Nosocomial Outbreak of *Exophiala jeanselmei* Fungemia Associated with Contamination of Hospital Water

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From December 1996 through September 1997, we diagnosed 19 cases of fungemia due to *Exophiala jeanselmei*. We conducted a matched case-control study in which we cultured specimens of blood products, intravenous solutions, and water from a hospital water system. Isolates from environmental cultures were compared to those recovered from patients by random amplification of polymorphic DNA (RAPD). Multivariate analysis showed that neutropenia, longer duration of hospitalization, and use of corticosteroids were risk factors for infection. Environmental cultures yielded *E. jeanselmei* from 3 of 85 sources: deionized water from the hospital pharmacy, 1 water tank, and water from a sink in a non–patient care area. Use of deionized pharmacy water to prepare antiseptic solutions was discontinued, and no additional cases of infection occurred. RAPD typing showed that isolates from case patients and isolates from the pharmacy water were highly related, whereas the patterns of isolates recovered from the 2 other sources of water were distinct.

In the past decade, fungi have emerged as important nosocomial pathogens [1]; *Candida* species account for >75% of cases of nosocomial fungal infection [2]. Among the other fungi that may cause nosocomial infections, the most common are *Aspergillus* species, which mostly infect immunosuppressed individuals [3]. In addition to these 2 etiologic agents, there is an extensive list of fungi that have been sporadically reported as agents of nosocomial infection, including *Fusarium* species, *Hansenula anomala*, *Malassezia* species, and others [4–6].

Potential sources for the acquisition of nosocomial fungal pathogens include the hands of health care workers [7], airborne conidia [8], and contaminated intravenous fluids and catheters [9, 10]. More recently, it has been suggested that hospital water may be a source of nosocomial fungal infection [11]. *Exophiala jeanselmei* is a dematiaceous fungus widely distributed in the environment, especially in the soil, wood, polluted water, and sewage [12, 13]. Until recently, this fungus had never been reported as a cause of nosocomial infection. In a 10-month period, we diagnosed 19 cases of fungemia due to *E. jeanselmei* [14]. However, the risk factors and epidemiology of these infections were not determined. In this study, we report risk factors for this infection and describe the investigation we performed to elucidate the source of this outbreak.

**PATIENTS AND METHODS**

**Setting.** The university hospital Universidade Federal do Rio de Janeiro is a 450-bed tertiary hospital with

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**References**

Coexisting risk factors

Underlying condition

- 1476 patients with fungemia due to Exophiala jeanselmei were studied. A detailed description of the case patients was published elsewhere [14]. Briefly, the majority of patients had a hematological malignancy or some degree of immunosuppression (due to receipt of steroids or chemotherapy, bone marrow transplantation, or agranulocytosis). All but 2 patients had received blood transfusions, 79% had received systemic antibiotics, and 74% had a central venous catheter. All intravenous catheters (peripheral or central) had been placed after admission to our institution. The first case of E. jeanselmei infection was diagnosed in December 1996, and the last case was diagnosed in October 1997. Figure 1 shows a time line of the occurrence of the cases, the culture surveys performed, and the measures taken to control the outbreak.

**Description of case patients.** Table 1 summarizes the characteristics of case patients. A detailed description of the case patients is provided elsewhere [14]. Briefly, the majority of patients had a hematological malignancy or some degree of immunosuppression (due to receipt of steroids or chemotherapy, bone marrow transplantation, or agranulocytosis). All but 2 patients had received blood transfusions, 79% had received systemic antibiotics, and 74% had a central venous catheter. All intravenous catheters (peripheral or central) had been placed after admission to our institution. The first case of E. jeanselmei infection was diagnosed in December 1996, and the last case was diagnosed in October 1997. Figure 1 shows a timeline of the occurrence of the cases, the culture surveys performed, and the measures taken to control the outbreak.

**Epidemiologic studies.** In order to identify risk factors for E. jeanselmei fungemia, we conducted a matched case-control study. Control subjects (2 for each case patient) were admitted to the hospital in the same month as case patients and had positive blood culture result (of a sample obtained from a peripheral vein or a central venous catheter) that was positive for E. jeanselmei during the study period, which was from November 1997 (the date of the first positive blood culture result) through September 1998 (the date of the positive blood culture result for the last case patient). To identify case patients, we reviewed the records from the mycology laboratory in the hospital.

