010363. Taking the morphology, biochemical, and nucleotide sequence data together, the final identification was reported as *P. aphidis* (Boekhout & Fell, 1998).

Discussion

Invasive disease due to ustilaginomycete yeasts is very unusual in humans. Although *Pseudozyma* species have not been clearly associated with disease in humans, the clinical presentation along with persistently positive cultures from the CVC suggest that *Pseudozyma* was the pathogen in this case. A review of the literature yielded only one report of *Pseudozyma* species and four reports of *Ustilago* species as documented adult clinical infections (Teo & Tay, 2006; Patel *et al.*, 1995; Sugita *et al.*, 2003). In relation to the *Ustilago* species infections, one patient had a chronic skin rash manifesting as scaly erythematous plaques over the nasal alae and philtrum and the other had a CVC infection (Teo & Tay, 2006; Patel *et al.*, 1995). In published reports, airborne *Ustilago* spores have been implicated in hypersensitivity pneumonitis, asthma and allergic rhinitis (Yoshida *et al.*, 1996; Santilli *et al.*, 1985). There is one report of *Pseudozyma* species isolated from the blood of

three adult patients, but the authors did not present the clinical characteristics of these patients or the clinical significance of the organisms (Sugita *et al.*, 2003). To the best of our knowledge, our patient is the only reported paediatric patient having invasive disease due to *P. aphidis*.

The mechanism of pathogenicity in this heterobasidiomycetous yeast is poorly understood (Perez-Martin & Castillo-Lluya, 2006; Kamper *et al.*, 2006). However, in recent years, *U. maydis* has become an attractive model for studying the relationships between cell cycle and virulence in pathogenic fungi (Perez-Martin & Castillo-Lluya, 2006; Kamper *et al.*, 2006). The genome sequence for *U. maydis* has been recently described, which may help with the elucidation of novel mechanisms of pathogenicity in biotrophic fungi and provide information about the pathogenicity of other related yeasts, including *Pseudozyma* species (Kamper *et al.*, 2006).

The present case has many similarities with the isolated case reported in the adult patient literature about *Ustilago* fungaemia (Patel *et al.*, 1995). Both patients had short gut syndrome secondary to partial small bowel resections. Both had CVCs and received chronic parenteral nutrition as well as suffering from multiple CVC infections. Since *Ustilago* species and *Pseudozyma* species are associated with corn in the environment (Martinez-Espinoza *et al.*, 2002; Miller & Harun, 1978), dietary history is important while evaluating patients with invasive disease caused by these yeasts. There was no unusual exposure to corn in the adult patient. In contrast, our patient admitted to have consumed large amounts of tortilla corn chips. In both patients, there was no history of consumption of huitlacoche, a popular Mexican delicacy made from *U. maydis*, and approved by United States Food and Drug Administration (Valverde *et al.*, 1995).

In our patient, the precise portal of entry of the *P. aphidis* infection is unclear. CVC contamination through hand-related transmission is possible. Alternatively, ingestion of tortilla corn chips containing the fungus could be the source of *P. aphidis* infection. The patient's short gut syndrome may have compromised the gastrointestinal mucosa and facilitated the passage of the fungal strain into the blood stream. Compromised gut integrity or anatomy, such as that caused by mucosal ulcerations or mucositis (e.g. in patients with cancer who may be receiving cytotoxic chemotherapy or undergo invasive procedures involving the gastrointestinal tract, or those with short-gut syndrome), may allow translocation of fungus across intestinal mucosal barriers, and lead to fungaemia and invasive disease.

Due to the rarity of *Pseudozyma* species infection in humans, data on management are extremely limited. Although no MIC breakpoints exist for this organism, limited susceptibility data suggested that some *Pseudozyma* species are resistant to flucytosine and susceptible to amphotericin B (Sugita *et al.*, 2003). Another report states that itraconazole and amphotericin B may be effective for

Ustilago species (Teo & Tay, 2006). Prompt removal of the CVC in our patient in conjunction with a short course of antifungal therapy resulted in a successful outcome. In conclusion, our case indicates that physicians caring for children with short gut syndrome need to be aware of these unusual yeasts as a potential source of fungaemia and CVC-associated infection.

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