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Peritoneal dialysis-associated peritonitis from pauci-septated mold: Life-threatening but curable

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ABSTRACT

Two cases of PD-associated peritonitis due to *Cunninghamella* (*C. bertholletiae* and *C. guizhouensis*) were reported here with favorable outcomes, albeit presenting with septicemia. Both patients presented with classic features of bacterial peritonitis, cloudy effluent with a neutrophil predominance, followed by fever and septicemia/septic shock. The pathogen species were confirmed and verified by molecular phylogeny using universal and specific fungal primers. All isolations were susceptible/intermediately susceptible to amphotericin B but resistant to other antifungal agents, including triazoles, caspofungin, and terbinafine. Both cases were successfully treated with timely PD catheter removal and antifungal medications for 2–4 weeks.

1. Introduction

Peritoneal dialysis (PD)-related peritonitis caused by fungal infections generally carries a high risk of morbidity and mortality rates [1–5]. Fungal infections are more challenging to treat and eradicate, leading to prolonged illness and an increased risk of complications compared to bacterial peritonitis [1–6]. While fungal infections from genera like *Rhizopus, Saksenaea, Rhizomucor*, and *Mucor*, within the Mucorales order, are life-threatening, they occur less frequently in patients with PD [7]. Only two cases of PD-related peritonitis due to *Cunninghamella* have been observed [8,9]. Both cases were infected with *C. bertholletiae* but successfully treated with PD catheter removal and antifungal medications. Whether the reported outcomes are genuinely favorable or the results from a reporting bias, we, therefore, describe the additional two cases of PD-related due to *Cunninghamella* (*C. bertholletiae* and *C. guizhouensis*) and confirm the favorable outcomes of this pathogen in PD patients if strictly managed according to the 2022 ISPD Peritonitis Guidelines [1].

2. Case presentation

We report two cases of PD-related peritonitis caused by *Cunninghamella*. The pathogen species were confirmed through molecular phylogeny using ribosomal DNA (internal transcribed spacer [ITS] regions and the large subunit [LSU] of the nuclear ribosomal DNA gene complex) with 100% query coverage and 93–100% identities. Antifungal susceptibility tests were performed using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute in 2020, with epidemiological cutoff values referred to *Aspergillus flavus*. (3rd edition CLSI M59 document, Clinical and Laboratory Standards Institute, Wayne, PA) [10]. Detailed clinical findings, antifungal susceptibility results, and outcomes are presented in Table 1.

2.1. Case #1

A 52-year-old female farmer with kidney failure from unknown etiology underwent continuous ambulatory PD (CAPD, 2-L 1.5% Dextrose bag x 4 exchanges/day) for 10 years. The patient did not have a history of peritonitis during her PD journey. She presented with severe

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Table 1
Baseline characteristics, laboratories, and clinical outcomes of patients with PD-peritonitis due to *Cunninghamella* spp. and review literature.

	Case #1	Case #2	Pimentel [8]	Bhutada [9]
Age, years	52	63	39	52
Onset after PD initiation, years	10	1	2	3
Immunocompromised status	No	No	No	No
Prior bacterial peritonitis episode(s)	–	1	1	–
Effluent cell counts				
- Leukocytes, cells/μL	1055	462	496	258
- Neutrophils, %	70	13	N/A	85
Galactomannan				
- Serum	Positive	Negative	N/A	N/A
- PD effluent	Positive	Positive	N/A	N/A
Species of *Cunninghamella*	*C. bertholletiae*	*C. guizhouensis*	*C. bertholletiae*	*C. bertholletiae*
Antifungal susceptibility test, MIC				
- Amphotericin B	4 (I)	2 (S)	4 (I)	N/A
- Voriconazole	8 (R)	2 (I)	>16 (R)	N/A
- Fluconazole	>64 (N/A)	>64 (N/A)	>256 (N/A)	64
- Itraconazole	>16 (R)	4 (R)	2 (R)	N/A
- Caspofungin	>16 (R)	>16 (R)	N/A	N/A
- Isavuconazole	8 (R)	8 (R)	N/A	N/A
- Posaconazole	8 (R)	8 (R)	N/A	N/A
- Terbinafine	8 (N/A)	16 (N/A)	N/A	N/A
Antifungal medication	AMB, 14 days	AMB, 28 days	VCZ, 6 weeks	IP FLC, 8 days, then ITC, 15 days
Follow-up time, months	36	12	3	1.5
Outcomes	Cure with resumed PD	Cure with maintenance HD	Cure, on-planned resuming PD	Cure with maintenance HD

