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Nosocomial Fungemia Due to *Exophiala jeanselmei* var. *jeanselmei* and a *Rhinocladiella* Species: Newly Described Causes of Bloodstream Infection

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Fungi have become increasingly important causes of nosocomial bloodstream infections. The major cause of nosocomial fungemia has been *Candida* spp, but increasingly molds and other yeasts have caused disease. *Exophiala jeanselmei* and members of the genus *Rhinocladiella* are dematiaceous moulds, which have been infrequently associated with systemic infection and have not been described as causes of fungemia. In this paper, the occurrence of 23 cases of fungemia due to these organisms over a 10-month period is reported and the clinical characteristics of patients and outcomes are described. The majority of patients were immunosuppressed; 21 of 23 (91%) had received blood products and 78% had a central venous catheter. All patients had at least one manifestation of fever, but only one patient had signs or symptoms suggesting deep-seated infection. Antifungal therapy was given to 19 of the 23 patients; of those who did not receive therapy, 3 died prior to the culture result and 1 had been discharged without therapy. Antifungal susceptibility of the organisms showed activity of amphotericin B, itraconazole, and the new triazole antifungals voriconazole and posaconazole. *E. jeanselmei* and *Rhinocladiella* species are potential causes of nosocomial fungemia and may be associated with systemic infection.

Systemic fungal infections are increasingly frequent in hospitalized patients (4). Whereas *Candida* species account for the majority of fungal infections, the spectrum of fungi that may cause infection is growing (2). *Exophiala jeanselmei* and *Rhinocladiella* species are dematiaceous fungi widely distributed in the environment, especially in soil, wood, polluted water, and sewage (7, 17). The clinical spectrum of infection caused by these organisms include mycetomas, chromoblastomycosis, and pheohyphomycosis, either superficial, cutaneous, subcutaneous, or systemic (10, 25). Deep-seated or systemic infections due to *E. jeanselmei* or *Rhinocladiella* are rare, with case reports of infection in the lungs (14, 26), brain (9, 30), peritoneum (1, 12, 22), and esophagus (6, 27). In addition, there is a single case of possible hematogenous dissemination of *E. jeanselmei* in a patient who developed endocarditis and arthritis (24). However, there have been no reports of fungemia due to these fungi. In this paper we report 23 cases of fungemia due to *E. jeanselmei* alone, *E jeanselmei* in combination with a *Rhinocladiella* species, or a *Rhinocladiella* species alone.

MATERIALS AND METHODS

The University Hospital of the Universidade Federal do Rio de Janeiro is a tertiary-care hospital with 540 beds, including a 6-bed bone marrow transplant unit, a 20-bed intensive care unit, and a 6-bed semi-intensive postoperative unit. Laboratory records were reviewed to identify patients with positive blood cultures from December 1996 through October 1997. In December 1996, *E. jeanselmei* was isolated from blood cultures of two patients. During 1997, 21 other patients had positive blood cultures for either *E. jeanselmei* or a *Rhinocladiella* species.

We reviewed the medical records of these 23 patients to determine the clinical characteristics and the outcome of this infection. Fungemia due to *E. jeanselmei* or a *Rhinocladiella* species was defined as the isolation of these fungi from at least one blood culture taken from a peripheral vein or a central venous catheter.

