Members of the genus Exophiala, which belong to the dematiaceous fungi, are ubiquitous in nature, especially in soil, water, sewage, rotting wood, and other plant matter [1–3]. These organisms have been described as pathogens in animals and are also recognized as being of increasing importance as pathogens causing human diseases [1, 2, 4, 5]. Clinical infections due to Exophiala species include mycetoma, subcutaneous phaeohyphomycosis, endocarditis, pneumonia, lung abscess, synovitis, arthritis, peritonitis, esophagitis, and keratitis [4, 6–14]. Most patients with clinical infection due to Exophiala species are immunocompromised or infected with HIV, but infections in previously healthy hosts have also been reported [1, 2, 4, 5].

Although strains of Exophiala species are widely distributed in the environment, nosocomial Exophiala jeanselmei pseudoinfection in thoracic disease patients has not been reported. In our chest sonography room, a pseudoepidemic due to E. jeanselmei was initially suspected in September to October 1996 after the identification of two isolates of the organism in pleural effusion specimens from two patients after sonography-guided aspiration. In the course of the investigation, a total of 16 isolates recovered from pleural effusion specimens were identified as E. jeanselmei.
specimens or biopsy of lung tissue via sonography-guided aspiration among 16 patients with thoracic disease between August 1994 and April 1998 were analyzed. Using epidemiologic surveillance methods coupled with microbiologic studies, we determined the relatedness of these isolates with regard to biotype, antimicrobial susceptibility, and genotype. The source of these pseudoinfections could not be traced because of the lack of recognition of the importance of this saprophytic fungus and, probably, due to the slow-growing nature of the organism, which makes timely environmental surveillance cultures not unfeasible. The purpose of this study was to describe the clinical characteristics of patients with *E. jeanselmei* pseudoinfection and to determine the microbiologic relatedness of the isolates.

**Materials and Methods**

**Study setting and microbiological culture records**

National Taiwan University Hospital is a tertiary-care teaching hospital in northern Taiwan. Each month, about 120 patients with various thoracic diseases undergo sonography examinations and about 70 to 80 patients undergo sonography-guided aspiration or biopsy of thoracic lesions in the chest sonography room. From September 1996 to October 1996, two patients who had pneumonia and parapneumonic effusion underwent sonography-guided aspiration of the pleural effusion for microbiologic and cytologic analysis. Ten to 14 days later, cultures of these two pleural effusion samples both grew *E. jeanselmei* without other microorganisms. These two patients received antibacterial agents but not antifungal agent therapy and both recovered. During this 2-month period, a total of 35 specimens, including pleural effusion and pulmonary parenchymal lesions, were collected via sonographic guidance and all but the samples of the two patients were negative for *E. jeanselmei*. A review of microbiologic culture records revealed that seven isolates of *E. jeanselmei* were recovered during the period from December 1, 1994, to September 1, 1996, and an additional 10 isolates were recovered from November 1, 1996, to October 31, 1998. All but three of these isolates were recovered from pleural effusion or lung mass specimens collected in the chest sonography room (Table 1).

**Epidemiologic surveillance**

Environmental samples were collected for surveillance cultures between October 1996 and November 1996 and between March 1997 and November 1998, to detect the presence of *E. jeanselmei* after each isolate of *E. jeanselmei* was discovered. These samples included 75% alcohol (4 samples), beta-iodine solution (4), lidocaine (4), swabs of collection tubes (14), syringes (10), machine surfaces (4), detection probes (6), linen (6), floor (4), and room air filter (4 times). Specimens from the skin of eight patients and the hands of one physician were also cultured for *E. jeanselmei* by plating onto Sabouraud dextrose agar plates (Difco Laboratories, Detroit, MI, USA) and incubating at 30°C for 4 weeks.

