Peritonitis Due to \textit{Curvularia inaequalis} in an Elderly Patient Undergoing Peritoneal Dialysis and a Review of Six Cases of Peritonitis Associated with Other \textit{Curvularia} spp.

Jason D. Pimentel, 1* Kumar Mahadevan, 2 Alan Woodgyer, 4 Lynne Sigler, 5 Connie Gibas, 5 Owen C. Harris, 1 Michael Lupino, 1 and Eugene Athan 3

Department of Microbiology, Pathcare Consulting Pathologists, 1 and Department of Renal Medicine 2 and Department of Infectious Diseases, 3 Barwon Health, Geelong, and Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne, Melbourne, 4 Australia, and University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada 5

Received 3 March 2005/Returned for modification 31 March 2005/Accepted 22 April 2005

Fungal peritonitis due to \textit{Curvularia} species in patients undergoing peritoneal dialysis is a very rare problem. We report a case of peritonitis caused by \textit{Curvularia inaequalis}. This is the first report in the English literature of this species causing human infection. We also review the six previously reported cases of continuous ambulatory peritoneal dialysis peritonitis caused by other \textit{Curvularia} spp.

CASE REPORT

An 85-year-old woman was on continuous ambulatory peritoneal dialysis for end-stage renal failure due to renovascular disease. Her past medical history included hypertension, hypothyroidism, cholelithiasis, left ventricular failure, and a left-sided stroke with minimal residual deficit. She had commenced continuous ambulatory peritoneal dialysis 2 years prior and performed peritoneal exchanges independently. During that period, there had been multiple episodes of suspected peritonitis, each treated empirically with a single dose of intraperitoneal (i.p.) antibiotics. However, one episode, in December 2003, was confirmed by culture as being caused by \textit{Staphylococcus epidermidis}. These episodes were believed to have been the result of poor aseptic techniques that led to contamination.

The patient presented on March 14, 2004, at the emergency department at Geelong Hospital in Victoria, Australia, with a 3-day history of abdominal pain, accompanied by fever and nausea, and cloudy peritoneal dialysis (PD) bags. On examination, she was found to be febrile (37.8°C) with a tense abdomen, rebound tenderness, and decreased bowel sounds. The Tenckhoff catheter site was clean with no sign of inflammation. Full blood examination showed an elevated white cell count (WCC): $15.1 \times 10^6$/liter with 84% neutrophils. The C-reactive protein level was raised to 90 mg/liter, and the albumin level was 24 g/liter. The urea level was $15.3 \times 10^6$ mmol/liter, and the creatinine level was $462 \times 10^6$ µmol/liter. The fluid in the PD bag was pale yellow, cloudy with fibrous material, and slow to drain. The PD fluid was sent for microscopy and culture and found to have a WCC of $2,328 \times 10^6$/liter with $108 \times 10^6$ red blood cells/liter. No organisms were observed with the use of the Gram stain.

The patient was admitted into the hospital with presumed bacterial peritonitis and started on a peritonitis protocol consisting of i.p. cephazolin, gentamicin, and heparin. On day 6 after admission, a single dose of i.p. vancomycin was given empirically for persisting peritonitis, turbid effluent with a rising WCC, and fever. Meanwhile, the patient continued to deteriorate, became progressively malnourished, and developed the complications of small-bowel ileus and paroxysmal atrial fibrillation. In light of the worsening prognosis, the patient and family decided to request that a not-for-resuscitation order be put in place.

During this patient’s stay, six samples of PD fluid were sent for microscopy and culture. No significant organisms were detected in the initial sample. Growth was detected in the second

* Corresponding author. Mailing address: Royal Darwin Hospital, P.O. Box 41326, Casuarina, NT, 0811, Australia. Phone: 61-8-8922-8022. Fax: 61-8-8922-8843. E-mail: pimentel@iprimus.com.au.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{\textit{C. inaequalis} UAMH 10438 showing septate conidia borne on a geniculate conidiophore.}
\end{figure}
sample, taken on the 9th day after admission, and microscopy revealed fungal hyphae. The WCC was 20,000 × 10⁶/liter. At this point, i.p. fluconazole was commenced and then was changed to intravenous (i.v.) amphotericin B (45 mg/day) the next day. Fungal hyphae were also identified in four subsequent PD fluid samples. On March 24, 2004, the PD catheter was removed and a central venous catheter was placed for hemodialysis.

On day 16 after admission, the patient became febrile and tachycardic. Oxygen saturation was <85% on high-flow oxygen, and bibasal crepitations were heard upon auscultation. Blood gases indicated the presence of mixed metabolic and respiratory acidosis (pH, 7.19; O₂ pressure, 66 mm Hg; CO₂ pressure, 35.2 mm Hg; HCO₃ level, 13.2 × 10⁻⁶ mmol/liter), and the WCC was 38.4 × 10⁶/liter. The patient died later that day due to respiratory failure.

