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The first human report of *Hyphopichia burtonii*, initially misdiagnosed as sterile peritonitis in a patient on peritoneal dialysis

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ABSTRACT

This is the first human infection caused by *Hyphopichia burtonii*, resulting in peritonitis in a patient on peritoneal dialysis initially diagnosed as sterile peritonitis, resulting in delayed diagnosis and treatment. This pathogen posed a challenging diagnosis, causing low-grade peritonitis and difficulty to culture with standard bacterial broth. Moreover, automated platforms for pathogenic yeast identification could not specify the species, but broad-range PCR targeting rDNA followed by DNA sequencing successfully solved the etiology.

1. Introduction

Fungal peritonitis is a severe life-threatening condition, causing high morbidity and mortality in patients on peritoneal dialysis (PD), particularly in those with delayed diagnosis and treatment [1]. However, the clinical presentation of fungal peritonitis is sometimes challenging since it is nonspecific and can be confused with a plethora of other infectious or non-infectious conditions, such as sterile peritonitis. Sterile peritonitis (also termed chemical peritonitis) is described as peritoneal inflammation caused by non-infectious agents, may present sporadically or in clusters [2], such as an outbreak caused by peptidoglycan-contaminated icodextrin bags in 2002 [3]. Various chemical compounds (such antibiotics, dialysis solutions, bag contaminants, etc.) have been reported causing chemical peritonitis [4].

Hyphopichia burtonii, formerly known as *Pichia burtonii*, is a widespread spoilage yeast, causing food and beverage spoilage, such as "chalk molds" defects on partially baked bakery products, cured meat, and cookies [5,6]. *Hyphopichia burtonii* can cause cutaneous infection in Barbastelle bats [7]; however, human infection has not yet been reported. We present the first human infection caused by *H. burtonii* in a patient on PD resulting in peritonitis and describe the challenges encountered in diagnosing this infection. The organism was successfully identified using broad-range PCR targeting rDNA and DNA sequencing and was successfully treated with PD catheter removal and a 2-week course of antifungal therapy.

2. Case report

A 43-year-old non-diabetic Thai farmer on PD with a regimen of 4 exchanges/day with 2L of 1.5% dextrose since 2 years presented with cloudy PD effluent (PDE) on day 0. He reported non-compliance with the handwashing technique, particularly during a daytime exchange after returning from agricultural work. PDE examination revealed a leukocyte count of 121 cells/mm^3 (55% neutrophils). Intraperitoneal cefazolin and ceftazidime (both 1 gm daily) was empirically started. PDE leukocyte counts were still elevated around 200 cells/mm^3 on subsequent days, resulting in the second examination of PDE on day +3. Bacterial cultures of the PDE revealed no organisms on both examinations. The provisional diagnosis was sterile peritonitis, and the attending clinician decided to withdraw the empirical antibiotics after the patient completed a 14-day course. Two days later (day +16), the patient was revisited with cloudy PDE and mild abdominal pain. PDE cell count was 301 cells/mm^3 (85%

