Acremonium kiliense: Reappraisal of Its Clinical Significance

Ziauddin Khan,1* Khaled Al-Obaid,2 Suhail Ahmad,1 Amal Abdel Ghani,2 Leena Joseph,1 and Rachel Chandy1

Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait,1 and Department of Microbiology, Mubarak Al-Kabeer Hospital, Ministry of Health, Jabriya, Kuwait2

Received 11 November 2010/Returned for modification 29 November 2010/Accepted 18 March 2011

A case of Acremonium kiliense peritonitis is described. Diagnosis was established by repeated isolation of the fungus from peritoneal dialysate and by its identification on the basis of morphological characteristics and sequencing of internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA). This report and available literature suggest that A. kiliense may have a greater clinical significance than hitherto recognized.

CASE REPORT

A 75-year-old Jordanian man with a long history of diabetes mellitus and hypertension developed end-stage kidney disease requiring continuous ambulatory peritoneal dialysis (CAPD). His course of CAPD was largely uneventful except for a single episode of bacterial peritonitis, for which he was hospitalized and treated with parenteral antibiotics, with an excellent response. In January 2010, he became febrile, with clinical evidence of peritonitis, and was admitted to the hospital. The peritoneal fluid was turbid, with a white blood cell (WBC) count of 2.1 × 109/liter. On initial microscopic examination, the presence of fungal elements in the peritoneal dialysate was missed. The peritoneal fluid sample was inoculated into aerobic Bactec blood culture bottles, which yielded a growth after 59 h of incubation. The Gram-stained smear from blood culture bottles showed branched hyphal elements. On Sabouraud dextrose agar (SDA; Oxoid Ltd., Basingstoke, England), the specimen yielded slimy colonies with a pinkish appearance (Fig. 1). Microscopic examination of the primary culture (isolate Kw441-2010) showed hyaline hyphae, with scanty sporulation. A provisional identification of Acremonium/Fusarium species was made, and the growth was subcultured on Sabouraud dextrose agar and oatmeal agar (OMA; oatmeal [30 g], agar [20 g], distilled water [1 liter]) for further identification and antifungal susceptibility testing. Subsequent cultures of the peritoneal fluid yielded the same fungus on three occasions. A serum sample was obtained for the detection of galactomannan (Platelia Aspergillus enzyme immunoassay [ELA] kit; Bio-Rad, Marnes-la-Coquette, France) and (1-3)-β-D-glucan (FungiTest; Associates of Cape Cod); the latter test was positive (253 pg/ml). An Etest performed on RPMI 1640 medium supplemented with 2% glucose revealed that the isolate was resistant to amphotericin B and caspofungin but susceptible to voriconazole and posaconazole, with MIC values of >32 μg/ml, >32 μg/ml, 0.064 μg/ml, and 0.75 μg/ml, respectively. The patient was started on voriconazole, with a loading dose of 400 mg, followed by a maintenance dose of 200 mg, given every 12 h via the oral route. Although the patient showed clinical improvement after 2 weeks of voriconazole therapy, the peritoneal dialysate remained turbid (WBC counts, 2.0 × 109/liter). Abdominal ultrasound examination did not reveal any evidence of intraperitoneal adhesions or organ invasion. Since the response to treatment was not adequate, the Tenckhoff catheter was removed and the patient was temporarily switched to hemodialysis. After 1 week of additional voriconazole therapy, the peritoneal dialysate became clear, and microscopic examination and culture were negative. The patient was discharged with advice to continue oral voriconazole (200 mg twice daily) for 1 month with regular follow-up in the CAPD unit. He remained symptom free for about 3 weeks but was readmitted with symptoms of severe septicemia due to Staphylococcus aureus and died of septic shock despite treatment.

Colonies of the isolate on SDA at 30°C were initially white and glabrous but became pinkish on further incubation. On microscopic examination, the growth showed mostly fasciculate mycelium, which gave rise to erect, slender phialides (18 to 54 by 1.6 to 3 μm) (Fig. 2), forming hyaline, thin-walled, slightly curved, cylindrical-to-ellipsoidal conidia (3 to 5 by 1.2 to 2.4 μm) at the tip, occurring mostly in groups (Fig. 2). On OMA medium, after 10 days of incubation at 24°C, the isolate formed adelophialides (Fig. 3A) and unicellular terminal and intercalary thick-walled chlamydospores (Fig. 3B). These phenotypic characteristics identified the isolate as Acremonium kiliense (30).

