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Eremothecium coryli bloodstream infection in a patient with acute myeloid leukemia: first case report of human infection

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ABSTRACT

Eremothecium coryli is a dimorphic fungus of the *Saccharomycetes* class. While species within this class are known to cause human infection, *Eremothecium* species have previously only been known as phytopathogens and never been isolated from a human sample. Here, we report the first known case of human *E. coryli* infection.

A 74-year-old woman presented to another hospital with diffuse pruritus. She was diagnosed with urticaria and prescribed prednisone. She was found to be anemic, prompting a bone marrow biopsy that revealed acute myeloid leukemia (AML) with 73% blasts. She was then transferred to our institution.

On hospital day 1 (HD1), a peripherally inserted central catheter (PICC) was placed. On HD3, she developed an isolated fever (39.4 °C) and cefepime was started. Routine blood cultures (BD BACTEC™ FX, Becton Dickinson, Sparks, Maryland) were negative. On HD4, vancomycin was added and cefepime was changed to piperacillin-tazobactam due to persistent fevers. Chest computed tomography demonstrated patchy consolidation with associated ground glass infiltrates in the right lower lobe in addition to a few spiculated pulmonary nodules bilaterally with surrounding ground glass (halo sign). Respiratory bacterial, mycobacterial, and fungal cultures were negative. Serum *Aspergillus* galactomannan was 0.08 (reference index range: negative <0.50, positive ≥0.50) and serum beta-D-glucan was 43 pg/mL (reference range: negative <60 pg/mL, indeterminate 60–79 pg/mL, positive ≥80 pg/mL). Intravenous posaconazole was added with subsequent defervescence. She simultaneously received induction chemotherapy with cytarabine and idarubicin. On HD5, vancomycin was discontinued. On HD17, bone marrow biopsy showed residual disease with 48% blasts. On HD20, the patient received re-induction therapy with granulocyte-colony stimulating factor, clofarabine, and high-dose cytarabine which was complicated by profuse watery diarrhea.

On HD28, she developed a low-grade fever (38.0 °C) and altered mentation. On HD29, 2 sets of routine blood cultures were obtained, including 1 set (2 BD BACTEC™ Plus Aerobic medium) from venipuncture which grew oval-shaped budding yeast with narrow-angle budding at 48 h of incubation (Fig. 1) while serum posaconazole trough level was 3350 ng/mL. On HD30, serum *Aspergillus* galactomannan remained negative (0.11) but serum beta-D-glucan was now >500 pg/mL. Clinical suspicion for candidemia from gut translocation was high in the setting of neutropenia, chemotherapy, and diarrhea. Out of concern for azole resistance since this infection developed while having a high posaconazole trough level, caspofungin was added. Posaconazole was continued concurrently for possible invasive pulmonary mold infection. On HD31, the PICC was removed and ophthalmic exam was negative for intraocular fungal infection.

The positive blood cultures drawn on HD29 were subcultured in potato flake agar (PFA) and CHROMagar Candida (Becton Dickinson, Sparks, Maryland) and incubated at 30 °C and 35 °C, respectively, per microbiology laboratory protocol. At 72 h of incubation, pale yellow,

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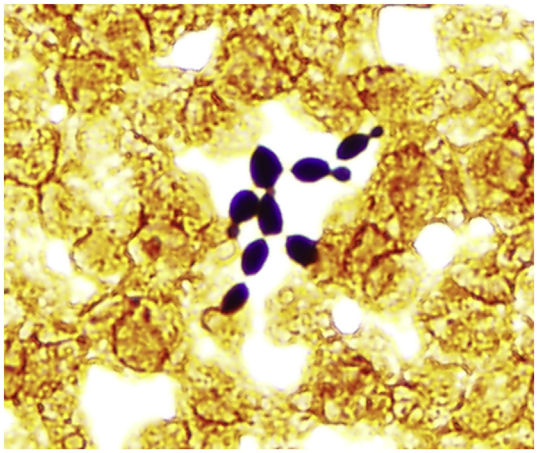

Fig. 1. Light microscopy (40× magnification) of Gram-stained smear taken from blood culture showing clusters of oval-shaped budding yeast, with narrow-angle budding, identified as *Eremothecium coryli*.

smooth, round colonies without dimorphism on PFA and mauve-colored colonies on CHROMagar were observed. Lactophenol cotton blue stain revealed small oval-shaped yeast with unilateral budding (Fig. 2). Hyphae and asci containing ascospores were not observed. Several identification attempts using phenotype and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Billerica, MA) were unsuccessful. Therefore, fungal sequencing was performed as previously described (Moncada et al., 2013). After fungal DNA extraction, real-time PCR was performed using pan-fungal primers for internal transcribed spacer-2 (ITS-2) and the D2 regions of the 28S ribosomal RNA (rRNA) gene. Amplicons were sequencing using cycle Sanger sequencing. Sequences were identified using the National Center for Biotechnology Information's (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/Genbank) and Westerdijk Fungal Biodiversity Centre (http://www.westerdijkinstitute.nl). Resolution to species and genus level was made with percentage identity score of ≥99.0% and ≥98.0% to <99.0%, respectively, per Clinical and Laboratory Standards Institute (CLSI) recommendations (Clinical and Laboratory Standards Institute (CLSI), 2008). Amplicon length was 364 and 296 bp for ITS-2 and D2, respectively. ITS-2 and D2 sequence similarity searches revealed 100% identity with *Eremothecium coryli* collection strain CBS 472.78 (GenBank accession number MH872931). Our sequence was deposited in NCBI GenBank (accession numbers: MH513670 and MH513609 for ITS-2 and D2 region, respectively). Antifungal susceptibility testing was performed using the Sensititre YeastOne panel (Trek Diagnostic Systems, Cleveland, OH) following CLSI M27 document guidelines. *Eremothecium coryli* demonstrated the following minimum inhibitory concentrations (MICs): amphotericin B: 2 μg/mL; anidulafungin: 0.03 μg/mL; caspofungin: 0.015 μg/mL; fluconazole ≥256 μg/mL; flucytosine ≥64 μg/mL; itraconazole ≥16 μg/mL; micafungin 0.03 μg/mL; posaconazole ≥8 μg/mL; and voriconazole ≥8 μg/mL. Interpretations were not provided due to the lack of established CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

