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First case report of bloodstream infection by *Rhizomucor pusillus* in a child with hemophagocytic lymphohistiocytosis

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ABSTRACT

We describe an unusual presentation of fatal infection due to *Rhizomucor pusillus* bloodstream infection in a 12-year old pediatric patient recently diagnosed with hemophagocytic lymphohistiocytosis. *R. pusillus* was isolated from one blood culture drawn on Day 11 of hospitalization.

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1. Introduction

Mucormycosis is a life-threatening fungal infection caused by fungi of the order *Mucorales*. Members of the *Mucoraceae* family within the order *Mucorales* that are implicated in human disease are the *Rhizopus, Mucor, Lichtheimia, Rhizomucor* and *Apophysomyces* species [1]. These fungi are ubiquitous in soil, decaying vegetation, manure and food. Infections in patients are most commonly a result of aerosolization of the spores, leading to invasion of tissue from the respiratory tract. Gastrointestional infections have also been reported due to ingestion of contaminated food [2]. In the immunocompromised host, mucormycosis is the second most common mold infection and increase in prevalence has been particularly evident in hematopoietic stem cell transplant (HSCT) and hematologic malignancy patients [3]. Contrary to other filamentous fungal pathogens, mucormycosis can be rapidly progressive and fatal in immunocompetent hosts as well, including individuals with diabetes mellitus [4] and intravenous drug users [5].

The risk factors attributed to invasive mucormycosis are generally similar among adult and pediatric population; major clinical characteristics include neutropenia, diabetes mellitus, bone marrow transplantation, and ketoacidosis. A major underlying cause of comorbid condition seen in pediatric patients with mucormycosis is prematurity. Prematurity is significantly associated with gastrointestional and cutaneous disease whereas clinical presentation seen in older children is more commonly pulmonary or sinuorbitaocerebral [6]. Common clinical patterns of mucormycosis in pediatric patients are cutaneous, gastrointestinal, rhinocerebral and pulmonary [7].

The signs, symptoms, and radiographic findings of mucormycosis are generally non-specific, and direct identification of the characteristic hyphae or recovery of the organism in culture is required for definitive diagnosis. The sensitivity and time to detection of conventional diagnostic tools remain suboptimal. This is particularly evident in the low recovery rate of *Mucorales* from blood cultures, despite the angioinvasive nature [8]. While automated blood culture systems have high recovery rates of bacteria and yeasts, recovery of most filamentous fungi from blood culture bottles is suboptimal and typically has no diagnostic value [9].

2. Case

A 12 year old girl with a history of hepatitis associated with Epstein Barr Virus (EBV) infection was presented to our institution

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(Day 0) with new onset fever and mild upper respiratory tract infection symptoms including cough and rhinorrhea. On examination, she was noted to have massive hepatomegaly and pallor. Initial labs were concerning for significant thrombocytopenia (platelet count of 32,000/uL), anemia (hemoglobin 6 g/dL, hematocrit 17%), evidence of liver failure (PTT > 150 s, fibrinogen < 40 mg/dL, albumin 2.2 g/dL, conjugated bilirubin 7.4 mg/dL, and AST 552 Units/L), elevated ferritin (21,300 ng/mL), and abnormal triglycerides (420 mg/dL). Her EBV viral load at presentation was > 5,000,000 copies/mL. Studies for methicillin resistant *Staphylococcus aureus* colonization, cytomegalovirus, and hepatitis (A, B, and C) were all negative. The patient met the clinical definition for hemophagocytic lymphohistiocytosis (HLH) associated with EBV infection.

On Day 2 of her admission, she experienced a generalized tonic–clonic seizure. She was intubated and required blood pressure support with continuous infusions of epinephrine and dopamine. At this time, she developed a fever to 39.4 °C. Two sets of blood cultures and urine cultures were drawn. Antibacterial coverage was initiated with piperacillin–tazobactam (400 mg/kg/day) and vancomycin (40 mg/kg/day). A computed tomography scan of her head with and without contrast was unremarkable. She was started on HLH 2004 modified protocol consisting of twice weekly etoposide and daily high-dose dexamethasone in an effort to bring her HLH under control. Femoral arterial and venous lines were placed.