Blood specimens were inoculated in bottles containing brain-heart infusion medium. The bottles were incubated at 37°C and examined daily for the first week and once a week until discharge. Blind subcultures were performed on the second day of incubation. *E. jeanselmei* was first identified as the growth of black colonies of yeasts from the subculture plate. The colonies were then isolated, plated onto Sabouraud dextrose agar, and incubated at room temperature. Species identification of *E. jeanselmei* was based on macroscopic, microscopic, and physiologic characteristics. All 23 isolates were initially identified as *E. jeanselmei* and sent to a reference laboratory for confirmation. Identification of all isolates was confirmed at the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio, Tex. Isolates for identification were subcultured onto potato flakes agar (PFA) slants, a PFA plate, and a PFA slide cultures (prepared in-house) (23). Colonies on PFA at 25°C were black and initially moist to mucoid with a yeast-like appearance. Microscopically, these young colonies consisted predominantly of the annellated black yeast synanamorph characteristic of several *Exophiala* species. After 2 weeks of incubation, the colonies were greater than 10 mm in diameter and were olivaceous black and velvety. The microscopic morphology examined by slide culture revealed medium-length annellophores, as well as annellides that were both terminary and intercalary (borne on short conidiogenous loci between septa). Annelloconidia accumulated in balls near the apex of the annellides and measured 2 to 3 by 4 to 8 μm. Temperature studies revealed no growth at 40°C, and nitrate was assimilated (20). On the basis of the above characteristics, most isolates were confirmed to be *E. jeanselmei* var. *jeanselmei* (29). *E. jeanselmei* var. *lecanii-corni* is differentiated from *E. jeanselmei* var. *jeanselmei* by having conidia being formed predominantly from intercalary conidiogenous loci and by forming a distinct cluster in an ITS1 phylogenetic tree (30). The other isolates identified as *Rhinocladiella* species were similar to those of *E. jeanselmei* var. *jeanselmei*, both macroscopically and physiologically, but they differed microscopically. The Colonies were initially black, mucoid, and yeast-like, displayed a black yeast synana-

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TABLE 1. Underlying conditions in 23 patients with fungemia due to *E. jeanselmei* or a *Rhinocladiella* sp.

Underlying condition	No. of patients
Hematological malignancies	
Lymphoma	6
Acute myeloid leukemia	3
Multiple myeloma	2
AIDS	3
Postoperative state	3
Systemic lupus erythematosus	1
Breast cancer	1
Agranulocytosis	1
Osteomyelitis	1
Thrombotic thrombocytopenic purpura	1
Unstable angina	1

morph, assimilated nitrate, and failed to grow at 40°C. Significant differences, however, were noted in the microscopic morphology of the filamentous forms for these two species. While both species contain intercalary conidiogenous loci (conidia formed from very short openings on the hyphae), the genera differ by the formation of balls of conidia at the apices of annellides in *Exophiala* and conidia borne on closely packed denticles in *Rhinocladiella*. All isolates identified as *Rhinocladiella* produced their conidia on crowded denticles, a feature not seen in *Exophiala* species. *Exophiala (Wangiella) dermatitidis*, another species displaying a black yeast synanamorph, is differentiated from the above by failing to assimilate nitrate and by having the ability to grow at 40°C.

Additionally, broth macrodilution MICs and MLCs of amphotericin B, itraconazole, voriconazole, and posaconazole (SCH59562) were obtained for nine of the clinical isolates of *E. jeanselmei* var. *jeanselmei* following National Committee for Clinical Laboratory Standards (NCCLS) procedures (16). Testing was performed by the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio.

RESULTS

Epidemiology. Between December 1996 and October 1997, 23 cases of fungemia due to *E. jeanselmei* or a *Rhinocladiella* species were diagnosed. The median age of the patients was 50 years, with a range between 8 and 76 years. There were 11 males and 12 females. Table 1 shows the underlying conditions of the patients. Cancer was the underlying disease in 12 patients (52%) and included 11 hematological malignancies and 1 case of breast cancer. An immunodeficiency was present in the other six patients: AIDS in three, agranulocytosis in one, and systemic lupus erythematosus and thrombotic thrombocytopenic purpura (both in patients receiving corticosteroids) in one each. Three patients were in the postoperative period (cardiac revascularization, Fournier syndrome, and gastric surgery), one had osteomyelitis, and one had unstable angina.

As shown in Table 2, 21 of the 23 patients (91%) had received a blood transfusion, either red blood cells (11 patients), red blood cells plus platelets (7 patients), red blood cells plus plasma (2 patients), or red blood cells plus platelets plus plasma (1 patient). In 78% of the patients a central venous catheter was in place, and 61% had received broad-spectrum antibiotics. Neutropenia was present in 13 (56%) of the patients, and 8 patients had undergone an autologous bone marrow transplantation. The positive blood cultures had been taken from peripheral blood in 14 patients, peripheral blood plus the central catheter in 3 patients, and the central catheter in only 6 patients. In addition, in one patient with positive blood cultures taken from a peripheral vein, *E. jeanselmei* grew from the bag of peripheral blood stem cells collected for transplantation. The median number of positive blood cultures was 1 (range 1 to 6; mean, 1.7).