The DNA from the isolate was prepared as described in detail previously (1). The entire internal transcribed spacer (ITS) region (containing ITS-1, 5.8S rRNA, and ITS-2) of the ribosomal DNA (rDNA) was amplified by PCR by using panfungal primers ITS1 and ITS4 as described previously (3). The amplicons were purified by using a PCR product purification kit (Qiagen, Hilden, Germany), and both strands were sequenced by using ITS1, ITS4, ITS1FS, ITS2, ITS3, or ITS4RS as sequencing primers as described in detail previously (13). For determining the sequence-specific identity of our isolate, pairwise comparisons were made by using ClustalW. The entire ITS region sequence (490 nucleotides) of our isolate exhibited 100% identity with the corresponding sequence from the type strain (MUCL 9724T) of A. kiliense. Based on previous observations that strains belonging to same species exhibit >99% nucleotide identity in the ITS-1 and ITS-2 regions of

* Corresponding author. Mailing address: Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat, Kuwait 13110. Phone: 00965-24986504. Fax: 00965-5318454. E-mail: zkhan@hsc.edu.kw.

† Published ahead of print on 30 March 2011.
their rDNA, the molecular identity of our isolate was determined as *A. kiliense* (30, 31).

*In vitro* susceptibility was determined by the Etest (AB Biodisk, Solna, Sweden) on RPMI 1640 medium supplemented with 2% glucose and buffered with morpholinepropanesulfonic acid. The test was performed according to the manufacturer’s instructions. Growth from a 7-day-old culture was uniformly suspended in 1 ml of normal saline. The clumps were allowed to settle, and the supernatant was used as an inoculum. The plates were inoculated by dipping a sterile swab into the growth suspension and streaking it uniformly across the surface of the agar. The plates were dried at ambient temperature for 15 min before Etest strips were applied. The plates were incubated at 35°C and read at 48 h. The Etest MICs were read at the point where dense colonial growth intersected the strip (24). The isolate was considered susceptible to voriconazole (0.064 μg/ml) and posaconazole (0.75 μg/ml) and resistant to amphotericin B (>32 μg/ml), fluconazole (>256 μg/ml), 5-fluorocytosine (>32 μg/ml), and caspofungin (>32 μg/ml).

**Discussion.** The last 2 decades have witnessed a steady increase in the spectrum of hyaline fungi incriminated as opportunistic pathogens in immunocompromised patients (5, 14, 28). Many soil saprobes and plant pathogens with no obvious pathogenic potential have now emerged as etiologic agents under a variety of clinical conditions, thus posing new diagnostic and therapeutic challenges (38). Although *Aspergillus* and *Fusarium* are two major pathogenic filamentous genera, the role of *Acremonium* spp. is also being increasingly recognized in both localized and systemic infections (5, 7, 28). In immunocompetent individuals, keratitis, endophthalmitis, mycetoma, onychomycosis, or cutaneous infections are the most familiar forms of localized infections (5, 38). On the other hand, in
TABLE 1. Summary of cases of peritonitis caused by Acremonium spp.a

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Year</th>
<th>Age/sex</th>
<th>Underlying condition(s)</th>
<th>Species</th>
<th>Treatment</th>
<th>Patient outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landay et al. (18)</td>
<td>USA</td>
<td>1982</td>
<td>68 yr/M</td>
<td>CAPD, ERD</td>
<td>Acremonium sp. and Klebsiella, A. kiliense</td>
<td>Amphotericin B</td>
<td>Died</td>
</tr>
<tr>
<td>Lopes et al. (21)</td>
<td>Brazil</td>
<td>1995</td>
<td>8 yr/M</td>
<td>Chronic renal failure</td>
<td>A. kiliense</td>
<td>Ketoconazole, catheter removal</td>
<td>Cured</td>
</tr>
<tr>
<td>Kendirli et al. (12)</td>
<td>Turkey</td>
<td>2008</td>
<td>7 mo</td>
<td>Hyponatremia, Down syndrome, congenital heart diseases</td>
<td>A. strictum</td>
<td>Fluconazole, amphotericin B, catheter removal</td>
<td>Died due to VAP, sepsis</td>
</tr>
<tr>
<td>Sener et al. (34)</td>
<td>Turkey</td>
<td>2008</td>
<td>47 yr/F</td>
<td>CAPD, ERD</td>
<td>A. strictum</td>
<td>Fluconazole, catheter removal</td>
<td>Cured</td>
</tr>
<tr>
<td>This study</td>
<td>Kuwait</td>
<td>2010</td>
<td>75 yr/M</td>
<td>ERD</td>
<td>A. kiliense</td>
<td>Voriconazole, catheter removal</td>
<td>Improved, died of Staphylococcus septicemia</td>
</tr>
</tbody>
</table>

