

## Graft-Related Endocarditis Caused by *Neosartorya fischeri* var. *spinosa*

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**The first case of endocarditis caused by *Neosartorya fischeri* var. *spinosa* is reported. The patient was a child who received a calf pericardium graft after removal of a previously inserted Dacron graft associated with deterioration of adjacent tissue. Copious vegetations removed from the heart were found to be composed of septate hyaline fungal filaments. The fungus was recognized in culture by its bivalved, winged, spiny ascospores, its *Aspergillus fischerianus* anamorph, and its thermotolerance.**

Members of the ascomycetous genus *Neosartorya* Malloch and Cain (11) have anamorphs similar to *Aspergillus fumigatus* and were placed in the *A. fumigatus* group by Raper and Fennell (15). *Neosartorya fischeri* (Wehmer) Malloch and Cain (anamorph: *A. fischerianus* Samson and W. Gams; syn., *A. fischeri* Wehmer) is the most common member of this genus and occurs in soils and decaying vegetation in tropical, subtropical, and temperate parts of the world (3). It shares with *A. fumigatus* not only its uniseriate, greenish tinted, phialidic conidial heads with pyriform vesicles and columnar conidial masses but also its thermotolerance. It differs by forming large numbers of pale colored ascospores bearing equatorially furrowed ascospores with prominent crests arising along the margins of the furrow. In vitro, *N. fischeri* grows at up to 51°C (3) and its exceptionally heat-resistant ascospores may remain viable after several minutes at 100°C (14). Hence, it is most commonly isolated as a contaminant in canned goods, especially canned fruit products (8).

Despite the strong affinity to *A. fumigatus*, *N. fischeri* has seldom been reported in association with human or animal disease. Gerber et al. (6) reported an apparent case of pulmonary infection caused by *N. fischeri* var. *spinosa* (Raper and Fennell) Malloch and Cain in a patient with no known predisposing factors. Infection was suggested by repeated sputum isolations, response to amphotericin B, and a strong serological response to *N. fischeri* antigen but not to other fungal antigens (except a weak probable cross-reaction to *Histoplasma capsulatum*), but fungal tissue invasion was not demonstrated. More recently, the spontaneous formation of superficial fungal masses by *N. fischeri* in the nasal cavities of two laboratory rats was reported by Nyska et al. (13). *N. fischeri* var. *fischeri* was documented from a case of mycotic keratitis by Coriglione et al. (2). The present report describes a case of graft-related endocarditis caused by *N. fischeri* var. *spinosa*.

A male child born in June 1982 was referred to Sainte-Justine Hospital at 2 months of age for evaluation of a heart murmur. Cardiac catheterization and angiography performed in January 1985 revealed the presence of Fallot's tetralogy with obstruction to right ventricular outflow at both

the infundibular and valvular levels, a ventricular septal defect, a right-sided aortic arch, and aberrant implantation of the right pulmonary artery on the ascending aorta. Total correction and implantation of the right pulmonary artery on the main pulmonary artery were carried out in May 1985. Repeat cardiac catheterization in September 1988 demonstrated severe stenosis of the right pulmonary artery and valvular pulmonary insufficiency.

The patient was admitted in October 1989 because of a draining pustular lesion located at the right sternoclavicular area. Bone scans revealed osteomyelitis of the superior third of the sternum. Culture of the draining pus yielded *Staphylococcus auricularis*, *Streptococcus sanguis* II, *Streptococcus MG intermedius*, and *Acetivibrio lwoffii*, and the patient was treated with intravenous cloxacillin and ampicillin. Draining pus persisted after 3 weeks of treatment, and open drainage of a retrosternal abscess extending to the pulmonary artery was carried out. The patient was treated with vancomycin, rifampin, and ticarcillin, and drainage ceased. Two weeks later, the Dacron infundibular material was removed and a calf pericardium graft was inserted; inspection revealed a fistula extending from the Dacron material to the myocardium. Culture of the removed Dacron graft was negative. A pectoralis major flap was used to cover a residual opening of the sternum. Ten days later, echocardiography demonstrated a vegetation on the pulmonary valve. Seven blood cultures performed by lysis-centrifugation were negative.

Counterimmunoelectrophoresis studies based on the technique of Fung and Tilton (5) revealed the presence of precipitins against antigens of *Aspergillus niger* but not against antigens of *A. flavus*, *A. fumigatus*, *A. nidulans*, and *A. terreus* (antigens were from Diagnostics Pasteur, Marnes-la-Coquette, France). Treatment was begun by using intravenous ceftazidime, tobramycin, cloxacillin, and amphotericin B (total dose, 59 mg [3.1 mg/kg]), but progression of the vegetation was demonstrated by repeat echocardiography 6 days later. Surgery revealed extensive vegetations obstructing the infundibulum and the pulmonary arteries, with disinsertion of the calf pericardium graft. Necrosis of the left myocardium and pulmonary arteries was observed. Vegetations were removed and an attempt was made to replace the calf pericardium graft, but the patient expired after diffuse bleeding and cardiac arrest.