Clinical manifestations. All patients presented with at least one manifestation of infection at the time a positive culture was drawn. Fever was the most frequent clinical manifestation of the fungemia, occurring in all but one patient, who presented with hypotension. This manifestation also occurred in five other cases. Only one patient presented signs suggestive of a deep-seated infection. The patient had the first positive blood culture for *E. jeanselmei* during a period of neutropenia due to the administration of chemotherapy for the treatment of a relapsing large-cell non-Hodgkin's lymphoma. She had a Hickman catheter in place, and since the only positive blood cultures had been collected from the catheter and the patient had no complaints, the device was removed and no antifungal treatment was given. Two weeks later she was admitted for an autologous peripheral blood stem cell transplantation. There was no sign of infection, and the chemotherapy was started. After 3 days of neutropenia, she developed fever and empirical antibiotic therapy was started. The blood cultures taken from a new Hickman catheter, as well as from peripheral blood, grew *E. jeanselmei*. The patient had positive blood cultures for 22 days, despite catheter removal and the use of amphotericin B (1 mg/kg daily). She subsequently developed thoracic pain, dry cough, and dyspnea. A chest radiograph showed nodular lung opacities. The patient developed respiratory failure and died after having received 975 mg of amphotericin B. Autopsy was not performed.

Species identification. The isolates were identified as *E. jeanselmei* var. *jeanselmei* in 19 patients, *E. jeanselmei* var. *jeanselmei* plus a *Rhinocladiella* species in 1 patient, and *Rhinocladiella* species in 3 patients.

Therapy and outcome. Table 3 shows the treatment and outcome of the 23 patients. Four patients did not receive any treatment: three patients died before the blood culture become positive, and one patient was discharged before the blood culture become positive. This patient was admitted for the treatment of thrombotic thrombocytopenic purpura. She had no central venous catheter in place, and the blood culture had been taken because of one spike of fever. Since no new fever developed and the patient was well, she was discharged. Follow-up evaluation up to 6 months after discharge did not show any abnormality. Among the 18 patients with a central venous catheter in place, the device was removed in 15. This was the sole treatment in seven patients. Amphotericin B was given to

TABLE 2. Coexisting exposures of 23 patients with fungemia due to *E. jeanselmei* or a *Rhinocladiella* sp.

Coexisting exposure	No. (%) of patients[a]
Blood transfusion	21 (91)
Central venous catheter	18 (78)
Antibiotic use	14 (61)
Neutropenia	13 (56)
Chemotherapy	12 (52)
Bone marrow transplantation	8 (35)
Foley catheter	5 (22)
Total parenteral nutrition	4 (17)

[a] A total of 23 patients were involved.

TABLE 3. Treatment and outcome of 23 patients with fungemia due to *E. jeanselmei* or a *Rhinocladiella* sp.

Patient	Underlying condition	CVC[a]	Treatment	Outcome
1	Hodgkin's disease	Yes	Itraconazole + CVC removal	Alive
2	Multiple myeloma[b]	Yes	CVC removal	Alive
3	Systemic lupus erythematosus	Yes	CVC removal	Alive
4	AIDS	Yes	CVC removal	Dead
5	Unstable angina	Yes	CVC removal	Alive
6	Postoperative state	Yes	Amphotericin B + CVC removal	Dead
7	Acute myeloid leukemia	Yes	Amphotericin B + CVC removal followed by itraconazole	Alive
8	Non-Hodgkin's lymphoma	Yes	Amphotericin B + CVC removal	Alive
9	AIDS[c]	Yes	Amphotericin B + CVC removal followed by itraconazole	Dead
10	Osteomyelitis	Yes	Amphotericin B + CVC removal followed by itraconazole	Dead
11	Breast cancer	Yes	Amphotericin B + CVC removal followed by itraconazole	Alive
12	Multiple myeloma	Yes	CVC removal	Alive
13	Postoperative state	Yes	No	Dead
14	Postoperative state	Yes	Amphotericin B + CVC removal	Alive
15	Acute myeloid leukemia[b]	Yes	No	Dead
16	Non-Hodgkin's lymphoma	No	Itraconazole	Alive
17	Agranulocytosis	No	Amphotericin B	Alive
18	Non-Hodgkin's lymphoma	Yes	Amphotericin B	Dead
19	Thrombotic thrombocytopenic purpura	No	No	Alive
20	Non-Hodgkin's lymphoma	Yes	CVC removal	Alive
21	Non-Hodgkin's lymphoma	No	No	Dead
22	AIDS	No	Amphotericin B	Dead
23	Hodgkin's disease[b]	Yes	Catheter removal	Alive

[a] CVC, central venous catheter.
[b] Isolate identified in reference laboratory as a *Rhinocladiella* sp.
[c] Mixed infection with *E. jeanselmei* and a *Rhinocladiella* sp.