a Abbreviations: M, male; F, female; CAPD, continuous ambulatory peritoneal dialysis; DM, diabetes mellitus; ERD, end-stage renal disease; i.p., intraperitoneally; i.v., intravenously; VAP, ventilator-associated pneumonia.

severely immunocompromised patients, Acremonium species may cause disseminated infections involving multiple organs following fungemia (9, 10, 38). In one autopsy-proven study of invasive mold infections in cancer patients, 10% of the cases were caused by Acremonium spp. (14).

The present case of peritonitis highlights the clinical importance of A. kiliense in human infections. The diagnosis was established by repeated isolation of the fungus from peritoneal dialysate in culture and its identification by typical microscopic characteristics and sequencing of the ITS regions of rDNA. So far, nine cases of Acremonium peritonitis (including the present case) have been reported and are summarized in Table 1 (12, 18, 21, 22, 25, 34). Three of these cases were caused by A. kiliense and two by Acremonium strictum; in the remaining four cases, the Acremonium species were not identified (Table 1). Patients of all age groups (7 months to 75 years) were affected. Four patients were cured following antifungal therapy and/or removal of their Tenckhoff catheter and three died, and for two patients, the outcome was not known. Of the three patients who did not survive, two apparently died of bacterial sepsis; the other patient probably had parenchymal invasion, and the total dose of amphotericin B (105 mg) administered was probably not sufficient for a favorable outcome (18). For the remaining two patients (22), who were treated with itraconazole (400 mg/day), the cultures became negative for the period of follow-up; however, the final outcome was not reported. The source of Acremonium infection in patients on peritoneal dialysis is difficult to ascertain; however, considering the manipulations involved in the procedure, environmental contamination is highly possible. Generally, the management of Acremonium peritonitis includes catheter removal and systemic antifungal therapy. Although amphotericin B has been used with favorable outcomes in some early reports, voriconazole or posaconazole may be better alternatives, particularly in patients with renal insufficiency. Presently, the clinical experience with the latter drugs in the treatment of Acremonium infections is limited (23, 36).

In a review of published cases of Acremonium infections other than peritonitis since 1981 for which etiologic species were identified to the species level, 18 of them were reportedly caused by A. kiliense (Table 2). Five of these cases were reported from the United States (4, 8), four each were reported from Brazil (15, 16, 20, 29) and India (6, 11, 37), two each were reported from Argentina (2, 26) and France (17, 19), and one was reported from Hungary (35). Only three of these cases occurred in immunocompromised patients, and the fungus was isolated from heart and brain tissue (15), blood (17), and lung tissue (29). Of these patients, one died (15) and two survived the infection (17, 29). In the remaining 15 patients, who were apparently immunocompetent, the infections were localized and were treated with antifungal agents with or without surgical debridement of the infected tissue (Table 2).

Considering the difficulties in identifying clinical Acremonium spp., it is probable that the etiologic species described in some of the case reports may have been misidentified. In fact, A. strictum, which has been described as the etiologic agent in most of the case reports (5, 9), could be one such species; its etiologic role in human infections appears to be uncertain (10). Since there are close morphological similarities between A. strictum and A. kiliense, it is possible that these cases were actually caused by the latter species or by some other species of the genus. In this context, attention may be drawn to two recent case reports (9, 27) where sequenced isolates identified as A. strictum in fact belonged to the Acremonium sclerotigenum-Acremonium egyptiacum group (30). In an attempt to differentiate A. kiliense from A. strictum, Perdomo et al. (30) observed that isolates of A. kiliense form unicellular chlamydospores and adelphialides (reduced forms of phialides without a basal septum) in the vegetative or substrate hyphae (not in aerial hyphae) when grown on oatmeal agar at 24°C for about 2 weeks. These morphological structures were also observed in our isolate, which confirmed its identity as A. kiliense.

