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Osteomyelitis Caused by *Neosartorya pseudofischeri*

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The first case of osteomyelitis caused by *Neosartorya pseudofischeri* is reported. The patient, a 77-year-old male with a history of silicosis and tuberculosis, on X-ray examination revealed lytic lesions of L_2 and L_3 vertebrae suspicious for metastatic lesions. Histologic examination of biopsy specimens from vertebral bodies showed short, distorted, extra- and intracellular, hyaline hyphal fragments. The culture from the biopsy tissue produced numerous, evanescent asci containing eight ellipsoidal ascospores with two distinctive equatorial bands ca. 1 μm wide. When examined by a scanning electron microscope, ascospores exhibited a convex surface ornamented with raised flaps of tissue, in shape resembling triangular projections or long ridge lines. The conidial state (anamorph) was identified as *Aspergillus thermomutatus* on the basis of conidial columns which were smaller and less tightly packed as well as of a lighter shade of green than those observed in *Aspergillus fumigatus*. On the basis of the morphologic features of the ascospores, the teleomorph was identified as *N. pseudofischeri*.

Aspergillus species rarely cause osteomyelitis. Infection may follow trauma or a surgical procedure, but most frequently, extension from a contiguous site, such as sinuses, lung, or vascular graft (1, 3, 10, 16), causes such infection. The infection is generally associated with defects in host defense which may be inborn (chronic granulomatous disease) or acquired as in leukopenia or treatment with corticosteroids or due to the presence of a foreign body. In children, the spread of the infection from lungs to vertebrae involves hematogenous spread (1). The species of *Aspergillus* causing osteomyelitis are *Aspergillus fumigatus* (3, 16), *Aspergillus flavus* (3), *Aspergillus nidulellus* (= *Aspergillus nidulans*) (3), *Aspergillus niger* (16), and *Aspergillus terreus* (8). In one case, the causal agent was identified as belonging to *Aspergillus* section *Flavipedes* (= *Aspergillus flavipes* group) (6, 11).

Some species of *Aspergillus* section *Fumigati* produce sexual ascosporic states (teleomorphs) in culture belonging to the genus *Neosartorya* Malloch and Cain, in addition to their asexual conidial states (anamorphs) (12, 13). Even though they are members of *Aspergillus* section *Fumigati*, species of *Neosartorya* have seldom been reported as etiologic agents of human aspergillosis. Gerber et al. (7) reported a pulmonary infection caused by *Neosartorya fischeri* var. *spinosa* (as *spinosus*) (Raper and Fennell) Malloch and Cain, in a patient with no known predisposing factors. The diagnosis was based on the repeated isolation of the fungus from sputum, response to amphotericin B treatment, and serologic testing using complement fixation, immunodiffusion, and immunoelectrophoresis showing a high degree of specificity for *N. fischeri* var. *spinosa*. A second human infection, due to *Neosartorya fischeri* var. *fischeri* (anamorph *Aspergillus fischerianus* Samson and Gams) (12), causing mycotic keratitis and having apparently no history of trauma in a 62-year-old man from Italy, was recently described (2). Summerbell et al. (15) described the first case of endocarditis caused by *N. fischeri* var. *spinosa* in a child who received a calf pericardium graft after removal of a previously inserted Dacron graft associated with adjacent tissue deterioration. We describe the first case of osteomyelitis of the L_2 and L_3 vertebrae caused by *Neosartorya pseudofischeri* Peterson (9).

A 77-year-old male was admitted to the hospital with low back pain of 3 months' duration, onset having occurred during a previous hospitalization for complications secondary to pneumonia. The X-ray examination before admission revealed lytic lesions of L_2 and L_3 vertebrae suspicious for metastatic

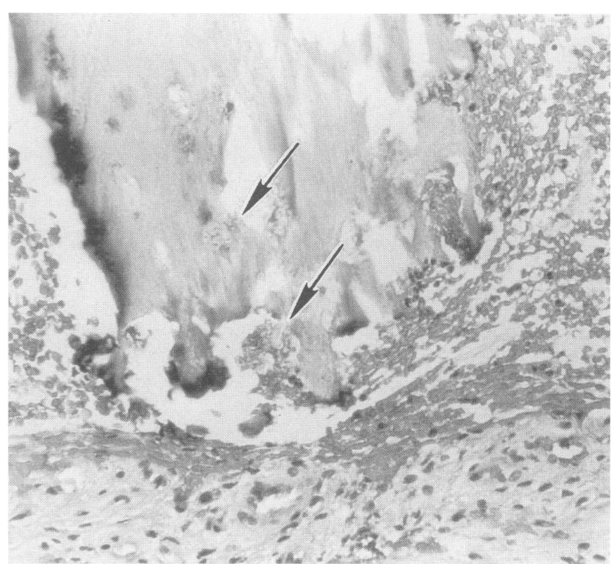

FIG. 1. Invasive aspergillosis caused by *N. pseudofischeri*. Note lysis and invasion of trabecular bone by compact clusters of atypical, poorly stained hyphae and hyphal fragments (arrows). Hematoxylin and eosin; magnification, ×150.