10 patients, with a median duration of 3 days (range, 3 to 15 days). The daily dose of amphotericin B varied between 0.5 and 1 mg/kg. Itraconazole was given to six patients; in four of these the azole was given after some days of amphotericin B use, and in two it was given alone.

Of the 23 patients, 9 (39%) died. The underlying diseases were AIDS (3 patients), postoperative period (2 patients), non-Hodgkin's lymphoma (2 patients), acute myeloid leukemia (1 patient), and osteomyelitis (1 patient). The median time between the positive blood culture and death was 25 days (range, 2 to 95 days). In eight of the nine patients who died, the death was attributed to the underlying condition or other complications rather than the fungemia. The patient whose death was attributed to the fungemia was the one who developed nodular opacities in the lungs (see above). No autopsy was performed.

Antifungal susceptibility. Antifungal susceptibility results for nine of the clinical isolates of *E. jeanselmei* are shown in Table 4. The MICs of each agent tested for all isolates were very similar to each other, and the MLCs were not increased. Significant antifungal activity was demonstrated against all strains tested with amphotericin B as well as itraconazole and the newer triazole antifungals voriconazole and posaconazole.

DISCUSSION

Fungi have increased in importance as nosocomial pathogens in the last decade, and the most dramatic increases have occurred in the rates of fungemia (3). While *Candida* species account for the vast majority of cases, fungemia due to other fungi has been increasingly reported. This study extends the list of dematiaceous genera known to incite fungemia. To our knowledge, fungemia due to *E. jeanselmei* or *Rhinocladiella* species has not been previously reported.

Infections due to dematiaceous fungi are usually restricted to the skin and soft tissues, but dissemination may occur (19). Catheter-associated fungemia due to *E. (W.) dermatitidis* has, however, been occasionally reported (11, 15, 28). In the present series, fungemia occurred in association with a wide range of underlying conditions, the majority of them in patients with some degree of immunodeficiency either due to the underlying disease itself such as cancer, agranulocytosis, and AIDS or due to the treatment (use of steroids). In addition, many of the coexisting exposures usually associated with nosocomial fungemia were present: postoperative state, central venous catheter, use of broad-spectrum antibiotics, and neutropenia. Infections were also associated with blood product transfusion in all but one of the patients, suggesting a potential role of contaminated transfusions in acquisition of infection (21). This outbreak appeared to be related to contaminated

TABLE 4. Antifungal susceptibility of *E. jeanselmei* var. *jeanselmei* isolates performed by NCCLS macrobroth testing

Isolate	NCCLS macrobroth MIC of:							
	Amphotericin B		Itraconazole		Voriconazole		Posaconazole	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
R-2976	0.5	0.5	0.125	1	1	1	0.25	0.5
R-2977	0.5	0.5	0.125	1	1	1	0.5	0.5
R-2978	0.5	1	0.25	2	1	1	0.5	0.5
R-2979	0.5	0.5	0.125	1	0.5	1	0.25	0.5
R-2980	0.25	1	0.125	0.5	1	1	0.25	0.5
R-2981	0.5	0.5	0.25	1	0.5	0.5	0.25	0.25
R-2991	0.5	1	0.125	0.25	1	1	0.25	0.5
R-2995	0.5	1	0.25	0.25	0.5	0.5	0.125	0.25
R-2996	0.5	1	0.125	0.25	0.5	0.5	0.125	0.25

deionized water from the hospital pharmacy. The water was used in the preparation of antiseptic solutions, and when the procedure for preparing these solutions was changed, no new cases occurred (M. Nucci, F. Silveira, T. Akiti, G. Barreiros, S. G. Revankar, B. L. Wickes, D. A. Sutton, and T. F. Patterson, Program Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1174, p. 417, 2000). In all but six patients the positive cultures had been taken from peripheral blood. In the other six patients the blood cultures had been obtained from a central venous catheter, all of which were surgically implanted catheters in patients with cancer. Four of these patients had undergone autologous bone marrow transplantation, and two were in remission-induction for acute myeloid leukemia. At the time the blood cultures were drawn, all six patients were neutropenic and febrile.

