Nosocomial Outbreak Caused by *Scedosporium prolificans* (inflatum): Four Fatal Cases in Leukemic Patients

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Four cases of fatal disseminated *Scedosporium prolificans* (inflatum) infection occurring in neutropenic patients are reported. Because of hospital renovation, the patients were cared for in a temporary hematology facility. *S. prolificans* (inflatum) was isolated from blood cultures of these four patients, two of whom underwent full necropsy, and revealed abundant vegetative hyphae and ovoid conidia with truncate bases in many organs. In vitro susceptibility testing of fungal strains showed all isolates to be resistant to amphotericin B, fluconazole, miconazole, ketoconazole, flufenazole, and itraconazole, with MICs greater than 16 μg/ml. The reported infections, two in each of two rooms, occurred over a period of 1 month, with very similar clinical outcomes. Circumstantial evidence suggested a nosocomial outbreak, but the environmental samples collected from the rooms, corridors, and adjacent areas did not yield *S. prolificans* (inflatum). Nevertheless, circumstantial evidence suggested a nosocomial outbreak of *S. prolificans* (inflatum).

Since the description of *Scedosporium inflatum* (Malloch and Salkin) as a human pathogen (14), investigators have suggested that the appropriate species epithet is *Scedosporium prolificans* (8, 11) reports of several cases have enlarged the clinical spectrum of this fungus (22, 29, 32, 33). Furthermore, it seems that *S. prolificans* causes a spectrum of clinical illness similar to that produced by *Scedosporium apiospermum* (30) but broader than was previously thought, especially among immunocompromised patients (5, 7, 10, 13, 26, 28).

The natural habitat of *S. prolificans* remains unknown, but it has been isolated from flower pots and is most likely a soil saprobe (6, 27).

Outbreaks of nosocomial infections caused by contaminating environmental fungi when the number of spores are increased by activities such as nearby building construction or the presence of contaminated air-conditioning systems (11, 12, 31). A nosocomial outbreak involving four cases of fatal disseminated infection by *S. prolificans* in leukemic patients is reported. The four cases occurred sequentially during reconstruction work at the hospital, over the short period of 28 days in two rooms in the provisional hematology unit.

The provisional hematology unit was located on the fifth floor of the hospital in an ordinary hospitalization unit, and two single rooms were designated for the isolation of immunocompromised patients. These two rooms were at the entry to the unit, opposite the construction work on the same floor. The barrier between the construction work and the unit was two sets of double doors, and the distance was 20 m. There was no trafficking of construction workers between the construction area and the provisional unit.

**MATERIALS AND METHODS**

**Case identification.** Between 26 November and 14 December 1992, four cases of disseminated *S. prolificans* infection were identified in the hematology department of Hospital Central de Asturias, a large, tertiary care community teaching hospital, built 30 years ago. Hospital renovation was being carried out, and the Hematology Department was provisionally housed in a nonprotected area (ordinary single rooms). The patients received treatment in private rooms of the inverse isolation class (mask and hand washing required). See Fig. 1 for a comparison of the clinical outcomes.

**Clinical samples.** Samples of urine, exudate, and feces, etc., were processed in the microbiology laboratory following standard methods in order to detect the presence of aerobic bacteria, anaerobic bacteria, and fungi (2).

Blood samples (10 ml) were inoculated into aerobic and anaerobic bottles (Bactec; BBL). They were incubated at 37°C, and daily readings with a Bactec NR-730 automatic detector (Beckton Dickinson, Microbiological Systems, Cockeysville, Md.) were made until the detection of growth. If after 8 days the cultures were still negative, the samples were subcultured on plates of chocolate agar. These plates were then incubated at 37°C in anaerobic jars (Oxoid) at 10% CO₂ for 48 h.

When fungal infection was suspected, the blood sample (10 ml) was also processed by a lysis-centrifugation technique (Isolator 10; Merck) by subculturing the sediment on plates of Sabouraud dextrose agar (SDA), sheep blood agar, and chocolate agar; the plates were incubated at 37°C for 8 days, and the samples were also subcultured in a biphasic medium (HEMOLINE; bioMérieux) and incubated at 30°C for 40 days.

**Environmental surveillance.** Saline-moistened sterile cotton swabs were used for collecting dust samples from the floors, ceilings, and walls of the rooms, the corridors, and the nurses' station in the affected unit and reconstruction areas. The swabs were inoculated onto SDA without and with amphotericin B at a final concentration of 10 μg/ml. The plates were incubated at 30°C for 10 days. No air samples were taken.

Dust samples were taken several times: the same day that the first blood culture fungus growth was identified, simultaneously with the fourth death, the next day, and during the following month.

**Mycology.** Each fungal colony detected was subcultured on two plates of SDA (Difco), Mycosel agar (BBL), malt extract agar (Oxoid), and potato dextrose agar (Merck), and the two plates of each set were incubated for 2 weeks at 30°C.