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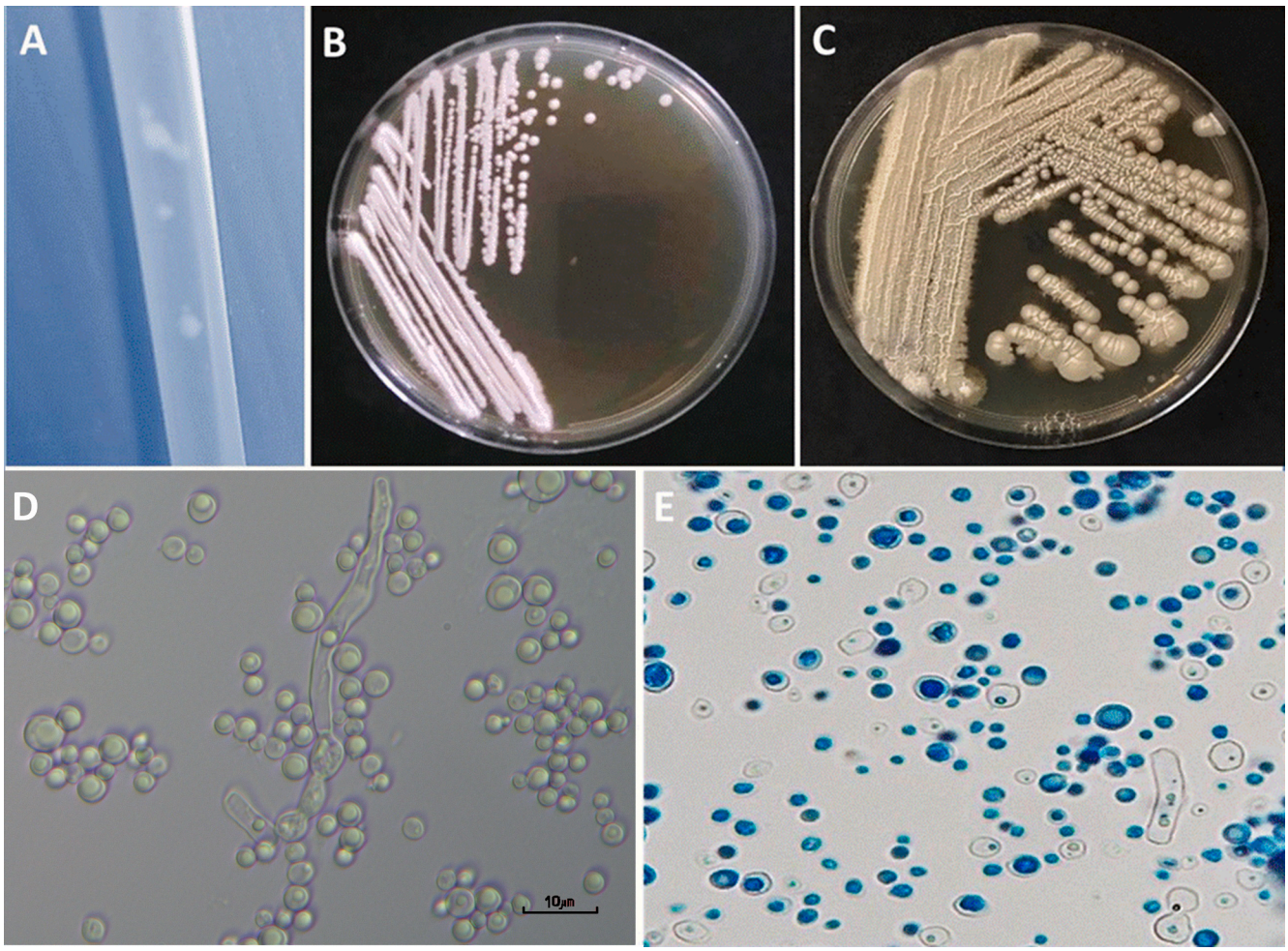


Fig. 1. *H. burtonii* colonies visible inside removed PD catheter (A); on Sabouraud dextrose agar on day 14 (B) and on day 21 (C); and KOH and Lactophenol cotton blue staining of the colonies on day 3 (D) demonstrating hyphae, denticulate conidiogenous cells and conidia; as well as yeast cells, partly conjugating, forming asci, and hat-shaped ascospores (D and E); 1000x magnification.

neutrophils). Intraperitoneal cefazolin and ceftazidime were resumed, with the third negative PDE culture on day +19. With meticulous inspection, small white spots were observed inside the PD catheter lumen (Fig. 1A), raising a suspicion of fungal colonization. The PD catheter was removed (day +21), and 2-week oral fluconazole was started at a dosage of 200 mg daily. The patient was transferred to temporary hemodialysis for 1 month. The PD catheter and PDE were submitted to a central microbiology laboratory to identify the microorganism. A new PD catheter was inserted on day +67, and the PD was resumed without relapsing peritonitis within 12 months after the diagnosis of infection.