Over the next few days, she developed anuria requiring continuous renal dialysis support. Blood and urine cultures from the onset of her fever were negative. Vancomycin was discontinued, but piperacillin–tazobactam was continued throughout her hospital course. By Day 5 of admission, she was afebrile and no longer required epinephrine or dopamine for blood pressure support. Her absolute neutrophil count dropped to below 150 cells/μL where it remained through the remainder of her hospital course. She intermittently required platelet transfusions and cryoprecipitate for bleeding. She was on minimal ventilator settings and looked much improved over the next few days.

The patient developed new low-grade fevers to 38.1 °C on Day 9 of hospitalization. Three sets of blood cultures were sent (2 from lines and 1 peripheral), all eventually were negative at Day 5. Clinically she appeared stable though she remained intubated and required continued dialysis. On Day 11 of admission, she acutely decompensated requiring maximal respiratory and blood pressure support. She developed profound hypothermia and bloody secretions from her endotracheal tube. Another aerobic blood culture was drawn. Before antibiotic coverage could be broadened, she experienced cardiac arrest and died despite resuscitation efforts. Family declined autopsy.

Fungal growth was detected at 48 h from the aerobic blood bottle collected on Day 11 of admission using the BacT/Alert (bioMérieux, Marcy l'Etoile, France) automated blood culture system. The system initially alarmed within 24 h but no organism was noted on Gram stain so bottle was subcultured onto blood agar and chocolate agar and placed back into the BacT/Alert. The bottle was flagged three more times over the next 24 h period and procedure was repeated; all subcultures were incubated at 35 °C in 5% CO_2 and no growth was seen in all sets of plates. At Day 3 of incubation in BacT/Alert, probable fungal balls were visualized in the blood culture bottle (Fig. 1) and a few broad hyphae were seen using calcofluor white stain. The organism was subcultured to sabouraud agar and incubated at 30 °C in ambient air. Greyish-brown, velvety colonies grew after 48 h incubation and were transferred to potato dextrose agar. Thermotolerance test was performed and the isolate grew best at 42 °C and 35 °C followed by 30 °C but was unable to grow at 25 °C. Growth on media was atypical of the Zygomycetes where the colonies did not fill the agar plate, even at the preferential