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37°C, respectively. The microscopic examination of the isolates was carried out by using a previously described technique (19).

Antifungal susceptibility testing. Susceptibility testing was performed according to the broth dilution micromethod described by Schmitt et al., with minor modifications (24).

The six antifungal drugs used in the susceptibility testing procedure were amphotericin B (Squibb), flucytosine (Hoffman–La Roche Laboratories), ketoconazole and itraconazole (Janssen Pharmaceutica), fluconazole (Pfizer), and miconazole (Sigma). Stock solutions of ketoconazole, fluconazole, and miconazole were prepared at 12.8 mg/ml in 100% dimethyl sulfoxide. Amphotericin B and itraconazole (1.6 mg/ml) were solubilized in 100% dimethyl sulfoxide, and flucytosine (1.6 mg/ml) was dissolved in sterile distilled water. All these solutions were stored at −70°C.

The medium used for susceptibility testing was RPMI 1640 with l-glutamine (Gibco) buffered with morpholinepropanesulfonic acid (MOPS) buffer (Sigma) to a final molarity of 0.165, adjusted to pH 7.0 by using 10 M NaOH (25) and supplemented with 18 g of glucose (RPMI–2% glucose) per liter (20). We employed RPMI–2% glucose as a testing medium because it provides good cell growth, the endpoints are also more easily read, and it is both easy to prepare and economical and does not contain antagonists for flucytosine activity.

Sterile plastic microtiter plates containing 96 round-bottom wells each, with their corresponding covers (Greiner), were employed. The concentrations of ketoconazole, fluconazole, and miconazole ranged from 128.0 to 0.25 μg/ml; itraconazole, amphotericin B, and flucytosine were used at concentrations of 16.0 to 0.03 μg/ml. The plates contained 10 twofold serial dilutions of each antifungal agent in each row and a drug-free medium in wells 11 and 12 for sterility check and growth control, respectively.

Homogeneous inoculum suspensions were prepared from SDA (Oxoid) cultures grown at 28°C for 7 days, according to the method of Espinel-Ingroff and KerKering (4). Briefly, suspensions were made by rubbing the surface of SDA slants with a loop after the addition of sterile water, washing the scappings, and then transferring the suspensions to a sterile tube. After the heavy particles of the suspensions were allowed to settle, the upper homogeneous suspensions were adjusted with a spectrophotometer set at 530 nm to 78 to 82% transmission to obtain final suspensions containing 1 × 10³ to 5 × 10⁹ CFU/ml. Colony counts on the SDA plates were determined. The suspensions were diluted 1:10 with RPMI–2% glucose, and 10 μl was dispensed in each of the wells (ca. 10⁴ CFU/ml).

After inoculation, the microtiter plates were incubated at 35°C for 48 h in a humid atmosphere. The plates were then read macroscopically with a mirror, and the MIC was defined as the lowest concentration of the antifungal agent that completely inhibited fungal growth.

Aspergillus fumigatus ATCC 9197 and Aspergillus versicolor ATCC 11730 were used as controls in all plates. For comparative purposes, one strain of S. apiospermum from our collection was also included.

Thus, because of the scarcity of reported cases of systemic infections by S. prolificans, we have deposited an isolate from each of the patients in the Unidad de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda Madrid, Spain (no. M93/1, M93/2, M93/3, and M93/4), and the first of these was also deposited in the Mycological Reference Laboratory of the Public Health Laboratory Services, National Collection of Pathogenic Fungi, London, England (no. 7108).

Autopsy. A complete macroscopic and microscopic study was performed for two cases, including lungs, liver, kidneys, spleen, thyroid glands, and lymph nodes. The histological sections were placed in 10% Formol and then stained with hematoxylin-eosine, periodic acid-Schiff stain, and methenamine-silver.

CASE REPORTS

Case 1. A 27-year-old woman was admitted with acute myeloid leukemia. On the 12th day after admission she became febrile, and a treatment of ceftazidime plus amikacin was started. On the 19th day, she became febrile again, and a chest radiograph revealed perihilar infiltrate. Amphotericin B (1 mg/kg of body weight per day) plus imipenem was started, but her condition worsened. She died 31 days after admission. Two blood cultures (Bactec) obtained on this day were positive for S. prolificans.

An autopsy was performed. Microscopic study revealed extensive and hemorrhagic necrotic zones with thrombosed vessels. Hyphae branched at acute angles and were accompanied by rounded, small elements observed around and within vessels as well as in the lung parenchyma. These same structures were also visualized in the liver, suprarenals gland, kidneys, and spleen. Samples for culturing were not taken.

