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Catheter-Associated Fungemia Caused by *Fusarium chlamydosporum* in a Patient with Lymphocytic Lymphoma

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A case of catheter-associated fungemia caused by *Fusarium chlamydosporum* is described in a patient with lymphocytic lymphoma. The fungus, which has been isolated from soil but not reported to cause human infection, characteristically produces microconidiophores that are polyphialides bearing microconidia that are spindle-shaped but never globose. Results of in vitro antimicrobial susceptibility tests depended on the test conditions used.

Fusarium species, which are common soil saprophytes (6, 12), have been reported to cause a variety of infections. These include keratitis (11), endophthalmitis (8, 14, 19), osteomyelitis (3), brain abscess (22), leg ulcers (25), hand lesions (5), facial granuloma (2), and disseminated infections (1, 4, 9, 15, 23, 24). Mycotoxins produced by *Fusarium* species contaminating grains and cereals cause the systemic illness called alimentary toxic aleukia in Russia (10) or akakabi-byo in Japan (20).

Several of the isolates from the infections listed above were identified and found to be either *Fusarium moniliforme* (5, 11, 24), *Fusarium oxysporum* (3, 9, 19, 22, 23, 25), or *Fusarium solani* (4). *Fusarium* species reported to produce mycotoxins causing alimentary toxic aleukia and akakabi-byo are *Fusarium sporotrichioides*, *Fusarium poae*, *Fusarium graminearum*, *Fusarium nivale*, and *F. oxysporum* (10, 20). Reports indicating that *F. nivale* produces toxins are in error, since the fungus originally was identified incorrectly and is *F. sporotrichioides* (12).

Many of the *Fusarium* species have not been tested for susceptibility to antifungal compounds in vitro, but of those tested, four were resistant to amphotericin B (4, 11, 19, 23) and two were sensitive (3, 22).

We report here a case of catheter-associated fungemia caused by *Fusarium chlamydosporum* in a patient with lymphocytic lymphoma. To our knowledge, this species has not previously been reported from human infections. Identifying characteristics of the fungus and results of antifungal susceptibility tests are discussed.

MATERIALS AND METHODS

Case report. A 33-year-old woman with poorly differentiated lymphocytic lymphoma was admitted to Memorial Sloan-Kettering Cancer Center on 18 May 1983 for the fourth time because of fever, shaking chills, and pain in the left chest and upper arm. She had developed lymphadenopathy in May 1982, and lymphocytic lymphoma was diagnosed in August 1982. She was treated with the L-17 protocol (intensive polydrug chemotherapy program divided into induction, consolidation, and maintenance phases). In October 1982 she had a Broviac catheter and an Ommaya reservoir placed. She had been on prednisone from 3 May 83 to 10 May 83. On the day before admission she had a shaking chill and temperature to 101°F (ca. 38.3°C) as well as pain in the left chest and upper arm. On physical examination, she appeared acutely ill, but there were no specific abnormalities found except an erythematous left ear canal and a perforated drum. The site of the Broviac catheter was not inflamed. Laboratory evaluation revealed a mild anemia and a normal leukocyte count and the following: hemoglobin, 9.5; hematocrit, 29.5; erythrocytes, 3.37; leukocytes, 4.7; neutrophils, 95%; bands, 1%; lymphocytes, 1%; monocytes, 3%; platelets, 217,000. Urinalysis showed 10 to 15 erythrocytes per high-power field but was otherwise normal. Bilirubin was 1.3, serum glutamic oxalacetic transaminase was 28, and radiographs revealed that the patient had evidence of pulmonary infiltrates which on a lung perfusion-ventilation scan did not appear to be pulmonary emboli. She was treated with moxalactam and gentamicin. Blood cultures taken on admission yielded *Escherichia coli* and *Pseudomonas aeruginosa*. After an initial response the patient was intermittantly febrile. Subsequent blood cultures were negative until 27 May, when a culture drawn through a peripheral arm vein was positive for a mold. Three more cultures taken through the Broviac catheter on 29 May, 30 May, and 2 June also grew the mold. On 2 June, the catheter was removed, and because of the positive cultures for mold, amphotericin B was started. The tip of the Broviac catheter also yielded mold. On 10 June, the amphotericin B was stopped after a total dosage of 371 mg had been given. The patient became afebrile, was discharged, and had no evidence of recurrent infection at last follow-up in August 1984.