**Culture surveys and microbiologic methods.** In September 1998, we started to perform cultures of samples of potential sources of fungemia. We cultured 52 randomly selected blood product samples (RBCs, plasma, and platelets), as well as 3 samples of each available lot of the following intravenous solutions: 5% dextrose, 0.9% saline, distilled water, 20% sodium chloride, 10% potassium chloride, Ringer lactate, and heparin. Samples of each material were plated onto Sabouraud dextrose agar and incubated at room temperature for 4 weeks. In addition, we cultured samples from 85 different sources of water. The selection of the sources was based on a map of the water distribution system of the hospital, and the selection was made to be representative of the whole hospital water system. We collected municipal water, water from the 3 main storage tanks, water from sinks and showers in patient bathrooms, and water from sinks used by staff for hand-washing. In addition, we collected water from the distillers and deionizers in the pharmacy, which were used to prepare antiseptic solutions (i.e., 70% ethyl alcohol and alcoholic chlorhexidine). Because the water was stored in 5-L bottles after distillation or deionization, we also sampled water from the bottles.

We collected water from each source on 2 different days. On each of these days, we collected 200 mL of water in 2 sterile 100-mL bottles. A sample of 10 mL from each bottle was then plated onto a Petri dish containing Sabouraud dextrose agar,
Figure 1. Time line showing the occurrence of 19 cases of *Exophiala jeanselmei* fungemia, the culture surveys performed, and the measures taken to control the outbreak.

RESULTS

Risk factors. Although the majority of case patients (58%) were admitted to the bone marrow transplant unit or the hematology unit, the case patients were distributed through all inpatient wards, including both surgical and medical wards, on all floors. The cases occurred throughout the 11-month study period, with more cases occurring in June 1997 (4 cases) and September 1998 (6 cases) than in other months (figure 1). Case patients were more likely to be neutropenic (\(P < .0001\)), to have a diagnosis of cancer (\(P = .003\)), to have received a bone marrow transplant (\(P = .006\)), to have a central venous catheter (\(P = .005\)), to have received blood transfusions (\(P = .001\) and/

Table 2. Risk factors for *Exophiala jeanselmei* fungemia, by multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>398</td>
<td>ND</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Longer duration of hospitalization(*)</td>
<td>1.13</td>
<td>1.06–7.61</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Use of corticosteroids</td>
<td>20.1</td>
<td>1.39–291</td>
<td>.02</td>
</tr>
</tbody>
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NOTE. ND, not determined.

\(*\) Incremental for each excess day of hospitalization.
or corticosteroids (P = .0006), and to have a longer duration of hospitalization (P = .008). In the multivariate model (table 2), risk factors for fungemia due to E. jeanselmei were neutropenia (OR, 398; 95% CI not determined), longer duration of hospitalization (OR, 1.13 for each excess day of hospitalization; 95% CI, 1.06–7.61), and use of corticosteroids (OR, 20.1; 95% CI, 1.39–291).

**Culture surveys.** All cultures of blood products and intravenous solutions were negative for E. jeanselmei. Thirty-eight of the 85 cultures of environmental samples grew mold (table 3). E. jeanselmei grew purely and repeatedly from cultures of samples from only 3 sources: water obtained from 1 of the 3 storage tanks, water obtained from a sink in a bathroom located in a non–patient care area, and deionized water obtained from the hospital pharmacy (both from the deionizer and from the stored deionized water). The tank for which cultures were positive provided water to showers and sinks in the bone marrow transplant unit and the intensive care unit, and 10 of the 19 patients with E. jeanselmei fungemia were admitted to these 2 wards. The water was not used for preparing food or for drinking. The deionized water from the hospital pharmacy was used to prepare antiseptic solutions (i.e., 70% alcohol and alcoholic chlorhexidine) that were used for skin disinfection before venous puncture and for catheter care (i.e., catheter site care, catheter site dressings, and connections of intravenous administration sets) in all the hospital wards.

**DNA typing.** RAPD analysis of multiple clinical specimens (figure 2) showed that E. jeanselmei isolates recovered from all case patients were highly related. Results were consistent with use of both primers (only results using primer M13 are shown in figure 2). In addition, these isolates had a banding pattern similar to that of the isolates from the deionized pharmacy water, but different from that of the isolates recovered from storage-tank water (figure 3).

**Outbreak control measures.** As soon as we had the results of the environmental survey, the use of deionized water in the pharmacy was discontinued, and no additional cases of infection occurred. We also cleaned the hospital tank, and subsequent cultures were negative for E. jeanselmei.