**Identification of isolates**

Specimens (pleural effusion, wound pus, and tissue) were plated onto Sabouraud dextrose agar plates. The plates were incubated at 30°C and 37°C, and were inspected daily for growth. Cornmeal agar (BBL Microbiology Systems, Cockeysville, MD, USA) slide cultures were also used in the identification process. Identification of the fungal isolate to the species level was based on the colonial morphology and microscopic picture as previously described [2, 3]. Strains were stored at -70°C in trypsin soy broth (BBL Microbiology Systems) supplemented with 15% glycerol until testing.

**Assimilation test**

The API ID32C System (BioMerieux, Marcy-l’Etoile, France) was used to determine the assimilation of different carbon substrates by these isolates. The system was used according to the manufacturer’s instructions with modifications including a higher inoculum (10⁶ colony-forming units, CFU/mL) and incubation of the strip at 30°C for 5 days [15]. All isolates were tested twice to verify the reproducibility of the tests.

**Cellular fatty acid composition**

Cellular fatty acid analysis was performed for 10 isolates (not isolate I3). Each isolate was harvested after 5 days of incubation at 28°C on Sabouraud dextrose agar plates. Lysis of cells, saponification, methylation of cellular fatty acids, and extraction of fatty acid methyl esters (FAMEs) were performed according to the manufacturers’ instructions and a previous description [15]. FAMEs were separated by gas chromatography and were analyzed with the Microbial Identification System (MIS) of MIDI (Microbial ID, Inc., Newark, DL, USA) [15, 16]. The analysis was performed twice with different subcultures for these isolates. The difference between the two isolates was expressed as a Euclidean distance, which is the distance in two-dimensional space between two isolates when their FAME compositions are compared [15, 16].
Antibiotypes

Antifungal susceptibility testing of the isolates was performed by the E-test in accordance with the manufacturer’s suggestions for testing strains of *Aspergillus*, *Fusarium*, and *Rhizopus* species. RPMI 1640 (American Biorganics, Buffalo, NY, USA) supplemented with 2% glucose, morpholinepropanesulfonic acid (MOPS; Difco Laboratories, Detroit, MI, USA), and 1.5% Bacto agar (BBL Microbiology Systems) was used as the growth medium. Five agents, including amphotericin B, fluconazole, itraconazole, and ketoconazole, were tested. *Candida parapsilosis* ATCC 22019 and *Paecilomyces variotii* ATCC 22319 were used as control strains. After 5 days of incubation at 30°C, the minimum inhibitory concentrations (MICs) of the agents against the isolates were read where the inhibition ellipse intersected the MIC scale.

Random amplified polymorphic DNA patterns

Random amplified polymorphic DNA (RAPD) patterns of the 11 isolates were generated by arbitrarily primed polymerase chain reaction (APPCR). Strains were cultured on Sabouraud dextrose agar for 7 days at 30°C. Two 10-µL loops of culture material were suspended in 300 µL of trypticase solution (Sigma, St. Louis, MO, USA). Chromosomal DNA of the isolates was extracted using a commercial kit, Puregene D-6000 (Gentra Systems, Inc., Minneapolis, MN, USA). Five arbitrary oligonucleotide primers selected from two commercial kits B (OPB-01 to OPB-20) and H (OPH-01 to OPH-20) were used: OPB-15 (5'-GGAGGGTGTT-3'), OPB-18 (5'-CCACAGCAGT-3'), OPH-12 (5'-ACGCGCATGT-3'), OPH-15 (5'-AATGGCGCAG-3'), and OPH-18 (5'-GAATCGGCCA-3') (Operon Technologies, Inc., Alameda, CA, USA). Amplification was performed in a PTC-100 thermocycler (MJ Research Inc., Watertown, MA, USA) and consisted of the following steps: predenaturation for 4 minutes at 94°C, 40 cycles of 30s at 94°C, 1 minute at 34°C, and 2 minutes at 72°C, and a final extension for 5 minutes at 72°C. The amplification products were separated by electrophoresis on 1.5% agarose gel. Gels were photographed and interpreted by visual examination. A 1-kb (kilobase) ladder (Gibco BRL Products, Gaithersburg, MD, USA) was used in each gel as a DNA fragment size marker. All experiments were performed in triplicate.