Culture methods. Since the original requisition did not specify fungus culture, all specimens were originally processed in the bacteriology section of the laboratory where cultures are incubated at 35°C, not at 30°C. Blood from a peripheral vein and from the Broviac catheter was inoculated equally into two bottles containing 50 ml of Columbia broth supplemented with cysteine (1 g/liter) (BBL Microbiology Systems, Cockeysville, Md.), and one of the bottles was transiently vented. Bottles were incubated at 35°C, and when visibly positive, broth was inoculated onto chocolate agar and Columbia base agar plates with 5% sheep blood. The Broviac catheter tip was placed in 5 ml of Trypticase soy broth (BBL), the broth tube was vortexed for 15 s, and a saturated swab of the liquid was applied to a blood agar

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plate. All agar plates were incubated in 5% CO_2 in air at 35°C. After initial isolation, the fungus was transferred to the mycology section of the laboratory and inoculated onto Sabouraud glucose agar for subculture at 30°C; material for microscopic examination was prepared in lactophenol cotton blue mounts. Final morphological observations were made from cultures grown on carnation leaf-water agar (7).

Antifungal susceptibility testing methods. All tests were performed at 35°C since the fungus grew rapidly at that temperature in all media. The isolate was grown on Sabouraud glucose agar at 35°C for 7 days. The agar plate was flooded with 15 ml of 0.1% Tween in saline, and the surface was gently raked with a sterile loop. Cells were washed twice by centrifuging the resulting suspension at 1,500 × *g* for 5 min, discarding the supernatant, and resuspending the cell sediment in 5 ml of sterile water. A 0.1-ml portion of the washed suspension was diluted 100-fold in water, and the fungal cells, primarily microconidia, were counted with a hemacytometer.

The isolate was tested for susceptibility to amphotericin B, flucytosine, and ketaconazole by a microtiter broth method and to amphotericin B by an agar dilution method. Tests by the microtiter method were performed in both Sabouraud glucose broth (pH 5.2) and yeast nitrogen base broth with 0.1 M glucose (YNG; pH 7.4). Both low (10^3 cells per ml) and high (5.0×10^4 cells per ml) inocula of the mold were tested. Doubling dilutions, ranging from 100 to 0.05 μg/ml, of the antifungal agents were prepared. Amphotericin B (Fungizone, E. R. Squibb & Sons, Princeton, N.J.) was diluted in 0.2 mM sodium desoxycholate, flucytosine was diluted in water, and the three highest concentrations of ketoconazole were prepared individually by dilution in water from a solution of the drug at 2.0 mg/ml in dimethylsulfoxide; lower concentrations were prepared by serial twofold dilutions in water. Portions (0.1 ml) of the drug solutions were added to microtiter tray wells. The inoculum was diluted in double-strength media to the desired cell number, and 0.1-ml portions were added to the microtiter wells containing the drug solutions. The minimum concentrations that inhibited visible growth were recorded after 24 and 48 h of incubation at 35°C.

Plates for the agar dilution susceptibility tests were prepared as follows. Serial twofold dilutions of amphotericin B were made in 0.8 mM sodium desoxycholate, and 2-ml portions of each dilution were combined with 18-ml portions of sterile molten antibiotic medium no. 3 (pH 7.4; Difco Laboratories, Detroit, Mich.) in petri plates (100 by 15 mm). Final concentrations of amphotericin B ranged from 0.05 to 100 μg/ml. Agar plates were inoculated with 0.01 ml of a suspension of the isolate at 104 cells per ml. The minimum concentration of the drug that prevented visible growth was determined after 48 h of incubation at 35°C. All susceptibility tests were performed in duplicate.

RESULTS

The mold grew visibly in blood broth bottles after 4 days of incubation and appeared as nondispersed clumps. Growth from the catheter tip occurred on chocolate and blood agar plates after 4 days of incubation. Subcultures of blood bottles or agar plates showed a rapidly growing white cottony mold that turned slightly yellow and then pink. The mold grew on Sabouraud glucose agar at 25, 30, and 37°C. Morphology of the structures was initially observed in a lactophenol cotton blue mount of a cover slip from a Sabouraud agar slide culture after 8 days of culture. Numerous one-celled microconidia were borne directly from the tip of the conidiophore singly and in groups of two or three. Rare 2- to 3-celled, slightly curved macroconidia with smooth thin walls were attached to the hyphae by a foot cell. Numerous intercalary chlamydoconidia were seen within the septate hyaline branching hyphae.