**Microbiology.** Peritoneal fluid was collected and processed according to the normal laboratory protocol. A 50-ml sample of dialysate fluid was centrifuged (Labofuge GL, Heraeus Christ, Germany) at 3,000 rpm for 15 min, and the supernatant was removed. The deposit was then resuspended in 5 ml of normal saline and inoculated onto Columbia horse blood agar (Oxoid, Basingstoke, Hampshire, United Kingdom) incubated at 35°C in 5% CO₂ and onto Sabouraud agar plates (Oxoid) incubated at 30°C. In addition, 3 ml of the deposit was inoculated into a BACTEC Peds Plus/F (Becton Dickinson, Sparks, MD) blood culture bottle and the bottle was incubated in a BACTEC 9240 incubator (Becton Dickinson) at 35°C. A Gram stain was made from the spun dialysate, and the cell count was determined for the noncentrifuged specimen.

No fungi were recovered from the primary culture plates. However, when the BACTEC 9240 signaled positive growth, fungal colonies were visually observed within the BACTEC Peds Plus/F bottles after Gram staining of a syringe-drawn sample revealed no bacterial organisms. The time to detection of positive growth ranged from 2 to 4 days. In each instance, a wet preparation confirmed the presence of hyphae. The fluid from these bottles was then subcultured on horse blood agar and Sabouraud agar plates incubated at 30°C. These plates grew colonies of a phaeoid fungus. The same fungus was isolated from five separate samples of PD fluid.

A microscopic mount from the colony on Sabouraud agar revealed septate, slightly curved, brown conidia borne singly on a geniculate conidiophore. The morphology was characteristic of species of the genus *Curvularia*, and identification as *Curvularia lunata* or *C. geniculata* was considered; however, the morphology and size of the conidia were not wholly typical of either species. One isolate (03-04) was therefore referred to...
the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Canada, where it was deposited as UAMH 10438. The isolate was grown on potato dextrose agar (Difco Laboratories, Detroit, MI) and on tap water agar (1.8 g UAMH 10438. The isolate was grown on potato dextrose agar (UAMH), Edmonton, Canada, where it was deposited as the University of Alberta Microfungus Collection and Herbarium (UAMH). The isolate showed it to be 99% similar (484 of 487 bases) to Curvularia inaequalis (AF120261 and 98% similar to Curvularia inaequalis). To identify the isolate, it was considered to be a contaminant.

Discussion. Peritonitis is one of the most common and serious complications of PD, and most episodes are caused by staphylococci (11). Fungal peritonitis (FP) has a prevalence of 2% to 10.2%, with Candida albicans being the predominating etiologic agent. However, FP caused by filamentous fungi is smaller, slightly paler end cells. The conidia were only slightly curved (Fig. 1 and 2) and not geniculate as is C. geniculata. The distal cell was quite broadly rounded, and the basal cell was not, or only slightly, protuberant. The isolate was identified as C. inaequalis according to the key provided by Ellis (8). To confirm the phenotypic identification, sequencing of the internal transcribed spacer regions (ITS) of nuclear rRNA genes was performed. Procedures for DNA extraction, amplification, and sequencing followed the protocols outlined by Gibas et al. (10). A BLAST (1) search using the ITS sequence for this isolate showed it to be 99% similar (484 of 487 bases) to C. inaequalis AF120261 and 98% similar to C. inaequalis AF163081 (486 of 493 bases) and AF313409 (479 of 487 bases).