Abbreviations: AMB, amphotericin B; FLC, fluconazole; IP, intraperitoneal; ITC, itraconazole; MIC, minimal inhibitory concentration; N/A, not applicable; PD, peritoneal dialysis; VCZ, voriconazole; *(I)*, intermediate; *(R)*, resistance; *(S)*, susceptible; and *(N/A)*, non-available.
Remarks: Antifungal susceptibility patterns of the *Cunninghamella* against common antifungal medications were assessed by broth dilution technique (according to the CLSI document M57 protocol). Interpretation of the susceptibility of *Cunninghamella* isolates employing the Minimum Inhibitory Concentration (MIC) reference to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Epidemiological Cutoff (ECOFF) level that capture ≥97.5% of the wild-type *Aspergillus flavus* populations. The ECOFF is a microbiological term used to differentiate between wild-type strains (those with no acquired resistance mechanisms) and strains that may have acquired resistance. Susceptibility, Intermediate, and Resistance strains if the MICs are below, equal, and above the ECOFF, respectively.

abdominal pain, cloudy PD effluent, and septicemia and was admitted on day +1. The PD effluent revealed a leukocyte count of 1055 cells/μL with 70% neutrophils. Empirical treatment with intravenous vancomycin and levofloxacin was initiated. The PD effluent culture was negative for bacterial growth but later tested positive for pauci-septated filamentous mold (day +4). Positive serum (0.8, cutoff level ≥0.56) and PD effluent galactomannan index (GMI) (1.5, cutoff level ≥0.5) were established. DNA barcoding of ITS (ITS1/ITS4 primer), 28S rDNA region (U1/U2 primer), *and Cunninghamella*-specific primers (Cu1/Cu2 primer) confirmed the species of the isolation of *C. bertholletiae* (FJ345351.1/93%identity, MH873434.1/99%, and LR215930.1/100%, respectively). Treatment with 1 mg/kg/day of amphotericin B was initiated for 14 days. The PD catheter was timely removed on day +4, and extended hemodialysis (HD) was prescribed 2 times/week. Four months later, PD was resumed without relapsing fungal peritonitis during the 3-year follow-up period.

2.2. Case #2

A 63-year-old unemployed male with chronic obstructive pulmonary disease and kidney failure from hypertensive nephropathy underwent CAPD (2-L 1.5% Dextrose bag x 4 exchanges/day) for 2 years. His wife, an agricultural worker, was his caregiver for the PD exchange. The patient had only one episode of culture-negative peritonitis, which was treated with a 2-week course of intraperitoneal cefazolin and ceftazidime one month ago. He presented with abdominal pain, cloudy effluents, nausea, vomiting, and hypotension (day +1). The PD effluent revealed a 462 cells/μL leukocyte count with 13% neutrophils. Empirical antibiotic with intravenous meropenem was initiated and subsequently replaced with intravenously amphotericin B at a dosage 1 mg/kg/day for 28 days (from day +4 to day +31) after the result of effluent culture was positive for filamentous mold. The PD effluent GMI was positive but negative serum GMI (0.6 and 0.2, respectively). DNA barcoding of 28S rDNA region (U1/U2 primer) and *Cunninghamella*-specific primers (Cu1/Cu2 primer) confirmed the species of the isolation of *C. guizhouensis* (MN908599.1/100%, both). The PD catheter was removed on day +5, and dialysis was transferred to maintenance hemodialysis. The patient was well without relapsing peritonitis for 1-year follow-up (Table 1).