In order to unequivocally establish the molecular identity of our isolate, we retrieved the ITS region sequences from type or reference strains of well-known Acremonium spp. of clinical relevance which have recently become available in the DNA
sequence database (30). These included *A. kiliense* (CBS 122.29/ATCC 34716/MUCL 9724T, GenBank accession no. AJ 621775), *Acremonium zaeae* (CBS 800.69T, accession no. FN691451), *A. strictum* (CBS 346.70/ATCC34717T, accession no. FN691453), *Acremonium implicatum* (MUCL 4112, accession no. FN70653), *Acremonium glaucum* (CBS 796.69T, accession no. FN691451), *A. sclerotigenum* (CBS 280.86 and CBS 281.80, accession no. FN706551 and FN706549, respectively), *A. egyptiacum* (CBS 114785T, accession no. FN706550), and *Acremonium persicum* (CBS 310.59T, accession no. FN706554).

As mentioned above, the entire ITS region sequence (490 nucleotides) of our isolate exhibited 100% identity with the corresponding sequence from *A. kiliense* MUCL 9724T. However, it differed at 15 or more nucleotide positions from the ITS region sequences from type or reference strains of the other *Acremonium* spp. mentioned above. With the availability of ITS region sequences of type or reference strains in GenBank and with an improved understanding of the morphological characteristics of individual *Acremonium* spp., it should be possible to identify clinical isolates to the species level with greater accuracy.

Due to the difficulties previously described for the phenotypic identification of *Acremonium* spp., most clinical laboratories identify isolates only to the genus level. Thus, the relative etiologic role of an individual *Acremonium* sp. under different clinical conditions remains under-documented. Moreover, morphologically similar fungal isolates belonging to other genera may be misidentified as *Acremonium* spp. (30). To clarify taxonomic uncertainties about the identification of *Acremonium* spp. and to assess their relative etiologic significance in human infections, Perdomo et al. (30) reexamined 75 phenotypically identified clinical isolates by studying morphological characteristics and comparing their observations with 29 type/reference strains available in the Centraalbureau voor Schimmelcultures (CBS-KNAW, Netherlands) and the Mycotheque de l'Université Catholique de Louvain (MUC, Belgium). These investigators also sequenced ITS regions of rDNAs of these clinical isolates and compared them with type/reference strain sequences. This comprehensive study provided new insight into the etiologic spectrum of *Acremonium* spp. associated with human infections. Contrary to the generally held view, it was *A. kiliense* (30%), and not *A. strictum*, *Acremonium recipiens*, or *Acremonium potronii*, which was the predominant species, followed by *A. sclerotigenum-A. egyptiacum* (22%), *A. implicatum* (14%), *A. persicum* (14%), and *Acremonium atregrides* (8%) among the 50 clinical isolates that were identified by morphological and molecular methods. Of the 15 *A. kiliense* isolates, four came from eye, three from blood, three from respiratory tract, and one each from cerebrospinal fluid (CSF), vertebral disc, sinus, foot mass, and scalp specimens, but none came from nails.

Another interesting finding of the study was the identification of several species that were not previously incriminated in human infections. It remains to be investigated if clinical isolates from