The issue of considering whether fungemia represents a true-positive result when the fungus was isolated from a central catheter remains a matter of controversy. In a large series of catheter-associated fungemia in patients with cancer, neither the death rate nor the rate of disseminated infection was different whether the source of blood was the catheter or a peripheral vein (13).

E. jeanselmei and *Rhinocladiella*, like other black molds, are considered fungi of low virulence, since they can persist in skin tissue of normal hosts for months to years without disseminating to other organs (19). Accordingly, in the present series, only one patient seemed to have a disseminated disease. The patient died due to multiple-organ failure but had pulmonary infiltrates on the radiograph. Although there were no nodules with cavitation and the computed tomograph was not obtained to see if there was the halo sign, the clinical picture was similar to cases of infections caused by angioinvasive fungi, with signs of pulmonary infarction (5). Whether the pulmonary signs were due to the fungus is not known, since autopsy was not performed. In the literature there are a few cases of systemic infection due to *E. jeanselmei* or *Rhinocladiella* spp. Roncoroni et al. (24) reported a case of systemic infection that appears to have been disseminated via the hematogenous route. The patient had undergone cardiac surgery for the correction of a ventricular septal defect and developed endocarditis. A single case of pneumonia was reported in a diabetic patient who developed a masslike infiltrate in the lower lobe with a protracted clinical course that evolved to hemoptysis (14). Another patient had a bronchopulmonary sequestration complicated by infection due to *E. jeanselmei* (26). Since there was no tissue invasion, the fungus was considered to be an opportunist analogous to *Aspergillus* species, which colonize previous lung cavities. If our patient had a pulmonary infection due to *E. jeanselmei*, the infection probably occurred by the hematogenous route since the patient had multiple positive blood cultures over many days.

While most of the patients received antifungal therapy, treatment regimens were very heterogeneous and were influenced by the clinical status of the patients at the time of the diagnosis. In general, the infection seemed to be mild, confirming the impression that this fungus has a low virulence. In patients with fungemia and a central venous catheter, removal of the device is associated with a better outcome (13, 18). Therefore, as a rule, in all patients with a catheter in place at the time of the diagnosis, the physicians attempted to remove the device, and the only three patients who did not have their catheters removed died before the diagnosis of the fungemia. Some patients had their catheters removed because of persistent fever before the positive blood culture. At the time of the diagnosis of the fungemia, they were afebrile and received either no further treatment or itraconazole. Patients who were neutropenic at the time of the diagnosis received amphotericin B and their catheters were removed if possible. In addition, five patients received itraconazole; in four of them this followed a short course of amphotericin B. Given the heterogeneity of the treatment, it is difficult do draw conclusions about this issue.

Regarding the identification of two distinct fungi, we do realize the pleomorphic nature of these closely related genera, the fact that *Rhinocladiella* species also have black yeast synanamorphs similar to those for *Exophiala* species, and the difficulties that can be encountered when identifying isolates (either phenotypically or by molecular studies). The cases presented here, however, as examined by our methods, appear to have been caused by two distinct genera of dematiaceous moulds.

Antifungal susceptibility testing of *E. jeanselmei* isolates demonstrated uniform susceptibility for each of the agents tested. This organism demonstrated susceptibility to itraconazole and the newer triazole antifungals voriconazole and posaconazole, suggesting a possible role for these agents in treating clinical disease. Although the death rate was 39%, in only one patient was the death possibly attributed to the fungemia. Since this impression was based on data collected from a careful review of the clinical charts and since no autopsy was performed, we cannot rule out the possibility that the fungus caused the deaths.

In summary, 23 cases of fungemia due to *E. jeanselmei* or *Rhinocladiella* spp. were identified. These cases were associated with signs and symptoms associated with infection, and the majority of patients responded to removal of a central venous catheter and administration of antifungal therapy. One patient died of apparent disseminated infection. This study demonstrates the potential of these organisms to cause fungemia in a nosocomial setting, which can be associated with systemic infection.

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