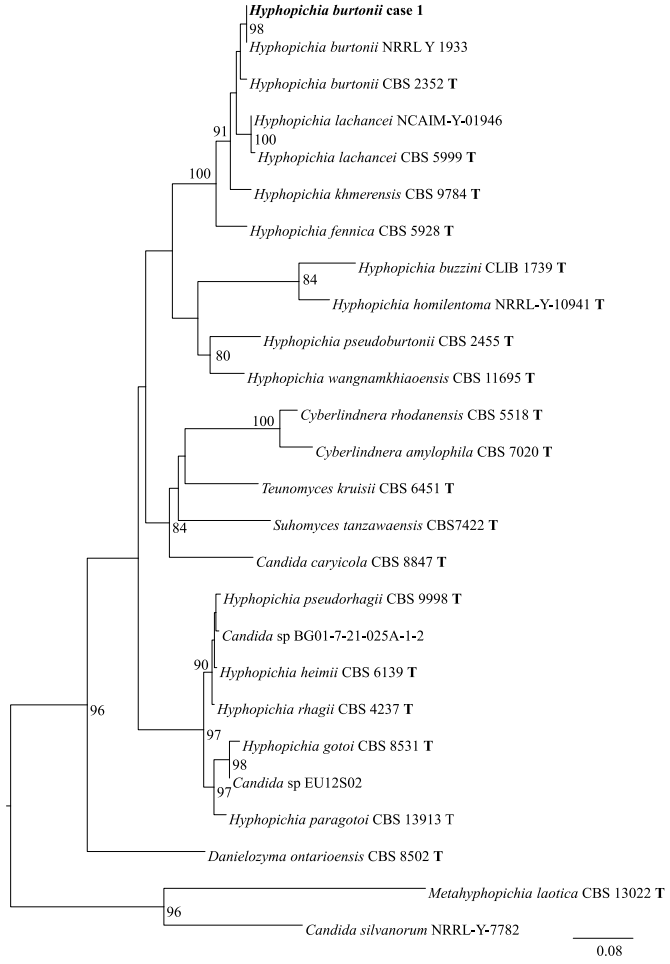
The specimens were inoculated onto several media, including broths, blood agar, Sabouraud Dextrose agar (SDA), trypticase soy agar (TSA) plates, and incubated at 25 °C and 35 °C. A spotted swab from the PD catheter displayed higher and faster colony growth than the PDE at 25 °C, but faint growth could be seen in both specimens at 37 °C. On day +35, the colonies obtained were cream-colored mucoid with a radiating fringe at the periphery (Fig. 1B), while on day +42, the surface of the colonies became arid (Fig. 1C). Wet mounts of the colonies demonstrated partly conjugating yeast cells, branching filamentous hyphae, and large quantities of small (3–5 µm), oval to rounded spores located inside and outside the asci, which attached to the fungal hyphae laterally on fine, shortened denticles (Fig. 1D–E). Duplicated examinations on the VITEK-2 yeast identification system failed to identify the yeast pathogen, giving a numerical number code of 6752144065301370 ('Unidentified organism'). Ancillary assessment with VITEK-MS (IVD Knowledgebase v.3.0) (bioMérieux, Marcy l' Etoile, France) using Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology also proved to be unsuccessful to identify the pathogen. Broad-range polymerase chain reaction (PCR) targeting the internal transcribed spacer (ITS1/ITS4 primer) [8] and large-subunit region (LSU primer) [9] of ribosomal DNA (rDNA) and DNA sequencing were employed. A blast search of the sequence data against the nucleotide database in GenBank revealed a similarity of 93.64% (577/577, accession number MH532416.1) and 100% (2028/2028, accession number NG054819.1) to *H. burtonii* in ITS and LSU rDNA gene regions respectively (https://blast.ncbi.nlm.nih.gov). The phylogenetic tree for the identified organism is shown in Table 1. Using the Epsilometer test (E-test) (bioMérieux, Marcy l' Etoile, France), the pathogen was highly susceptible to amphotericin B, voriconazole, fluconazole, itraconazole, and caspofungin with minimal inhibitory concentrations (MICs) of 0.023, 0.032, 4.0, 0.023, and 0.75 µg/ml, respectively.