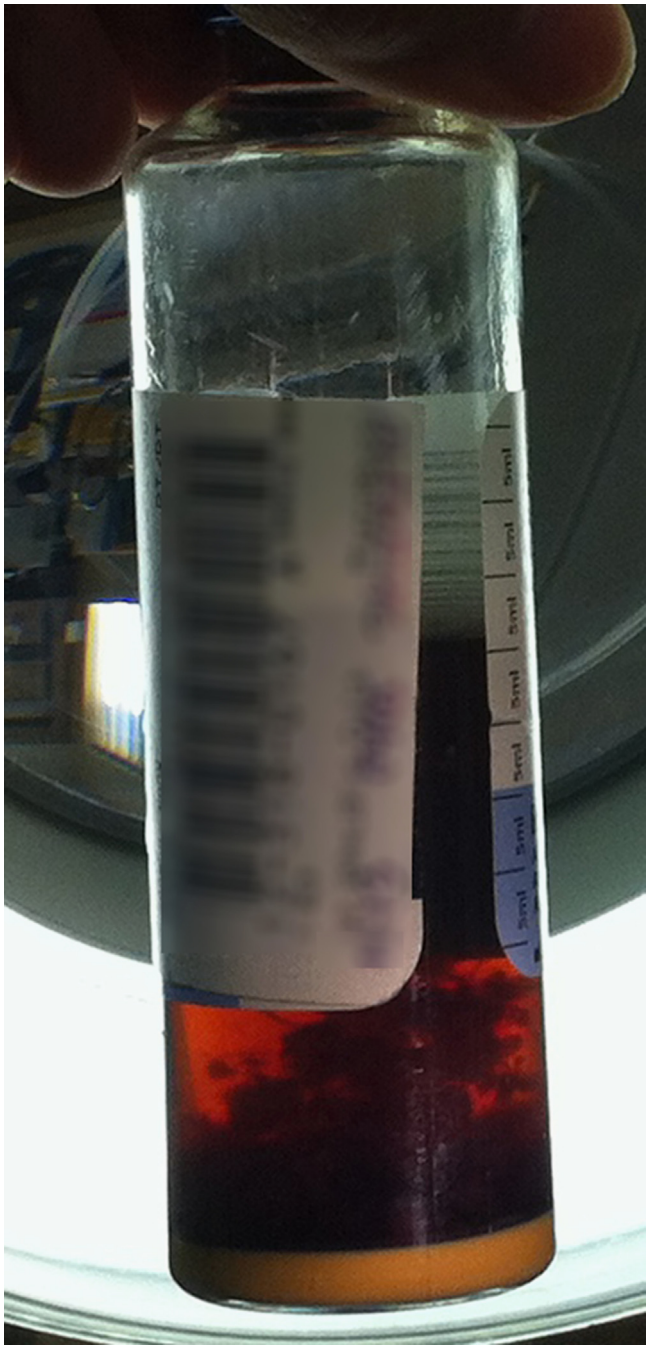

Fig. 1. BacT/Alert aerobic blood culture bottle. Demonstration of fungal balls growing after 72 h of incubation.

temperature of 42 °C (Fig. 2a). Slide cultures revealed a mold with large, non-septate hyphae, hyaline, branched sporangiophore (8–11 μm), subglobose, yellowish-brown sporangia approximately 70 μm in diameter, and subglobose columellae (20–35 μm); rhizoids were visualized but scarce (Fig. 2b). Based on microscopic morphology, organism was reported as *Rhizomucor* spp.

Definitive identification of the fungi was obtained by amplifying the internal transcribed spacer (ITS) 1 and 2 regions (ITS1 and ITS2) followed by bidirectional sequencing of the PCR product [10]. Resulting sequence were submitted to a GenBank Basic Local Alignment Search Tool software (BLAST) and > 99% similarities to *Rhizomucor pusillus* [AJ853748] sequence was obtained. In addition, an ITS-BLAST search (www.mycologylab.org) also confirmed the GenBank result [WM 04.476]. The sequencing results