Table 1. Characteristics of 17 patients with various types of thoracic disease who had positive cultures for *Exophiala jeanselmei* during the period from 1994 to 1998

<table>
<thead>
<tr>
<th>No.</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Date (d/mo/yr)</th>
<th>Source</th>
<th>Designation</th>
<th>Biotype</th>
<th>Antibiotyp pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50/F</td>
<td>Acute myelocytic leukemia, pneumonia</td>
<td>12/8/1994</td>
<td>Pleural effusion</td>
<td>A</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>2.</td>
<td>60/F</td>
<td>Thoracic empyema</td>
<td>21/2/1995</td>
<td>Empyema fluid</td>
<td>B</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>3.</td>
<td>71/M</td>
<td>Thoracic empyema</td>
<td>23/2/1995</td>
<td>Empyema fluid</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.</td>
<td>60/M</td>
<td>Infective endocarditis</td>
<td>15/10/1995</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5.</td>
<td>68/M</td>
<td>Metastatic adenocarcinoma</td>
<td>18/10/1995</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6.</td>
<td>67/M</td>
<td>Lung carcinoma</td>
<td>17/6/1996</td>
<td>Lung mass</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7.</td>
<td>74/F</td>
<td>Fever of unknown origin</td>
<td>27/7/1996</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8.</td>
<td>43/M</td>
<td>Non-Hodgkin lymphoma, pneumonia</td>
<td>19/9/1996</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9.</td>
<td>69/M</td>
<td>Pneumonia</td>
<td>5/10/1996</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10.</td>
<td>56/M</td>
<td>Lung carcinoma, pneumonia, septic shock</td>
<td>20/3/1997</td>
<td>Pleural effusion</td>
<td>C</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>11.</td>
<td>59/M</td>
<td>Lung carcinoma</td>
<td>11/2/1998</td>
<td>Pleural effusion</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>12.</td>
<td>66/M</td>
<td>Liver cirrhosis</td>
<td>11/2/1998</td>
<td>Pleural effusion</td>
<td>D</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>13.</td>
<td>40/M</td>
<td>Lung tuberculosis</td>
<td>25/2/1998</td>
<td>Pleural effusion</td>
<td>E</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>14.</td>
<td>51/M</td>
<td>Maxillary carcinoma with lung metastasis</td>
<td>27/2/1998</td>
<td>Pleural effusion</td>
<td>F</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>15.</td>
<td>83/M</td>
<td>Pulmonary tuberculosis</td>
<td>27/3/1998</td>
<td>Pleural effusion</td>
<td>G</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>16.</td>
<td>54/M</td>
<td>Lung carcinoma</td>
<td>8/4/1998</td>
<td>Pleural effusion</td>
<td>H</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>17.</td>
<td>63/M</td>
<td>Cardiac transplant, cutaneous phaeohyphomycosis (right hand)</td>
<td>11/9/1998</td>
<td>Necrotic tissue</td>
<td>I-1</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9/10/1998</td>
<td>Wound pus</td>
<td>I-2</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25/10/1998</td>
<td>Skin biopsy</td>
<td>I-3</td>
<td>II</td>
<td>II</td>
</tr>
</tbody>
</table>

*a* Isolates were not preserved for study. RAPD = random amplification polymorphic DNA; d = day; mo = month; yr = year; F = female; M = male.

**Exophiala jeanselmei** *Pseudoepidemic*
Definition
Antibiotypes of the isolates were considered identical if the MICs of all agents tested were identical or within a two-fold dilution discrepancy. Isolates with RAPD patterns that differed by one or more discrete bands were categorized as different. Otherwise, the patterns were considered identical. Isolates were defined as the same strain or derived from a single clone if they had identical antibiotypes, biotypes, and RAPD patterns.