3. Discussion

We report two cases of PD-related peritonitis caused by *Cunninghamella*, which manifested aggressively but were treatable with timely PD catheter removal and appropriate antifungal medication. Both pathogens' species were confirmed using DNA barcoding with both universal (ITS1/ITS4 and U1/U2 primers) [11] and specific primers for *Cunninghamella* (Cu1/Cu2 primer) [11]. Notably, *C. guizhouensis* causing human infection is reported for the first time here.

Cuninghamella is in phylum Mucoromycota, subphylum Mucoromycotina, order Mucorales [7,12]. The molds in this class have a unique character of having pauci-septa separating individual cells, also known as coenocytic molds [7]. *Cuninghamella* is commonly found in the environment, particularly in soil and decaying organic matter [8,9]. *Cuninghamella*, mainly *bertholletiae*, were previously described as causing pulmonary infection and destructive sinusitis [13,14] in patients with defective immunities, such as those with poorly controlled diabetes, having organ transplants, taking immunosuppressive medications, and receiving chemotherapy [8,9,13]. However, it can affect normal hosts after trauma or receiving surgery [9]. However, none of our patients recently experienced such injuries. The environmental molds might enter the peritoneal cavity by breaching the aseptic technique or spillage of fungal spores during the PD bag exchanges [4,15–17] since both cases were related to agricultural activities.

The initial symptoms of fungal peritonitis usually do not differ from bacterial peritonitis, including abdominal pain, fever, nausea, vomiting, and less common diarrhea [1,8,9]. If left untreated, the infection can spread rapidly, leading to complications such as sepsis, organ failure, and death, as observed in our 2 cases and the previous two reported cases [8,9].

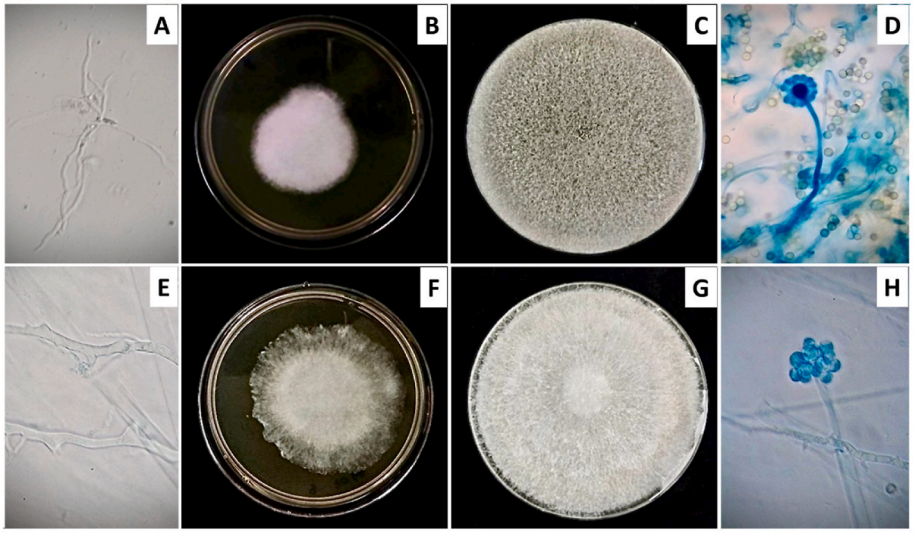

Fig. 1. Wet smear mounted with potassium hydroxide (KOH) demonstrates branching pauci-septate filamentous mold 400x [A] and 1,000x [E] Fungal colonies of *Cunninghamella bertholletiae* and *C. guizhouensis* on Sabouraud Dextrose Agar (SDA) agar plates at day 2 (B, F) and day 6 (C, G), respectively. Sporangiola on branched sporangiophores of *Cunninghamella bertholletiae* and *C. guizhouensis* (lactophenol cotton blue staining, LPCB x400) [D, H], respectively.