Table 1. Summary of details of seven cases of FP caused by Curvularia species

<table>
<thead>
<tr>
<th>Year, reference</th>
<th>Patient’s age (yrs), sexa</th>
<th>Identity of isolate</th>
<th>Time on PD</th>
<th>Preexisting condition(s)b</th>
<th>Presenting complaint(s)c</th>
<th>Locations of visible, dark fungal material</th>
<th>Signs of peritonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985, DeVault et al. (6)</td>
<td>60, F</td>
<td>C. lunata</td>
<td>2 yrs</td>
<td>PKD</td>
<td>Tenckhoff obstruction</td>
<td>Bag, catheter wall</td>
<td>No</td>
</tr>
<tr>
<td>1988, Brackett et al. (3)</td>
<td>46, M</td>
<td>C. lunata</td>
<td>2 yrs</td>
<td>DM</td>
<td>Black material in effluent</td>
<td>Bag, catheter, effluent</td>
<td>No</td>
</tr>
<tr>
<td>1989, Guarner et al. (12)</td>
<td>53, M</td>
<td>Curvularia species</td>
<td>1.5 yrs</td>
<td>DM, HTN, CHF</td>
<td>Abdo pain, fever, N/V, diarrhea</td>
<td>Catheter, effluent</td>
<td>Yes</td>
</tr>
<tr>
<td>1990, Ujhelyi et al. (20)</td>
<td>28, M</td>
<td>Curvularia species</td>
<td>1.5 yrs</td>
<td>MPGN</td>
<td>Abdo pain, black flakes in effluent</td>
<td>Bag, catheter</td>
<td>Yes</td>
</tr>
<tr>
<td>1994, Lopes et al. (15)</td>
<td>63, M</td>
<td>C. lunata</td>
<td>3 yrs</td>
<td>DM</td>
<td>Abdo pain, vomiting, fever, turbid effluent</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>2001, Canon et al. (4)</td>
<td>11, M</td>
<td>Curvularia species</td>
<td>9 mos</td>
<td>ICGN</td>
<td>Tenckhoff obstruction, cloudy dialysate</td>
<td>Catheter, transfer set</td>
<td>No</td>
</tr>
<tr>
<td>2005 (present case)</td>
<td>85, F</td>
<td>C. inaequalis</td>
<td>2 yrs</td>
<td>RVD</td>
<td>Abdo pain, turbid effluent</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Note: a F; female; M, male.
b PKD, polycystic kidney disease; DM, diabetes mellitus; HTN, hypertension; CHF, congestive heart; failure; MPGN, membranoproliferative glomerulonephropathy; ICGN, immune complex glomerulonephritis; RVD, renovascular disease.
c Abdo, abdominal; N/V, nausea and vomiting.

In our review of the literature, we found no other cases of clinical infection due to C. inaequalis and only six other cases of a Curvularia species complicating PD (Table 1) (3, 4, 6, 12, 15, 20). The mean age of the patient group was 49.4 ± 18.1 years (range, 11 to 85 years). The average time on dialysis was 21.5 ± 6.5 months (range, 9 to 36 months; n = 6). The preexisting causes of renal failure included diabetes mellitus (n = 3), glomerulonephropathy (n = 2), polycystic kidney disease (n = 1), and renovascular disease (present case). Six of the seven patients had had previous episodes of peritonitis. The presenting symptoms in these cases included clinical signs of peritonitis (n = 4), Tenckhoff catheter obstruction (n = 2), and abdominal effluent (n = 5). In five cases, black fungal material was grossly visible in the lumen of the Tenckhoff catheter. DeVault et al. (6) confirmed catheter wall invasion by histological examination of the catheter tubing; however, Guarner and coworkers (12) were not able to demonstrate tube invasion when they examined histologic sections. We did not find fungal material in the catheter. Antifungal therapy most frequently consisted of administration of i.v. amphotericin B (n = 5). Guarner et al. (12) administered i.v. flucytosine, and DeVault et al. (6) gave no antifungal treatment. Brackett et al. (3) initially used i.p. amphotericin B without success and changed to i.v. amphotericin B. In all cases, the dialysis catheter was ultimately removed. Catheter removal was a temporary measure in four cases. Ujhelyi et al. (20) and Lopes et al. (15) opted for their patients to remain on hemodialysis. In all the previous cases, the patients recovered and survived the acute episode. Our patient died despite receiving i.v. amphotericin therapy. Curvularia species have been shown to be susceptible in vitro to amphotericin B, itraconazole, and ketoconazole (13), and a favorable response to oral itraconazole has been reported in locally invasive phaeohyphomycosis (18).

The treatment of FP remains controversial. Microbiologic cures with amphotericin B, flucytosine, econazole, miconazole,
and ketoconazole have been described (2, 7, 20). However, the dose of medication, the route and duration of treatment, and the use of combination antifungal therapy are debated. In addition to treatment with antifungals, catheter removal is believed to be essential for cure in cases of FP (2). However, controversy still exists on the best timing for catheter removal. The International Society of Peritoneal Dialysis recommends antifungal chemotherapy for 4 to 6 weeks and catheter removal is addition to treatment with antifungals, whereas the only trial of i.p. therapy failed. Discriminating features in our case are the much older age of the patient (85 years), the lack of response to i.v. therapy and the fatal outcome, and the etiology attributed to Curvularia disease is very often considered to be associated with soil contact. Cases include that of a football player (17) and those of gardeners and of children who played outdoors (9).

In conclusion, several important points arising from our review of Curvularia FP cases are that (i) pieces of dark material present in the effluent or catheter may indicate the presence of a phaeoid fungus and should be examined microscopically, (ii) removal of the PD catheter is a crucial element of treatment, and (iii) cure is achieved most frequently with i.v. antifungals, whereas the only trial of i.p. therapy failed. Distinguishing features in our case are the much older age of the patient (85 years), the lack of response to i.v. therapy and the fatal outcome, and the etiology attributed to C. inaequalis.