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Yr</th>
<th>Age/Sex</th>
<th>Underlying condition</th>
<th>Site(s) of disease or type of infection</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacaz et al. (15)</td>
<td>Brazil</td>
<td>1981</td>
<td>47 yr/M</td>
<td>Prosthetic heart valve</td>
<td>Heart, brain</td>
<td>Surgery, AB (local) + FC</td>
<td>Died</td>
</tr>
<tr>
<td>Brabender et al. 4</td>
<td>USA</td>
<td>1985</td>
<td>35 yr/M</td>
<td>Head trauma</td>
<td>Head (osteomyelitis)</td>
<td>Surgical debridement (craniectomy), AB, KE</td>
<td>Cured</td>
</tr>
<tr>
<td>Lacroix et al.</td>
<td>France</td>
<td>1988</td>
<td>32 yr/F</td>
<td>Esophageal cancer</td>
<td>Septicemia, blood, catheter, feces?</td>
<td>AB, FL, NYS</td>
<td>Cured</td>
</tr>
<tr>
<td>Simon et al. (35)</td>
<td>Hungary</td>
<td>1991</td>
<td>11 yr/M</td>
<td>Myeloma</td>
<td>Myotic esophagitis</td>
<td>IT, NMY, NYS, surgery</td>
<td>Cured</td>
</tr>
<tr>
<td>Venugopal and Venugopal (37)</td>
<td>India</td>
<td>1995</td>
<td>24 yr/M</td>
<td>Trauma</td>
<td>Right foot</td>
<td>KE, DE</td>
<td>Cured</td>
</tr>
<tr>
<td>Lopez et al. (20)</td>
<td>Brazil</td>
<td>1995</td>
<td>4 yr/M</td>
<td>None</td>
<td>Back and perineum</td>
<td>Scalp</td>
<td>Cured</td>
</tr>
<tr>
<td>Fridkin et al. (8)b</td>
<td>USA</td>
<td>1996</td>
<td>73 yr/F</td>
<td>Cataract extraction</td>
<td>Endophthalmitis</td>
<td>FL, topicaly, orally, AB</td>
<td>Cured</td>
</tr>
<tr>
<td>Le Guen et al. (19)</td>
<td>France</td>
<td>1997</td>
<td>73 yr/M</td>
<td>Cataract extraction</td>
<td>Keratomycosis</td>
<td>FL, TER</td>
<td>Cured</td>
</tr>
<tr>
<td>Lacaz et al. (16)</td>
<td>Brazil</td>
<td>1999</td>
<td>?/M</td>
<td>NA</td>
<td>Podaic mycetoma</td>
<td>IT</td>
<td>Cured</td>
</tr>
<tr>
<td>Gupta et al. (11)</td>
<td>India</td>
<td>2003</td>
<td>NA</td>
<td>Cataract surgery</td>
<td>Endophthalmitis</td>
<td>Surgery, AB</td>
<td>Cured</td>
</tr>
<tr>
<td>Pastorino et al. (29)</td>
<td>Brazil</td>
<td>2005</td>
<td>25 days/M</td>
<td>CGD</td>
<td>Pneumonia</td>
<td>IT (6 wk)</td>
<td>Cured</td>
</tr>
<tr>
<td>Negroni et al. (26)</td>
<td>Argentina</td>
<td>2006</td>
<td>NA</td>
<td>NA</td>
<td>Mycetoma</td>
<td>KE, IT</td>
<td>Cured</td>
</tr>
<tr>
<td>Albrecht et al. (2)</td>
<td>Argentina</td>
<td>2010</td>
<td>18 yr/M</td>
<td>Trauma</td>
<td>Onychomycosis</td>
<td>8% ciclopirox nail solution, IT orally</td>
<td>Cured</td>
</tr>
<tr>
<td>Das et al. (6)</td>
<td>India</td>
<td>2010</td>
<td>46 yr/M</td>
<td>Type 2 diabetes</td>
<td>Nodular swelling in soles, toenail</td>
<td>FL, TER</td>
<td>Cured</td>
</tr>
</tbody>
</table>

Abbreviations: AB, amphotericin B; EC, econazole; FC, 5-fluorocytosine; FL, fluconazole; IT, itraconazole; KE, ketoconazole; NA, not available; NTS, natamycin; NYS, nystatin; s.c., subcutaneously; TER, terbinafine; CGD, chronic granulomatous disease.

b The management of these cases was apparently described by Weissgold et al. (39).
other tropical and temperate geographic regions exhibit similar etiologic spectra or if they vary according to ecological or environmental conditions. It is also possible that individual Acremonium species prefer to cause a particular type of infection, as reflected by the apparent association between A. recifei and mycetoma (5).

Presently, there is a paucity of data on the antifungal susceptibilities of Acremonium spp. The reduced susceptibility of our strain to amphotericin B, 5-fluorocytosine, fluconazole, and caspofungin is consistent with findings of previously published studies (10, 30). Most of the information on antifungal susceptibility has originated from single isolates described in published studies (10, 30). In some other studies, only small numbers of Acremonium species isolates (without species-level identification) have been tested, yielding inconsistent results, particularly with reference to amphotericin B (7, 32, 33). Due to either strain variation or the susceptibility method used, different geographic regions are needed to elucidate the etiologic spectra or if they vary according to ecological or environmental conditions.


REFERENCES


