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Colonization of peritoneal catheter with a thermophilic fungus, *Thermoascus crustaceus*: a case report

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Thermoascus crustaceus is a thermophilic fungus and the teleomorph form of *Paecilomyces crustaceus*. *Thermoascus* spp. have been rarely isolated from human mycoses as etiological fungal agents. We believe that our patient is the first case of catheter colonization with *Thermoascus crustaceus*. In a 50-year-old male patient undergoing chronic peritoneal dialysis, the mold was isolated from three separate, consecutive dialysate fluid specimens and peritoneal catheter tip. The patient had slight clinical findings and he was treated by early catheter removal without antifungal treatment. Therefore this case was considered as the colonization of the peritoneal catheter rather than peritonitis. Consequently, we think that the human pathogen fungal spectrum will continue to enlarge.

Keywords *Thermoascus crustaceus,* peritoneal catheter, colonization

Introduction

Among the eukaryotes, only thermophilic fungi have a special ability to grow at high temperatures of 50–60°C, and the thermophilic fungi have a growth temperature minimum at or above 20°C and maximum at or above 50°C [1]. Thermophilic fungi have been found in self-heating organic materials such as hay, garden compost, manure, stored peat, and mushroom compost [2]. *Thermoascus crustaceus* is a thermophilic fungus and the teleomorph form of *Paecilomyces crustaceus* [3]. Microscopically, it produces superficial, subspherical to hemispherical, non-ostiolate, yellowish-orange to reddish orange ascomata surrounded by a loose cover of aerial hyphae and contained eight spored ascie. Ascospores are pale yellow to brown, ovoidal to ellipsoidal, thick walled, finely echinulate [3]. Although *Paecilomyces* spp. are uncommon pathogens, they can produce serious infections in immunocompromised patients, and the incidence of their infections is increasing in immunocompetent hosts [4,5]. The most common infections have been reported as keratitis, endophthalmitis and cutaneous infections [5–8]. Additionally *Paecilomyces* spp. have also been reported from peritonitis cases [9]. Hitherto, there are only two reports about its teleomorph form, *Thermoascus* spp., to our knowledge [10,11]. We think that our report is the first case of *Thermoascus crustaceus,* and the second by *Thermoascus* spp.

Peritonitis is one of the most serious complications of peritoneal dialysis and fungal peritonitis (FP) is observed rarely. However, FP is associated with the inability to continue on the peritoneal dialysis, increased rates of morbidity and mortality [12]. However, a few reports have been published about colonization of the peritoneal catheter by fungi without clinical peritonitis [13,14].

Case report

A 50-year-old man, with 7-year history of end stage renal failure due to malignant hypertension, had been treated with chronic peritoneal dialysis (CPD) to three times a week since 2002. So far, he had had eight peritonitis episodes; four bacterial episodes in January and July 2005, September 2006 and November 2007; three culture

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negative episodes in August 2006, January 2007 and May 2007; and *Aspergillus fumigatus* peritonitis in March 2007. On 28 January 2009, he was admitted to our hospital because of blurry dialysate and weakness. Usually, all membranous infections, including peritonitis have specific manifestation of mild or even severe pain; interestingly our patient had no abdominal pain or fever. Physical examinations were all within normal limits on admission, there was diffuse abdominal tenderness compatible with peritonitis. The external portion of the peritoneal catheter was clear. Laboratory data included 5800/mm^3 white blood cells (WBC), 8.5 g/dl hemoglobin, 216000/mm^3 platelets, 2.77 mg/dl C-reactive protein, 140 mm/h erythrocyte sedimentation rate, 1700/mm^3 dialysate WBC count and 600/mm^3 absolute neutrophil count. Empirical intraperitoneal treatment with 1.5 g/day cefazolin and 150 mg/day amikacin was started. Dialysate WBC count was 1700/mm^3 and the absolute neutrophil count was 1200/mm^3 at 48 h of treatment. Gram stain from the centrifuged dialysate material revealed numerous polymorphonuclear leukocytes and no microorganisms; Ehrlich-Ziehl-Nielsen staining was negative for acid-fast bacilli. Three separate, consecutive samples of dialysate fluid collected on 29 January, 5 February and 12 February 2009 were studied. Three dialysate samples were inoculated onto blood, chocolate, eosine metilen blue (EMB), Sabouraud dextrose agar (SDA) and Lowenstein-Jensen medium and incubated at 35°C. In addition, parts of the first two dialysate samples were also injected into BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) standard aerobic blood culture bottles. All bacterial cultures were negative. Two days later, initially white or pale yellow, spreading broadly mould colonies began to grow on all SDA plates, weakly; the older colonies became yellow-orange, buff colored. Growth signals in the automatic blood culture system for both bottles were detected at the 4th day of incubation, and the same mould colonies grew in pure culture on SDA plates from these bottle specimens. Despite intraperitoneal cefazolin and amikacin, peritoneal fluid leukocytosis and turbidity continued. Therefore empirical antibacterial treatment was stopped, but antifungal treatment was not started. Only the indwelling peritoneal (Tenckhoff) catheter was removed, and hemodialysis was thereafter maintained via a temporary central vein catheter. The internal tip of the peritoneal catheter filled with thick mucoid deposits, macroscopically. This material contained only fungal products such as mycelium comprising great fungal hyphae and polymorphonuclear leukocytes, microscopically. Mycological culture examinations revealed the same mould from these deposits. The follow-up of the patient observed no adjunct problem; his clinical findings disappeared within one week.