Nucleotide sequence accession number. The ITS sequence from the case isolate is deposited in GenBank under accession number AY941256.

We thank R. Shivis, Department of Primary Industries, Queensland, Australia, for providing data from the Australian Plant Pest Database. L. Sigler thanks the Natural Sciences and Engineering Research Council of Canada.

REFERENCES


TABLE 1—Continued

<table>
<thead>
<tr>
<th>Source of positive microscopy samples</th>
<th>Previous peritonitis</th>
<th>Catheter invasion</th>
<th>Coinfecting organism</th>
<th>No. of times Curvularia was isolated</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD fluid WCC (10⁶/liter) (%) N</td>
<td>Serum WCC (10⁶/liter) (%) N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1,474</td>
<td>Yes</td>
<td>Yes</td>
<td>1</td>
<td>RT for 2 wks</td>
<td>Infection resolved</td>
</tr>
<tr>
<td>49,600 (70)</td>
<td>10,700 (54)</td>
<td>Dark material</td>
<td>Yes</td>
<td>No</td>
<td>GNB</td>
<td>Infection resolved</td>
</tr>
<tr>
<td>704 (51)</td>
<td>10,500 (62)</td>
<td>Dark material</td>
<td>Yes</td>
<td></td>
<td>Proteus species*</td>
<td>Infection resolved</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,075 (78)</td>
<td>7,000 (71)</td>
<td>Dark material</td>
<td>Yes</td>
<td></td>
<td>Staphylococcus aureus</td>
<td>Infection resolved</td>
</tr>
<tr>
<td>2,328</td>
<td>15,100 (84)</td>
<td>Enrichment broth</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued

and ketoconazole have been described (2, 7, 20). However, the dose of medication, the route and duration of treatment, and the use of combination antifungal therapy are debated. In addition to treatment with antifungals, catheter removal is believed to be essential for cure in cases of FP (2). However, controversy still exists on the best timing for catheter removal. The International Society of Peritoneal Dialysis recommends antifungal chemotherapy for 4 to 6 weeks and catheter removal if there is no clinical improvement after 4 to 7 days (21).

Three prior reports of Curvularia FP identified C. lunata as the species involved, and the remainder identified the fungus to the genus level only. This is the first report concerning C. inaequalis. Curvularia species are similar in colonial morphologies, appearing cottony to felty, raised, and brownish-gray to black. The conidiophores are typically pale brown to brown, erect, and simple or branched, producing conidia sympodially on a geniculate rachis. The C. inaequalis conidia are evenly colored or have end cells that are slightly paler, are predominantly straight or only slightly curved, and are mostly five-celled, with the central cell larger than the others (8, 19) (Fig. 1 and 2). The C. lunata conidia are similar in size (20 to 32 μm long and 9 to 15 μm wide) (8) to C. inaequalis conidia but differ in being predominantly four-celled and more strongly curved due to the swollen subterminal cell (5, 8, 19). Our isolate was confirmed to be C. inaequalis by morphology and high ITS sequence similarity. A molecular phylogenetic analysis of ITS sequences showed that Cochliobolus species, including anamorphs placed in the genera Curvularia and Bipolaris, form a monophyletic group and that C. inaequalis and C. lunata are distinct species (16).

C. inaequalis has a broad distribution in temperate and subtropical areas, and it is associated mainly with forage grasses and grains (19). Data from the Australian Plant Pest Database showed that three of the four strains listed were from plants in the family Poaceae in South Australia; the fourth strain was isolated from soil in Queensland. The species is reported to have been isolated from the air and dust of one Canadian home (14). How our patient acquired her infection is unclear the case of a child who played in a wooded area (4) and that of a patient who would work in the garden prior to exchanges (20). These cases are consistent with the literature, which indicates that clinically relevant Curvularia disease is very often considered to be associated with soil contact. Cases include that of a football player (17) and those of gardeners and of children who played outdoors (9).

In conclusion, several important points arising from our review of Curvularia FP cases are that (i) pieces of dark material present in the effluent or catheter may indicate the presence of a phaeoid fungus and should be examined microscopically, (ii) removal of the PD catheter is a crucial element of treatment, and (iii) cure is achieved most frequently with i.v. antifungals, whereas the only trial of i.p. therapy failed. Distinguishing features in our case are the much older age of the patient (85 years), the lack of response to i.v. therapy and the fatal outcome, and the etiology attributed to C. inaequalis.

Nucleotide sequence accession number. The ITS sequence from the case isolate is deposited in GenBank under accession number AY941256.

We thank R. Shivis, Department of Primary Industries, Queensland, Australia, for providing data from the Australian Plant Pest Database. L. Sigler thanks the Natural Sciences and Engineering Research Council of Canada.