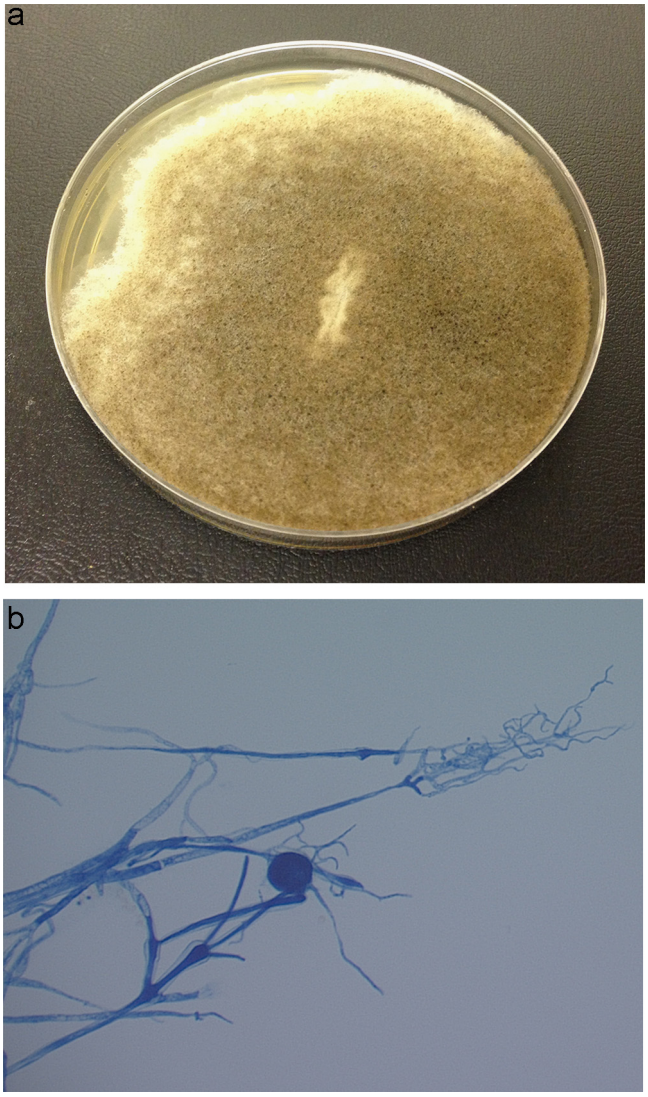

Fig. 2. (a) Macroscopic illustration of *Rhizomucor pusillus*. Preferential growth on potato dextrose agar after 48 h incubation at 42 °C. (b) Microscopic illustration of *Rhizomucor pusillus*. Presence of rare rhizoids on slide culture.

correlated with the microbiological findings in the microbiology laboratory for *R. pusillus*.

3. Discussion

To our knowledge, there have only been two previously reported cases of mucormycosis diagnosed by positive blood culture [11,12]. *Rhizopus rhizopodiformis* was isolated from routine blood culture obtained post-mortem from one pediatric patient [11] and *Mucor circinelloides* was isolated from central venous catheter (CVC) line of a 48 year old patient with short gut syndrome [12].

While the majority of localized cutaneous mucormycosis cases are successfully resolved with anti-fungal agents, rare cases of disseminated mucomycosis are associated with high mortality [1,6,7]. In a review of 929 reported cases of mucormycosis, generalized dissemination occurred in 2.7% of cases with 100% mortality rate [1]. Moreover, timely treatment is pertinent to overall survival as rate in patients not treated with anti-fungal therapy was 3% compared to 70% survival rate in patients treated with anti-fungal and surgery. A multi-variate regression analysis of 157 pediatric patient cases demonstrates that risk of death is 7 times more likely in disseminated cases [6]. In this study, receipt of anti-fungal therapy significantly reduced risk of death by 92%.

R. pusillus is a thermophilic saprophytic mucormycetes ubiquitous in air, soil, water and organic matter, including a variety of food items. *R. pusillus* is not commonly associated with human disease and typically occurs as opportunistic infections in susceptible hosts. Despite its low pathogenicity in humans compared to other agents of mucormycosis, *R. pusillus* is angioinvasive and thermotolerant, allowing for wide dissemination in febrile individuals [13]. To our knowledge, there have been 27 reported cases of *R. pusillus* related infections including pulmonary, soft tissue, rhino-orbital-cerebral, cerebral, intra-abdominal and disseminated infections; 25 out of the 27 patients (92.6%) had underlying conditions and overall mortality rate was 55.6%. 9 of the 26 reported cases were from the pediatric population, 7 of which had acute lymphoblastic leukemia, 1 had aplastic anemia, and 1 was immunocompetent [14–20]. Disease manifestations of the 9 cases were 3 disseminated, 2 rhinocerebral, 2 cutaneous, 1 pulmonary and 1 sinus-orbital case; 2 of the 3 patients with disseminated *R. pusillus* mucormycosis died [16,19,20]. Diagnosis of *R. pusillus* mucomycosis in all 9 patients was by direct culture of the site of infections and in the 3 disseminated cases, all blood cultures were negative. Our patient represents the third fatal case of *R. pusillus* bloodstream infection in the pediatric population and the first case of *R. pusillus* bloodstream infection diagnosed by positive blood culture.

Due to the absence of autopsy, it is impossible to know definitively the etiology of the patient's *R. pusillus* infection. We suspect that infection spreads from primary pulmonary or abdominal infections and rapidly progressed to dissemination and death. Unfortunately, her clinical presentation was non-specific enough that invasive fungal infection was not thought to be part of her presentation until just prior to death. The fact that this mold grew in routine aerobic blood culture suggests a high burden of disease. This case emphasizes the importance of mucormycoses as severe and often fatal infections in immunocompromised patients. Though rare, Gram stain negative blood culture bottles that are flagging positive in this patient population may benefit from further evaluation, specifically looking for mucormycosis or other molds.

Conflict of interest statement

None.

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