Case 2. A 45-year-old woman was admitted with acute myeloid leukemia. On the seventh day after admission, she had a fever and a neutrophilic count of <100 × 10⁹/liter. Ceftazidime plus amikacin treatment was started. After 48 h of treatment, she continued to be febrile, and the antibiotic treatment was changed to vancomycin plus aztreonam. On the 12th day, the patient’s condition worsened, and she reported thoracic pain characteristic of pneumonia. Amphotericin B (1 mg/kg/day) plus imipenem was started. An X-ray of the thorax showed diffuse pulmonary infiltrates bilaterally. The patient died 22 days after admission. Blood cultures obtained 48 h (three Isolator cultures) and 24 h (three Bactec cultures) before death were positive for S. prolificans. A postmortem examination was not authorized.

Case 3. A 79-year-old woman was admitted with acute lymphoblastic leukemia. After 1 week she became febrile. She responded well to treatment with ceftazidime plus amikacin, but on the 12th day she became febrile again. The neutrophilic
count was $<100 \times 10^9$/liter. A chest radiograph revealed bilateral pulmonary infiltrate.

On the 25th day, her condition worsened. A chest X-ray revealed right pleural effusion, and treatment with amphotericin B (1 mg/kg/day) plus imipenem was begun. The patient died 27 days after admission. Two blood cultures (Bactec) taken 48 h before death were positive for \textit{S. prolificans}. A postmortem examination was not authorized.

**Case 4.** A 54-year-old woman was admitted because of an acute myeloid leukemia. Chemotherapy with adriamycin plus 1-$\beta$-D-arabinofuranosyl cytosine was started. On the 8th day after admission, she had a fever of 38°C, treatment with cefazidime plus amikacin was begun, and teicoplanin was added after 48 h. During the third week after admission, she became febrile again, the neutrophilic count was $<100 \times 10^9$/liter, and treatment with amphotericin B (1 mg/kg/day) plus imipenem was started.

Three days later, the patient’s condition worsened, with bilateral ocular and muscular pain. An X-ray film of the chest showed a bilateral pulmonary infiltrate. The results of a computed tomography scan of the head were normal. The patient developed acute respiratory and renal failure and died 23 days after admission. A blood culture (Bactec), tracheal aspirate, and another blood culture (Isolator) obtained 96, 72, and 24 h, respectively, before death were positive for \textit{S. prolificans}.

An autopsy was performed. The histopathological findings revealed that the lungs had increased in weight. The parenchyma was solid, showing pinkish-to-red rounded areas. Microscopically, bronchopneumonic zones with little cellular reaction, alveolar masses of fibrinoid material, and extensive areas of thrombotic vessels and necrosis were observed (Fig. 2). In all these zones, spreading myriad mycotic structures formed by long hyphae, which branched at acute angles, and rounded structures with small sizes were seen (Fig. 3). This fungal invasion was also observed in the liver, spleen, kidneys (Fig. 4), thyroid glands, and lymph nodes. Samples for culturing were not taken.

**RESULTS**

\textit{S. prolificans} grew on chocolate agar after routine subculturing of apparently fungus-negative samples in Bactec bottles. The time required to detect \textit{S. prolificans} in blood culture (Bactec) always exceeded 7 days: 15, 10, 10, and 9 days, respectively, for the four cases, but with the Isolator, the time needed was 48 and 72 h in cases 2 and 4, respectively.

On subculture, the fungi grew rapidly on SDA, malt extract agar, and potato dextrose agar. The colony was flat, moist, spreading, olive-grey to black in color, and arachnoid to woolly in appearance. With age, the four isolates developed sections that were woolly in appearance (Fig. 5).

Microscopic examination revealed ovoid, smooth, one-celled, thin-walled conidia. The conidiogenous cells were annellides, which occurred isolated along the hyphae and in clusters of 2 to 5 at the apex of unbranched conidiophores. The conidiogenous cell showed the distinctive swollen base of \textit{S. prolificans}. None of the four isolates grew in cycloheximide-containing media. The overall appearance of the colony and the...
characteristic microscopic morphology were consistent with those of *S. prolificans*, as shown in previous reports (3, 7, 9, 10, 14, 15, 21, 22, 33).

The activities of the six antifungal drugs tested against the four isolates of *S. prolificans* and control strains are shown in Table 1.

*S. prolificans* failed to grow in any of the environmental samples processed for 2 months, although there was massive growth of other environmental fungi, such as *Aspergillus fumigatus* *Penicillium* sp., *Rhizomucor* sp., and, to a lesser extent, *Alternaria* sp. and *Cladosporium* sp. All dematiaceous fungus was subcultivated for identification.

**DISCUSSION**

Fungal infections are becoming an increasingly significant problem and are a major cause of mortality in immunocompromised patients. Although the major risk factor is granulocytopenia, other factors such as multiple antibiotic therapy, humoral and cellular immunodeficiencies, surgery, and indwelling catheters are related with these infections.