For the identification of this mould isolated from peritoneal fluids and the catheter tip, microscopic examinations with lactophenol cotton blue, temperature studies and additional subcultures were performed onto SDA, potato dextrose agar, Czapek Dox agar and malt extract agar. Microscopically, there were wide, long, septate and repeatedly branched hyphae, but we could not observe the specific conidial structures on any medium. The isolate was incubated at 20, 26, 30, 37, 45 and 50°C and the best growth was observed at 45°C. Additionally it was sent to the Mycology Reference Laboratory (MRL) Bristol, UK, where its final identification was made as *Thermoascus crustaceus* by PCR amplification of the D1-D2 portion of the 28S rRNA gene, and sequencing of the resulting amplicons. PCR amplification of fungal genomic DNA was achieved using the primers and conditions as described previously [15]. The resulting sequences (EMBL accession number FN675837) shared greater than 99% identity with isolates of *Thermoascus crustaceus* (anamorph *Paecilomyces crustaceus*) present in the public synchronized databases (*Thermoascus crustaceus* CBS 374.62, *Thermoascus crustaceus* NRRL 1563). The current isolate has been stored in the National Collection of Pathogenic Fungi (NCPF) housed at the UK Mycology Reference Laboratory, Bristol, with the unique identifier NCPF 7771.

This isolate was stored with culture plates at +4°C, in distilled water at room temperature and in medium containing skimmed milk, tryptone, glucose, and glycerine (STGG) at −20°C and −70°C. Approximately six months later, it was subcultured, but it could not be revitalized from any of the media. It was notified that the same problem was also experienced in MRL. Interestingly, it is a very fastidious fungus.

Discussion

Paecilomyces species are ubiquitous fungi in man's environment and they can be recovered from soil and air, and can cause the deterioration of grain, food and paper [3]. First, Apinis and Chesters isolated a thermophilic cleistothecial fungus from debris of coastal grasses and described as *Dactylomyces crustaceus* in 1965, later this fungus was transferred to the genus *Thermoascus* by Stolk in 1965 [10,16]. *Thermoascus* spp. includes *T. aegyptiacus, T. aurantiacus, T. crustaceus, T. taitungiacus*, *and T. thermophilus* [16]. *Thermoascus* spp. have been rarely isolated from human mycoses as an etiological fungal agent, and to date, we have seen only one human mycosis case caused by *Thermoascus* spp. in the literature; *Thermoascus taitungiacus* has been reported in a patient with peritonitis undergoing chronic peritoneal dialysis [11]. Also, *Thermoascus crustaceus* has been reported from monocyte cultures of three patients with AIDS [10].

One of the most frequent complications of peritoneal dialysis (PD) is peritonitis, and FP is responsible for 1–15% of episodes [12]. The most common cause of FP is *Candida*

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spp., especially *C. albicans* [12]. While filmentous fungi are a relatively rare cause of FP, it is reported that there is increasing recognition of *Paecilomyces* species as pathogens in such patients and there are 14 cases described as *Paecilomyces* peritonitis in the literature [9]. Thirteen of them were *Paecilomyces variotii* and one was *Paecilomyces taitungiacus (T. taitungiacus)*. We believe that our report is the first case caused by *Thermoascus crustaceus* (anamorph *P. crustaceus*) isolated from a patient undergoing chronic peritoneal dialysis.

Paecilomyces peritonitis has significant morbidity however fatalities have not been reported, in contrast with the high mortality rate of CPD-related peritonitis due to other fungi [9,11]. Although certain treatment of *Paecilomyces* peritonitis is unclear, early removal of the Tenckhoff catheter is essential [17]. The authors recommend for the Internal Society for Peritoneal Dialysis (ISPD) Guidelines that the catheter should be removed immediately after the diagnosis of FP is confirmed, antifungal treatment should be started and therapy should be continued after catheter removal [17].

Our patient had slight clinical findings for FP with blurry peritoneal fluid and a high WBC count. At the beginning, he was admitted as a peritonitis case. But he recovered by early peritoneal catheter removal without antifungal therapy, and a Tenckhoff catheter tip filled with fungal products. Probably, there was catheter colonization and distribution of fungi via the catheter into the peritoneum by the flow of dialysate fluid, and once the catheter tip was removed, there was no longer any transient seeding of the dialysis treatment of the patient. Thus we considered that this case was not fungal peritonitis but catheter colonization by a fungus. Perhaps, due to its thermophile and fastidious nature and the low human pathogenicity of *T. crustaceus*, while visible colonization was observed in the catheter, prominent peritonitis did not occur in our patient.

Finally, the spectrum of pathogenic fungi for humans enlarges day to day. Previously many fungi known as unusual and non-pathogenic or contaminant have been increasingly reported as etiologic agents of human mycosis. The causes of this issue are the increase of the susceptible patient population to fungal infections because of medical and technological developments, and the widespread use of antibiotics. We think that the human pathogen fungus spectrum will continue to expand in parallel with technological developments.

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