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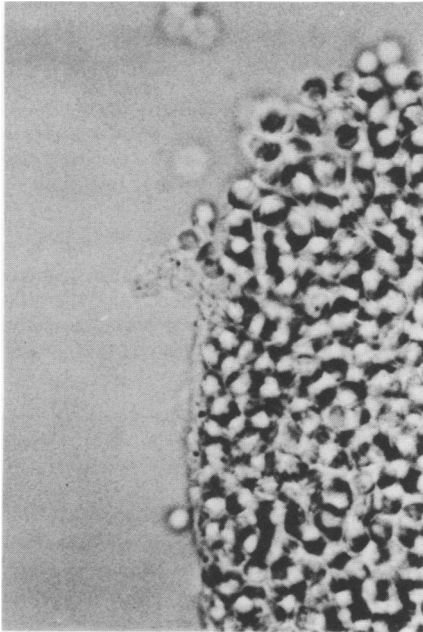


FIG. 1. Thin peridium of an *N. fischeri* ascoma torn open to release ascospores. Magnification,  $\times 400$ .

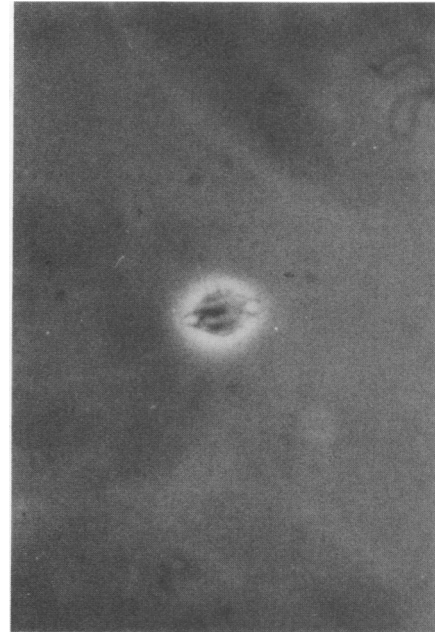


FIG. 2. *N. fischeri* var. *spinosa*: crested ascospore with spiny valves. Magnification,  $\times 1,000$ .

Vegetations examined by wet mount and after Gomori-methenamine silver staining (16) contained numerous septate hyaline hyphae, dichotomously branched at wide angles with terminal structures suggestive of vesicles but not forming phialides or conidia. No sporulating structures were seen. Culture on Sabouraud's peptone-glucose medium, amended with 40 mg of chloramphenicol per ml, showed abundant growth of a mold independently identified by both the CAB International Mycological Institute, Kew, United Kingdom, and one of us (R.C.S.) as *N. fischeri*. The isolate was deposited in the University of Alberta Microfungus Collection, Edmonton, Alberta, Canada, as UAMH 7109.

In detailed examination on modified Leonian's agar (10), the etiologic agent was fast growing, attaining a colony diameter of 60 mm after 7 days at 25°C. At first white and velvety, it rapidly became granular with formation of the predominant teleomorphic state. Conidial heads were not apparent macroscopically or microscopically, but coiled ascogonial initials developed within 7 days. Ascumata formed within 10 days and were cream colored, approximately 400  $\mu\text{m}$  in diameter, with a very thin, easily fractured, pseudoparenchymatous peridium (Fig. 1). Asci were subglobose and evanescent. Ascospores were ellipsoidal, 5 to 6  $\mu\text{m}$  long by 4 to 4.5  $\mu\text{m}$  wide, with two sinuous equatorial crests extending approximately 1  $\mu\text{m}$  beyond the spore body (Fig. 2). The ascospore valve surfaces were ornamented with prominent ridges, occasionally curving and anastomosing, and prominent spines extending up to 2  $\mu\text{m}$ . Colonies grown on modified Leonian's agar at 37°C produced a moderate number of conidial heads 50 to 150  $\mu\text{m}$  tall, with pyriform vesicles covered over the upper two-thirds by flask-shaped phialides 5 to 6  $\mu\text{m}$  long (Fig. 3). The cluster of phialides on each vesicle gave rise to a single columnar mass of grey-green to blue-green, ellipsoidal, smooth to delicately roughened conidia 2.5 to 3.0  $\mu\text{m}$  long by 2.0 to 2.5  $\mu\text{m}$  wide. The isolate grew vigorously at 45°C.