3. Discussion

We describe the first human infection, peritonitis, caused by *H. burtonii* in a patient on PD. The diagnosis was delayed due to the fact that standard microbiological assays initially didn't reveal the fungal pathogen, and identification of this rare yeast was not retrieved by automated fungal identification platforms using growth-based technology and mass spectrophotometry.

The prevalence of fungal peritonitis varies from approximately 1%–24% of all peritonitis episodes in people undergoing PD, ranging from

Table 1
The phylogeny tree of the identified organism is consistent with *H. burtonii*.



0.01 to 0.09 episode/patient-year [10,11]. The most common fungal pathogens are *Candida* spp. Non-*Candida* yeast account for less than 10% of fungal peritonitis episodes [12–14]. *Hyphopichia* belongs to the family Saccharomycetales genera incertae sedis. Many species in this family display only anamorphic phenotype and have an unclear teleomorphic relationship [15]. The presented case is the first human infection with this species. The source of infection was probably associated with contact contamination since the patient did not comply to the aseptic technique while performing "bag" exchange. Proper hand hygiene is crucial and should be periodically reemphasized to prevent peritonitis. Moreover, this genus has not been found in humans gut, albeit reports demonstrating that it can be found in animal feces, on the chicken egg's shell, and in food products [16,17]. It is unlikely that the infection was acquired by ingestion of contaminated food as no gastrointestinal symptoms were present.

Identification of yeast to the species level is essential to be informed about the antifungal susceptibility [18], and may provide clues to how the infection was acquired. Generally, PD-associated peritonitis is diagnosed based on clinical suspicion, a cloudy appearance of the PDE, and detection of microorganisms by culture or molecular detection. Using specific fungal media at first presentation will avoid delay diagnosis of fungal peritonitis, as demonstrated in this case report. Cultivation of this specific organism in media with a high water activity (>0.98) or at a higher temperature than 30 °C (maximal temperature of 37 °C) significantly slow its growth [16]; therefore, traditional bacterial broth (water activity, 0.99) incubated at 37 °C may prevent growth of this rare yeast. Appropriate media should be selected to detect fungal pathogens, and incubation at different temperature conditions are recommended (room temperature and 35–37 °C) [19].

The 2016 International Society for Peritoneal Dialysis (ISPD) Peritonitis Guidelines strongly recommend removing the PD catheter immediately after fungi are identified in PDE of patients with fungal peritonitis, followed by antifungal therapy for 2 weeks. No specific recommendations are made regarding the type and dose of antifungal medications to be administered [20]. However, these recommendations are based on studies of *Candida* peritonitis. Ram et al. [21] demonstrated that the mortality rate increases exponentially with the delayed onset of the catheter removal, 19% (1 day), 67% (1 week), and 94% (1 month). Attempting to treat fungal peritonitis with the catheter in situ might leave an ongoing source of infection and impair the effectiveness of antifungals. Prompt removal of the patient's PD catheter and administering of an antifungal agent successfully eradicated the infection, resulting in a clinical cure. The *H. burtonii* was susceptible to all common antifungal agents, and the infection was successfully treated with oral fluconazole for 2 weeks after PD catheter removal.

In conclusion, we reported the first human infection caused by *H. burtonii* resulting in fungal peritonitis in a patient on PD. Diagnosis was delayed due to challenges to diagnose this rare yeast. Most likely, the infection was introduced due to non-compliance with aseptic handling techniques around the PD process.

Declaration of competing interest

T.K. has received consultancy fees from VISTERA as a country investigator and current recipient of the National Research Council of Thailand and received speaker honoraria from Astra Zeneca and Baxter Healthcare.

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