Results

Patient characteristics
From August 1994 to October 1998, a total of 19 isolates of *E. jeanselmei* were recovered from 17 patients with thoracic diseases. The demographic data and underlying diseases of these patients, the sources and dates of recovery of the isolates, and the pathologic or cytologic findings of the aspirated or biopsy specimens of these patients are shown in Table 1. Sixteen of these patients underwent pleurocentesis due to the presence of pleural effusion or aspiration of the thoracic lesion in the chest sonography room. These patients had no evidence of fungal infection, received no antifungal therapy, and survived during the hospital stay. Another patient, who underwent a cardiac transplant in 1996, had an ulcerative wound over the right hand for 3 months. He had not visited the sonography room prior to diagnosis. *E. jeanselmei* was isolated from the necrotic tissue, pus, and biopsy specimen of the wound. Cutaneous phaeohyphomycosis was diagnosed in this patient and he underwent surgical debridement and received itraconazole (200 mg twice daily) for 1 month; he responded well. All 17 patients were notified that *E. jeanselmei* had been isolated from their specimens within 10 to 14 days after collection of specimens for culture.

Epidemiologic investigation and infection control measures
All specimens from environmental sources, patients’ skin, and the hands of the physician were negative for *E. jeanselmei*. Except for alerting the staff about the need for thorough and frequent hand washing among medical personnel working in the sonography room, no additional infectious control measures were taken.

Fungal isolates
On the Sabouraud dextrose agar and cornmeal agar subcultures, the colonies of the 11 isolates were black, 20 to 25 mm in diameter, raised or dome-shaped, and covered by short gray aerial hyphae after 2 weeks of incubation. The reverse of the culture dish was black. Microscopically, the fungus produced brown septate hyphae with cylindrical annellides that formed unicellular, subglobose, and ellipsoidal to cylindrical annelloconidia. The conidia, measuring 1.5 to 3 µm in diameter, were smooth and aggregated in masses in the tips of the annellides or slid down the conidiophore or along the hyphae. Based on the above findings, these isolates were presumptively identified as *E. jeanselmei*.

Biotypes
Assimilation profiles of the 11 isolates of *E. jeanselmei* determined by API ID 32C are shown in Table 2. All isolates assimilated melezitose, sucrose, sorbitol, and glycerol and had negative assimilation reactions for lactose, ribose, and inositol. Differences were found in the assimilation of galactose, raffinose, α-methyl-D-glucosidase, sorbose, and glucosamine. On the basis of the assimilation profiles of the five substrates, two biotypes were identified, biotypes I (8 isolates) and II (3 isolates; Table 2). The results of duplicate testing for each isolate were identical.

Cellular fatty acid profiles
The cellular fatty acid chromatogram of an *E. jeanselmei* isolate is shown in Fig. 1. Although the FAME profiles of the 10 isolates were not identified as *E. jeanselmei* by the MIS System, all these isolates had the five major FAMEs (> 3% of total FAMEs), including 18:1 w9c (cis-9-octadecenoic acid), 16:0 hexadecanoic acid, 18:2 octadecadienoic acid, 18:1 w9t (trans-9-octadecenoic acid), and 18:0 (octadecanoic acid, with respective percentage concentrations for each FAME of 35 to 40%, 23 to 30%, 13 to 20%, 8 to 14%, and 6 to 8%). The cluster analysis identified two clusters (isolates A and B, and Isolates C to I-1 and I-2). If we adopted the criteria of interpreting Euclidean distance for bacterial strains, all of these isolates belonged to the same subspecies (a
Exophiala jeanselmei Pseudoepidemic

Euclidean distance of < 6), and isolates C, F, G, and H belonged to the same strain (clone).

Antibiotypes
As shown in Table 3, two antibiotypes (antibiotypes I and II) were identified. Isolates belonging to antibiotype I (8 isolates) had amphotericin B, ketoconazole, and itraconazole MICs 10- to 16-fold higher than those for isolates of antibiotype II (3 isolates).