Several cases of disseminated infection caused by *S. prolificans* have been reported. The affected populations were predominantly immunosuppressed patients (5, 7, 18, 26, 28, 33) although there are two reported cases of endocarditis (5, 8) in nonimmunosuppressed patients. The clinical outcome is generally fatal. In Spain, eight cases of disseminated infection caused by *S. prolificans* in leukemic patients have been reported (7, 10, 16, 21, 28), all of which occurred in an isolated way. Death was the usual outcome, despite antifungal treatment.

To our knowledge, this is the first report of disseminated *S. prolificans* infections occurring as a nosocomial outbreak associated with renovation inside the hospital. At the time, the patients were cared for in a provisional unit without the standard protective measures for granulocytopenic patients; they were located in single rooms with restricted entry and obligatory use of a mask, wedge, and dressing gown. There were no air-conditioning or potted plants in this zone, and the unit was, in fact, closed off from the renovation zone. The four cases occurred in two adjacent rooms which were occupied successively by these four patients for 28 days.

When the fourth death occurred, simultaneously with the fungal identification of the positive blood cultures from the first two deceased patients, the two rooms were temporarily sealed and dust samples were processed several times. The hematology unit was moved to a different area (from the fifth floor to the second floor), until the permanent hematologic unit was completed, and no additional cases have been detected since.

The clinical symptoms and the radiological findings of X-ray of the thoraxes of the four patients suggested aerial transmission. Many investigators emphasize the importance of isolation during granulocytopenia in order to avoid disseminated mycoses (1, 16). However, the environmental samples taken were negative despite the fact that a selective medium for *S. prolificans* was used (SDA plus amphotericin B). Therefore, the source of this nosocomial outbreak and the transmission route of the fungus remain unknown.

The isolation of the etiological agent was closely premortem, as the blood cultures taken on the day of or 48 and 72 h before death were positive. Furthermore, only the lysis centrifugation...
method provided fungal growth within 48 to 72 h, as in cases 2 and 4, respectively. Fungi grew in the subcultures made from Bactec blood culture bottles after 7 days of incubation. After 7 days, Bactec blood culture bottles were subcultured in a blind fashion, and at this time the fungus was detected. Unfortunately, the identification of the fungus was made only after the patients’ deaths. In our laboratory, only two previous cultures of a Scedosporium sp. from clinical samples in the last 15 years were available, and they were S. apiospermum.

In cases when a biopsy can be performed, the observation of vegetative hyphae together with ovoid conidia in histological sections could suggest S. prolificans infection, (15, 21, 32), and our experienced pathologist had never before seen these elements. Fungal culture is always necessary for correct identification.

Even though an early diagnosis was reached, the therapeutic outcomes were disappointing. In previous investigations, Salkin et al. (22) found four isolates for which the MICs of

![Image](http://jcm.asm.org/)

**TABLE 1. Antifungal MICs of S. prolificans**

<table>
<thead>
<tr>
<th>Fungus</th>
<th>No. of samples tested</th>
<th>AB</th>
<th>F</th>
<th>KZ</th>
<th>FZ</th>
<th>IZ</th>
<th>MZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. prolificans</td>
<td>4</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>128</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>128</td>
</tr>
<tr>
<td>S. apiospermum</td>
<td>1</td>
<td>4</td>
<td>&gt;16</td>
<td>2</td>
<td>&gt;128</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>1</td>
<td>0.5</td>
<td>&gt;16</td>
<td>8</td>
<td>&gt;128</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>&gt;128</td>
<td>0.5</td>
<td>2</td>
</tr>
</tbody>
</table>

* AB, amphotericin B; F, fluocytosine; KZ, ketoconazole; FZ, fluconazole; IZ, itraconazole; MZ, miconazole.
itraconazole were lower than 0.018 μg/ml, and Wood et al. (33) found that one isolate of S. prolificans was susceptible to itraconazole at 25 μg/ml, with clinical response at high doses, but this was not seen in our case. Our data also confirm previously published results (21, 22) regarding the resistance of S. prolificans to amphotericin B, ketoconazole, and miconazole, compared with the lower MICs for S. apiospermum. Antifungal susceptibility tests for filamentous fungi remain unstandardized, and the in vitro results cannot be extrapolated to the in vivo outcome. However, many investigators agree that S. prolificans shows a high degree of resistance to antifungal drugs irrespective of the method of detection used.

Wood et al. (33) have reported the only case of an immunosuppressed patient without fatal outcome who responded to antifungal drugs plus granulocyte colony-stimulating factor. Whether that treatment can be successful remains unanswered, and further studies are needed (17, 23). At the present time, antifungal treatment cannot be expected to play a major role in recovery from S. prolificans infections.

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REFERENCES