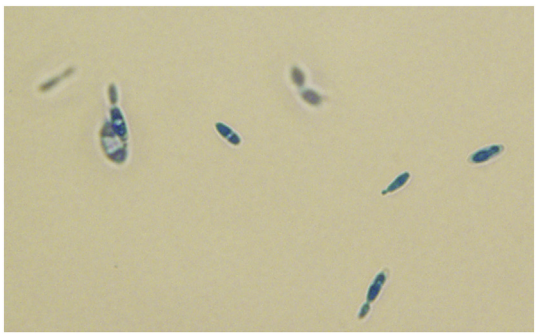

Fig. 2. Lactophenol cotton blue stain from PFA plate showed oval-shaped yeast with unilateral budding, at 100× magnification, latter identified as *Eremothecium coryli* by fungal ribosomal gene sequencing.

On HD35, the patient remained afebrile but her mentation did not improve, so blood cultures were repeated, growing yeast in 2 out of 6 blood culture bottles. Her altered mentation persisted, and she developed worsening anasarca, acute kidney injury, and transaminitis. Lumbar puncture was deferred due to thrombocytopenia. On HD37, repeat blood cultures again grew yeast in 1 out of 4 blood culture bottles. The persistent fungemia raised concerns for lack of source control and/or antifungal resistance. Posaconazole was changed to liposomal amphotericin B, while caspofungin was continued. On HD39, transthoracic echocardiogram was negative for endocarditis, and abdominal ultrasound did not reveal an unaddressed nidus of infection.

Because of persistent fungemia and progressive clinical decline in the setting of refractory AML, comfort-based measures were instituted on HD40. She died on HD41. Postmortem examination was declined.

Eremothecium coryli is a dimorphic fungus belonging to the genus *Eremothecium* (Gastmann et al., 2007), which includes dimorphic and filamentous fungi and constitutes clade 12 of the *Saccharomyces* complex (Kurtzman and Robnett, 2003; Wendland and Walther, 2011, 2014). It previously belonged to the genera *Ashbya*, *Holleya*, *Nematospora*, and *Crebothecium*. Other *Saccharomyces* species are known to cause human infection (Boyle et al., 2006; Enache-Angoulvant and Hennequin, 2005; Muñoz et al., 2005). While *Eremothecium* species are known to be phytopathogenic, they have not been previously associated with human infection (Prillinger et al., 1997). To our knowledge, this is the first reported human infection caused by *Eremothecium* species including *E. coryli*.

As a phytopathogen, *Eremothecium* species have been reported to cause stigmatomycosis, a fungal infection of crops such as cotton, soybeans, pecans, pomegranates, citrus fruits, and pistachios (Leeuw et al., 2006; Prillinger et al., 1997; Wendland and Walther, 2014). Stigmatomycosis frequently occurs in California pistachio orchards severely infested by hemipteran insects. Based on this information and the pathogenesis of *Saccharomyces* fungemia, it was postulated that our patient may have ingested foods contaminated with *E. coryli*. She had maintained a home vegetable garden, but it did not include any of the aforementioned plants and was disease-free. She washed and cooked her vegetables prior to consumption. She was not taking probiotics.

Without any existing medical literature regarding human *Eremothecium* infection, data were extrapolated from similar scenarios such as candidemia and *Saccharomyces* fungemia (Andes et al., 2012; Boyle et al., 2006; Enache-Angoulvant and Hennequin, 2005; Muñoz et al., 2005; Pappas et al., 2016). Central venous catheters were removed when feasible (Andes et al., 2012; Pappas et al., 2016). Although antifungal susceptibility testing was performed, there are no CLSI or EUCAST interpretive criteria. The existing medical literature regarding antifungal susceptibilities of *Saccharomyces* species was reviewed. Amphotericin B, flucytosine, posaconazole, and voriconazole generally had lower MICs, while fluconazole, itraconazole, and echinocandins had higher MICs (Enache-Angoulvant and Hennequin, 2005; Muñoz et al., 2005; Pfaller et al., 1997). Interestingly, acetylsalicylic acid possesses antifungal properties *in vitro* against some yeasts such as *E. coryli*, presumably *via* mitochondrial inhibition (Leeuw et al., 2007). Liposomal amphotericin B would have otherwise been the preferred empiric agent, but its use was preempted by renal failure and anasarca. Therefore, she initially received posaconazole and caspofungin.

With newer, advanced diagnostic techniques such as gene sequencing, we anticipate that emerging pathogens such as *E. coryli* will become increasingly identified and reported. A concomitant increase in antifungal susceptibility data can help establish clinical interpretive MIC

breakpoints. This knowledge will be invaluable in guiding antifungal therapy and improving patient outcomes.

Conflict of interests

The authors do not have any conflicts of interest to disclose.

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