**DISCUSSION**

E. jeanselmei is a black mold that causes superficial, cutaneous, subcutaneous, and, less frequently, systemic infection [17, 18]. Until our recent report [14], it had never been associated with fungemia. In this outbreak, cases occurred in all inpatient wards of the hospital during a 10-month period. This observation suggested a common source that was widespread in the hospital, but the risk factors for fungemia and the epidemiology of infection were not defined.

Our first strategy to investigate the outbreak was to conduct a matched case-control study, using the period of hospitalization as the only matching criterion, in order to determine possible risk factors for infection. By multivariate analysis, 2 host factors (neutropenia and use of corticosteroids) and 1 factor associated with the duration of exposure (duration of hospitalization) emerged as risk factors. These data suggested that patients with severe compromise of the host defenses who were exposed for prolonged periods to an environmental source were at higher risk of developing the infection. The occurrence of fungemia due to E. jeanselmei in a population of severely immunosuppressed patients supports the hypothesis that this fungus has a low virulence potential [19].

The pathogenesis of infection due to E. jeanselmei and other dematiaceous fungi is poorly understood. However, because sinus, pulmonary, and cutaneous infections are frequent forms of disease, inhalation of conidia and traumatic inoculation of the organism are possible routes of infection. In addition, some organisms, such as E. jeanselmei, can produce yeastlike synanamorphs that adapt to aqueous environments [19]. Indeed,

![Figure 2](https://academic.oup.com/cid/article-abstract/34/11/1478/367856) DNA fingerprints of clinical isolates from patients with fungemia due to Exophiala jeanselmei, determined by random amplification of polymorphic DNA with use of the M13 primer.
E. jeanselmei has been recovered from sludge from bathroom drainpipes [13]. Therefore, there is a reasonable possibility that fungemia due to this organism has an aqueous source.

Until recently, water has not been described as a source of nosocomial fungal pathogens. In 1997, Anaissie et al. [11] collected water samples from a hospital in which there were ongoing nosocomial cases of mold infection, and they isolated opportunistic fungi, including dematiaceous fungi, from 52% of the samples. They suggested that water could be a potential source of aerosolization of opportunistic fungi in the hospital setting. Based on these findings, we examined water in our hospital to assess its potential role in this nosocomial outbreak. Notably, samples from 3 of 85 water sources cultured during the time of the outbreak were positive for E. jeanselmei. The only source common to all of these positive water cultures was the deionized water from the hospital pharmacy, which was used to prepare antiseptic solutions. Because these solutions were applied to the skin of patients before venous punctures and used in the care of intravenous catheters, we hypothesized that the deionized pharmacy water may have been the source of infection.

The identification of highly related DNA patterns for E. jeanselmei strains recovered all patients strongly suggested a common source for the outbreak. This characteristic DNA pattern was also observed in strains cultured from the deionized water, but not in strains recovered from the 2 other sources identified. Therefore, the most likely explanation for this outbreak is that patients acquired the infection through inoculation of the organism into the bloodstream at the moment of catheter insertion or through the peripheral or central catheter during use, and that the likely source of contamination was the antiseptic solution made with deionized pharmacy water. This hypothesis is supported by fact that the outbreak was interrupted after discontinuation of the use of deionized water to prepare antiseptic solutions. We attempted to cultivate E. jeanselmei in 70% alcohol, but no growth was observed (data not shown). However, this observation does not rule out our hypothesis, because other factors, such as storage of antiseptic solutions that were made in the pharmacy, may have played a role in the growth of E. jeanselmei. Unfortunately, cultures of samples of the antiseptic solutions used for the patients who developed fungemia were not performed. Interestingly, one of the properties of Exophiala species is that they use ethanol as a source of carbon, which suggests that these fungi may be able to survive in alcoholic environments [20]. In summary, this study documents the occurrence of nosocomial E. jeanselmei fungemia in high-risk patients and confirms the potential role of contaminated water in nosocomial fungal infection.

Acknowledgment

We would like to thank Dr. Elias Anaissie for his thoughts about searching the hospital water system as a potential source of this outbreak.

References

11. Anaissie EJ, Monson TP, Penzak SR, Stratton SL. Opportunistic fungi

Figure 3. DNA fingerprints of clinical and environmental isolates of Exophiala jeanselmei, determined by random amplification of polymorphic DNA with use of the M13 primer. Lanes 2–5, Clinical isolates from patients with fungemia. Lanes 6 and 7, Isolates from the deionized water. Lanes 8 and 9, Isolates from the water tank. Lanes 10 and 11, Control isolates of E. jeanselmei from the laboratory at the University of Texas Health Science Center at San Antonio. Lane 12, Aspergillus fumigatus isolate.