Diagnosing *Cunninghamella* peritonitis primarily relies on identifying the pathogen in the PD effluent, which has a specific characteristic of pauci-septate mold. *Cunninghamella* colonies exhibited rapid growth on Sabouraud Dextrose Agar (SDA) agar, typically within 4 days, initially appearing as white and subsequently turning yellowish/brown colonies [18]. Their sporangiophores were broad, erect, mostly branched, irregular, and verticillate, with sporangia being oval/globose and terminal vesicles being globose/subglobose [18] (Fig. 1). Positive tests of PD effluent and serum GMI were also helpful in diagnosing mold peritonitis with sensitivities of 77% and 65%, respectively [19,20]. However, galactomannan is not a typical cell wall component in Mucorales fungi. Several case reports and cohorts demonstrated positive GMI [21–23] but were interpreted as suspicious of co-infections with *Aspergillus* spp. [21,22]. The mechanism behind the positive GMI in *Cunninghamella* specimens required further exploration. In our cases, fungal DNA barcoding employing universal fungal primers (ITS and LSU rDNA) was able to identify the species of causative pathogens [5]. Furthermore, the species were confirmed with the specific *Cunninghamella* primers (Cu1/Cu2 primer) [11].

The ubiquitous habitat of this environmental mold raises a concern for specimen contamination during PD bag collection and transfer. However, as part of the MycoPDICS cohort [2], a national registry designed to survey the incidence of PD-related infections with fungus or environmental organisms under the Nephrology Society of Thailand, our specimens were well collected and handled with aseptic techniques and conditions. Additionally, colonization of the fungus was observed inside the PD catheter collected from CASE #2. Subsequently, cultivation of the removed catheter confirmed its presence, thereby supporting its role as a genuine pathogen.

According to the 2022 International Society for PD (ISPD) Peritonitis Guidelines, prompt diagnosis, timely PD catheter removal, and appropriate antifungal medication for at least 2 weeks after catheter removal are crucial in treating fungal peritonitis [1,2]. Leaving the PD catheters in situ or delaying PD catheter removal may lead to dismal outcomes [2]. Although all cases with *Cunninghamella* peritonitis (Case #1, Case #2, and 2 previous reported cases) presented with life-threatening conditions, strict adherence to the guidelines contributed to curing the infection. However, the regimen used in the previous literature were heterogeneous, including fluconazole 200 mg daily for 8 days and followed by itraconazole 200 mg daily for additional 15 days in Bhutada et al. [9] and voriconazole 400 mg daily for 2 weeks in Pimental et al. [8], even though the isolations in both cases demonstrated resistance to these drugs (MIC >60 μg/mL by E-test and >16 μg/mL by broth microdilution method, respectively). Based on our MIC results and Pimental's MIC results, along with the 2019 Global Guideline for the diagnosis and management of mucormycosis, which is an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium [24], the first-generation of triazole antifungal agents (fluconazole and itraconazole) should be avoided.

Although the 2019 Global guideline for the diagnosis and management of mucormycosis [24] recommended isavuconazole and posaconazole (the second-generation triazoles) as the first-line treatment with patients with pre-existing kidney compromise, our cases demonstrated resistance (MIC 8 μg/mL) against both recommended triazoles. Bearing in mind that despite the antifungal susceptibility test promoting antifungal stewardship, it is challenging to interpret antifungal susceptibility testing for non-*Aspergillus* molds. Upon suspicion of PD-associated mucormycosis, a combination of high-dose liposomal amphotericin B and timely PD catheter removal is highly recommended. In contrast, a high dose (1–1.5 mg/kg/day) of amphotericin B deoxycholate is an alternative antifungal medication but may be the only option in resource-limited settings.

In conclusion, aseptic technique and proper hygiene should be maintained in patients with PD to minimize the risk of environmental-borne infection. Strict adherence to the 2022 ISPD Peritonitis Guidelines can salvage PD-related peritonitis caused by the deadly fungi.

Conflicts of interest

TK has received consultancy fees from VISTERRA, ELEDON, Otsuka OLE, and Otsuka VISIONARY as country investigators and is a current recipient of the National Research Council of Thailand and received speaking honoraria from Astra Zeneca and Baxter Healthcare. The other authors declare that they have no relevant financial interests.

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