RAPD patterns
Using the five primers, isolates A to H had identical RAPD patterns (pattern 1), which were obviously different from those for isolates of I-1, I-2, and I-3 (pattern 2). Fig. 2 shows the two RAPD patterns using primers OPB-15 and OPB-18.

Table 3. In vitro susceptibility data and antibiotypes of the 11 isolates of Exophiala jeanselmei, determined by the E-test

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>E-test MIC (µg/mL)* in each antibiotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>8</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>1.0</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>2</td>
</tr>
<tr>
<td>5-fluorocytosine</td>
<td>&gt; 32</td>
</tr>
</tbody>
</table>

MIC = minimum inhibitory concentration.

Discussion
The obscurity of the source of the nosocomial E. jeanselmei pseudoinfection in this series despite surveillance indicates the difficulty of identifying and controlling the sources of this slowly growing fungus. The lack of specimen collection from a wider group of staff might have contributed to the failure to recognize the sources and route of transmission of the organism. RAPD analysis coupled with antibiotyping and biotyping were highly discriminatory for the epidemiologic study of this pseudoepidemic caused by E. jeanselmei.

The assimilation assay using the API Yeast Identification System has potential usefulness for the differentiation of some of the dematiaceous fungi, including the black fungi, and for typing Exophiala dermatitidis strains [15, 17]. However, limitations of identification are related to the different technical procedures [15]. The MIS system, based on cellular FAME analysis by gas chromatography, was established to identify bacterial and fungal species and has been successfully used for clustering bacterial or fungal strains, including E. dermatitidis isolates [15, 16]. RAPD analysis, though the technique suffers from some limitations, is useful for typing several fungal isolates, and it can be used with cluster analysis of FAME profiles to accurately type E. dermatitidis strains [5, 15, 18, 19].

In the present study, two distinct RAPD patterns were identified by more than five primers (initially screened by 40 primers, OPB-1 to OPB-20 and OPH-1 to OPH-20). Two biotypes, differing by five different tests after duplicate testing using API ID 32C, were clearly recognized among the 11 isolates. Two E-test antibiotypes were also demonstrated, though this method is not cleared for clinical use with molds (laboratory use only) in the USA.
In conjunction with RAPD analysis, assimilation assay and the E-test antibiotyping method were used in this study. Two clones among the 11 *Exophiala* isolates were clearly identified. In contrast to the findings of Rath et al [15], cluster analysis of FAMEs of the 10 isolates failed to separate the two clones.

The in vitro susceptibilities of *Exophiala* species to several antifungal agents are variable [5, 15, 20]. In the present study, remarkable differences in MICs of amphotericin B, ketoconazole, and itraconazole were found in the two clone isolates. The patient with cutaneous phaeohyphomycosis underwent surgical excision of the lesion and responded well to itraconazole (MIC, 0.125 µg/mL). Several agents have been used successfully in combination with surgical intervention for treating phaeohyphomycosis [19, 21–23]. However, the efficacy of specific agents in treating this disease is not well documented.

Recovery of a rarely encountered organism from more than one patient over a short period of time might suggest the occurrence of an outbreak. The first two isolates in this series were found in 1995 in two patients with thoracic empyema. Failure to promptly initiate effective epidemiologic surveillance due to the slow-growing nature of *E. jeanselmei* might have allowed the organism to persist in the chest sonography room for more than 4 years. Extensive environmental surveillance cultures failed to identify *E. jeanselmei*. Although an inadequacy of specimen collection from hospital staff members in this study suggests that transmission by staff may have been overlooked, the origin of this organism associated with this pseudoepidemic was presumed to be procedure related due to a contaminated environment. This organism was not found in patients who visited the chest sonography room between November 1998 and May 2001.

In conclusion, *E. jeanselmei* should be included as a microorganism causing nosocomial pseudoinfection. The unidentified origin of the pseudoinfection in this series suggests that timely identification of rarely isolated organisms and prompt and adequate initiation of epidemiologic surveillance is crucial for tracing the origin of the organism and prevention of further nosocomial transmission.

**References**


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