Numerous spindle-shaped microconidia with zero to one septation and, rarely, two to three septations were produced on carnation leaf-water agar (Fig. 1a and b). Typical sickle-shaped macroconidia with three to five septa and a foot-shaped basal cell were rarely seen (Fig. 1c). Microconidiophores appeared as unbranched and branched monophialides and polyphialides (Fig. 1d, e, and f). Macroconidiophores also appeared as unbranched and branched monophialides. Chlamydoconidia were formed in culture on carnation leaf-water agar after 2 to 3 weeks. They were globose and were formed singly or in short chains (Fig. 1g, h, and i).

On the basis of these characteristics, this fungus was identified as *F. chlamydosporum* Wollenweber and Reinking by using the taxonomic system described by Nelson et al. (17). The distinguishing characteristic of this species is the presence of microconidiophores that are polyphialides bearing microconidia that are spindle shaped but never globose. This strain was deposited in the Fusarium Research Center Collection under the accession number T-660. The identification was confirmed by C. Booth of the Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.

Table 1 gives the antifungal susceptibility results at 24 h from the microtiter susceptibility determinations in which two broths, Sabouraud glucose broth and YNG, and both low and high inocula were used. The organism appeared resistant to amphotericin B in Sabouraud glucose broth but was sensitive when tested in YNG. The organism was resistant to flucytosine by all test methods. It was sensitive to ketoconazole in both media when a low inoculum was used. With every active drug, the MIC increased no more than one doubling dilution when tests were incubated for an additional 24 h. The isolate was considered sensitive to amphotericin B, with an MIC of 0.4, when tested by the agar dilution method.

DISCUSSION

F. chlamydosporum belongs to the section *Sporotrichiella* of the genus *Fusarium*, in the family *Tuberculariaceae*, order *Moniliales*, class *Hyphomycetes* of the *Deuteromycotina* (13). The section *Sporotrichiella* contains three other species in addition to *F. chlamydosporum*. The distinguishing characteristics of each species are as follows: *F. poae* (Peck) Wollenweber, microconidia are globose or oval in shape with zero to one septation, and microconidiophores appear as unbranched and branched monophialides; *Fusarium tricinctum* (Corda) Saccardo, microconidia are lemon to pear shaped or spindle shaped with zero to one septation, and microconidiophores appear as unbranched and branched monophialides; *F. sporotrichioides* Sherbakoff, microconidia are oval to pear shaped or spindle shaped with zero to one septation, and microconidiophores appear as unbranched and branched monophialides and polyphialides; *F. chlamydosporum* Wollenweber and Reinking, microconidia are spindle shaped but not globose with zero to one septation, and microconidiophores appear as unbranched and branched monophialides and polyphialides. The primary characteristics separating species in this section are the shape of the microconidia and the presence or absence of polyphialides (16). *F. chlamydosporum* has a higher opti-