Sensitivity of the isolate to amphotericin B, 5-fluorocy-

tosine, ketoconazole, and itraconazole was determined by a broth macrodilution method (17). The media used were antibiotic medium 3 for amphotericin B, yeast nitrogen base supplemented with 1% glucose for 5-fluorocytosine, and RPMI 1640 for ketoconazole and itraconazole. Serial two-fold dilutions were prepared for each antifungal agent, distributed in 1-ml aliquots, and inoculated with 50  $\mu\text{l}$  of a suspension containing  $10^6$  conidia per ml previously standardized by counting in a hemacytometer. Tests were per-

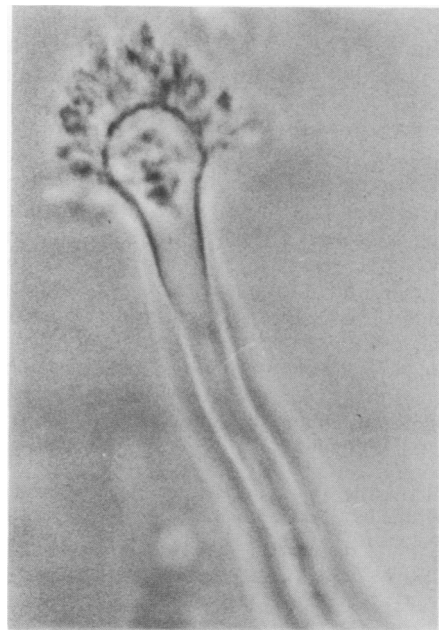


FIG. 3. Conidiophore of *A. fischerianus* anamorph. Magnification,  $\times 400$ .

formed in duplicate, and incubation was carried out at 37°C. Results were read after 48 h for amphotericin B and 24 h for the other drugs. The MICs were 0.08 µg/ml for amphotericin B, >100 µg/ml for 5-fluorocytosine, 3.13 µg/ml for ketoconazole, and 0.05 µg/ml for itraconazole.

The presence of prominent, discrete spines on the ascospore valves of the isolate revealed it to be a representative of *N. fischeri* var. *spinosa*. This relatively uncommon variety is distinguished from the more common *N. fischeri* var. *fischeri*, with ascospore valves bearing anastomosing ridges but not spines, and from *N. fischeri* var. *glabra* (Fennell and Raper) Malloch and Cain, with smooth valves, as well as *N. fischeri* var. *verrucosa* (Udagawa and Kawasaki) Malloch and Cain, with verrucosely warted valves. The scant data available reveal no reason to suspect that *N. fischeri* var. *spinosa* differs in virulence properties from the type variety, even though that variety has greater antigenic similarity to virulent *A. fumigatus* isolates (4). In general, the infrequency of *N. fischeri* in human and animal diseases, remarkable for a moderately common fungus so closely related to *A. fumigatus*, remains unexplained. The similarity between *N. fischeri* and *A. fumigatus* is not merely morphological but also extends to similar levels of thermotolerance and production of similar toxic metabolites, including the neurotoxins verruculogen and fumitremorgens A, B, and C (12, 19). Further comparisons of *A. fumigatus* and *N. fischeri* may be useful in elucidating the virulence factors of *A. fumigatus*.

Because *N. fischeri* var. *spinosa* has been very rarely isolated as a clinical contaminant or from soils in northern temperate areas (1, 3, 7), we speculated that the species might have been a contaminant of the calf pericardium graft material. This material, an Ionescu-Shiley pericardial closure patch (Shiley Inc., Irvine, Calif.), originated in California. The manufacturer kindly informed us that the graft was decontaminated in a glutaraldehyde solution and shipped in 4% buffered formaldehyde. To determine whether the notoriously resistant ascospores of *N. fischeri* could survive under these conditions, we exposed ascospores of our isolate to solutions based on information supplied by the manufacturer. Mature ascospores retrieved from both 25% glutaraldehyde (14 days, 25°C) and 4% formaldehyde (7 days, 25°C) failed to grow on modified Leonian's medium at 25 or 37°C. They also failed to respond to heat stimulation (60°C, 30 min) as recommended by Warcup and Baker (18) to stimulate ascospore germination. We concluded that the manufacturer's decontamination and shipping procedures were adequate and that the *N. fischeri* isolate in the patient likely originated as an aerial contaminant in Montreal. Our patient had three recognized predisposing factors for filamentous fungal endocarditis, namely, a pre-existing lesion, the presence of avascular foreign bodies (Dacron and calf pericardium grafts), and repeated subjection to open heart surgery (9). *N. fischeri* var. *spinosa*, although uncommon in northerly regions, appears able to cause potentially hazardous complications in such cases worldwide.

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