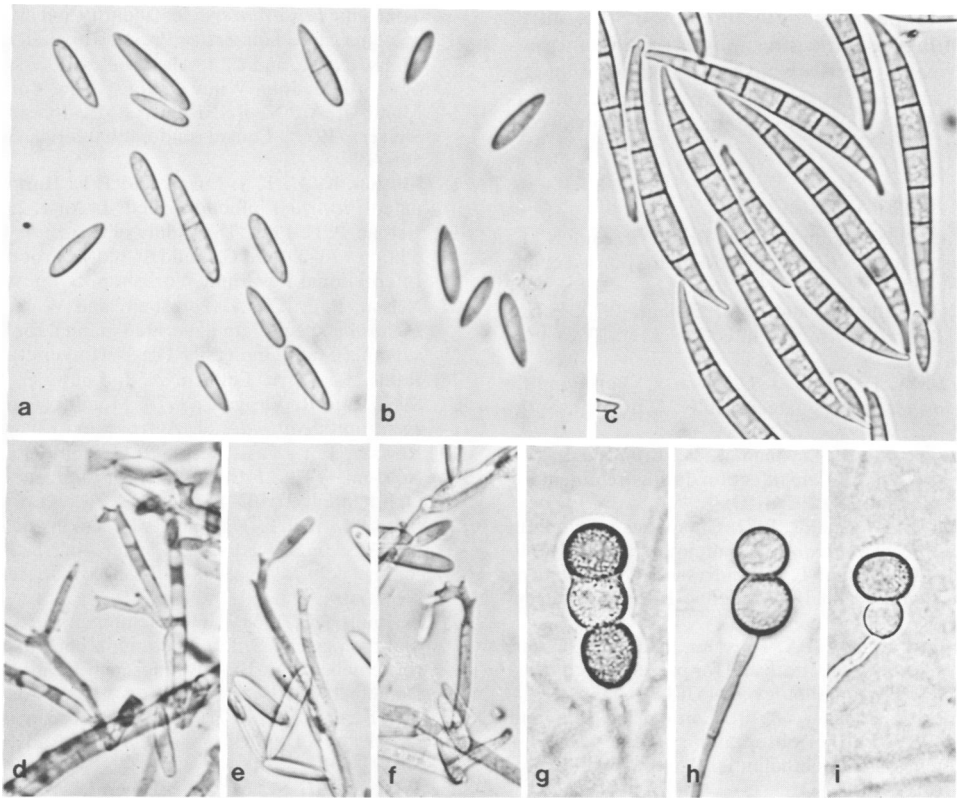

FIG. 1. *F. chlamydosporum*: a, b, microconidia; c, macroconidia; d through f, polyphialides; g through i, chlamydospores (all magnified ×750).

mum temperature for growth than other species in the section *Sporotrichiella* (21).

The source of the isolate found in our patient is not known. *F. chlamydosporum* occurs as a saprophyte in soil and on other substrates in tropical and subtropical areas including Australia, Central America, India, Iraq, Namibia, Pakistan, South Africa, and the Sonoran Desert of the southwestern United States (6, 12). *F. chlamydosporum* is also commonly associated with seeds of crops such as beans, millet, peanuts, and sorghum in areas such as Australia, India, and southern Africa (6, 12). Recently, one of the authors, P.E.N., has isolated *F. chlamydosporum* from soil collected from sorghum fields in Gainesville, Fla.; Starkville, Miss.; and Lajars and Isabela, P. R. The patient had not traveled outside of the United States except to the Caribbean 5 years before this episode.

The section *Sporotrichiella* contains toxigenic *Fusarium* species such as *F. sporotrichioides*, which produces T-2 toxin and other trichothecenes (12). A toxic isolate of *F. chlamydosporum* was obtained from millet from households of patients suffering from the hemorrhagic disease Onyalai in Namibia (18). The toxic compound produced by this strain was identified as moniliformin (6, 12).

TABLE 1. Antifungal susceptibility testing of *Fusarium chlamydosporum* by microtiter methods at 24 h

Antifungal agent	MIC (μg/ml)			
	Sabouraud glucose broth		YNG	
	Low inoculum[a]	High inoculum[b]	Low inoculum[a]	High inoculum[b]
Amphotericin B	6.25	6.25	0.8	0.8
Flucytosine	>100	>100	>100	>100
Ketoconazole	3.2	>100	12.5	>100

[a] 10^3 cells per ml.
[b] 5.0×10^4 cells per ml.

The results of antifungal susceptibility tests on the present isolate point out the difficulty in interpreting these results. We found that the in vitro susceptibility of the isolate depended on the test conditions used. The fungus was sensitive to amphotericin B by agar dilution with antibiotic medium no. 3 and by broth dilution with YNG; however, the organism was resistant when tested in Sabouraud glucose broth. Differences in susceptibility to amphotericin B may have been due to differences in composition or pH of the media. A low inoculum of organism was needed to demonstrate inhibition by ketoconazole. Flucytosine had no detectable activity by any method. Some strains of *Fusarium* species that have been tested were sensitive to amphotericin B, and others were resistant to the polyene. The test methods used in these studies have not always been clearly described; thus, it is not known whether variations in results are due to differences in methodology or to true differences in organism susceptibility. Standardization of procedures for antifungal susceptibility testing, particularly for molds, and proper interpretation of results should be studied further. For our patient, removal of the infected catheter appears to have been sufficient treatment. It is doubtful that any immunological abnormality from her lymphocytic lymphoma contributed to this catheter-associated infection.

In summary, we have described a case of catheter-associated fungemia caused by *F. chlamydosporum* in a woman with lymphocytic lymphoma. We have also described the

morphological and taxonomic characteristics of this infrequently recovered fungus. Difficulties in interpreting antifungal susceptibility results were discussed. As microbiology laboratories isolate more of these organisms, complete identification of the isolates will help define the spectrum of disease they can cause.

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