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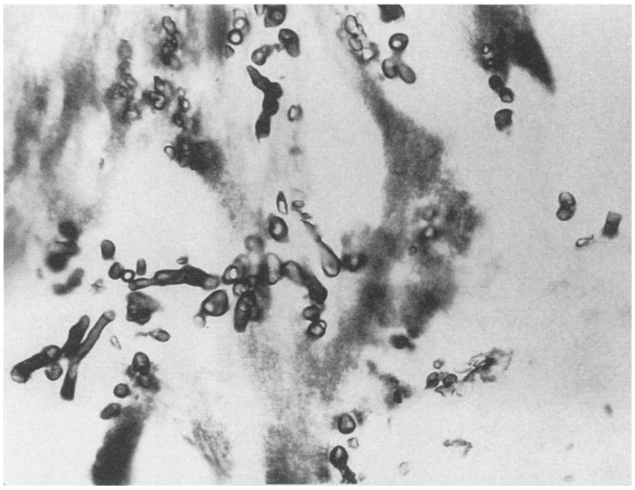

FIG. 2. Paratrabecular collections of *N. pseudofischeri*. Many hyphal elements have fragmented and have nonparallel contours. Gomori's methenamine silver; magnification, ×300.

disease. Computed tomography scan of the spine after admission suggested an inflammatory process of multiple vertebrae. The patient underwent aspiration of L_2 disc space and Craig needle biopsy of the superior aspect of the body of L_3 vertebra. Cultures and histopathology were negative for routine bacteria, acid-fast bacilli, and fungi. The patient was treated empirically with cephalosporin intravenously by Hickman catheter in the superior vena cava for 4 weeks but was readmitted to the hospital approximately 2 weeks later because of increased back pain. Because of a history obtained at that time of silicosis and tuberculosis, antitubercular therapy was initiated and the patient underwent open biopsy of L_2 and L_3 vertebrae as well as bone marrow aspiration and biopsy of the right posterior superior iliac spine. Histopathology of the tissue from L_2 and L_3 vertebrae revealed a mycotic osteomyelitis, and cultures developed into powdery, white fungal colonies. Both histopathologic slides and cultures were sent to the Fungus Reference Laboratory of the Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, for specific identification.

FIG. 3. Elongated branched and septate hypha of *N. pseudofischeri* invading trabecular bone of vertebra. Gomori's methenamine silver; magnification, ×150.

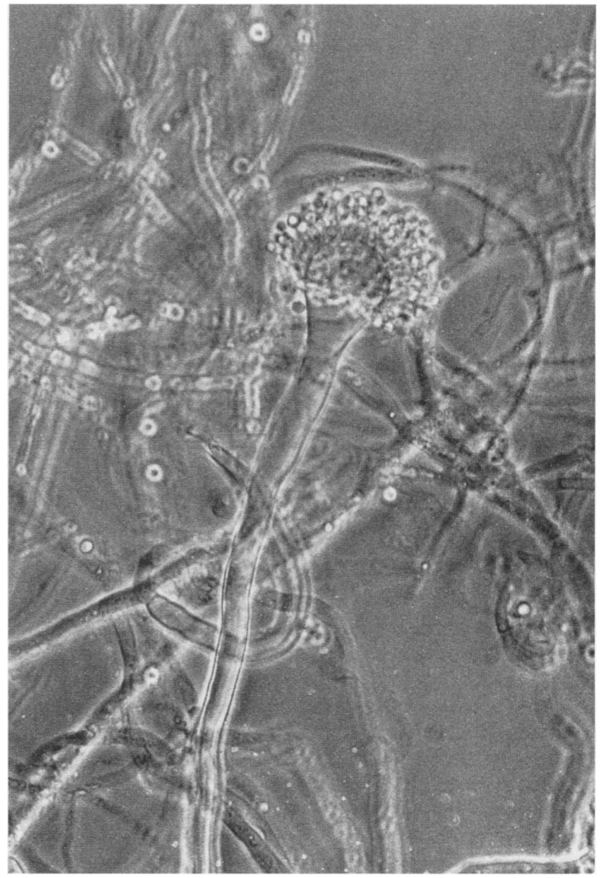

FIG. 4. Smooth conidiophore and subglobose vesicle of *N. pseudofischeri* (B-4812) covered on the distal two-thirds area with ampulliform phialides. Magnification, ×450.

Biopsy specimens from the vertebral bodies of L_2 and L_3 stained with hematoxylin and eosin and Gomori's methenamine silver procedures revealed multifocal and confluent granulomatous osteomyelitis and lysis of trabecular bone (Fig. 1). The granulomas were composed of epithelioid cells, lymphocytes, fibroblasts, and multinucleated giant cells of both the foreign body and Langhans' types. Occasional neutrophils were scattered among the epithelioid and giant cells, and there was stromal fibrosis with formation of dense collagenous connective tissue.

Short, distorted, extracellular and intracellular hyaline hyphal fragments, some of which contained bulbous dilatations measuring up to 10 μm in width, were scattered within foci of granulomatous inflammation (Fig. 2). The aberrant hyphal fragments were sparse and infrequently branched and usually lacked septa. Necrotic trabecular bone and cartilaginous fragments contiguous to the granulomas also contained individual and clustered atypical hyphae as well as occasional elongated hyphae that were dichotomously branched, septate, 4 to 6 μm in diameter with parallel contours, and morphologically suggestive of an *Aspergillus* species (Fig. 3). Although hyphae were pale, eosinophilic, and poorly delineated in hematoxylin-and-eosin-stained sections, they were intensely and uniformly ar-

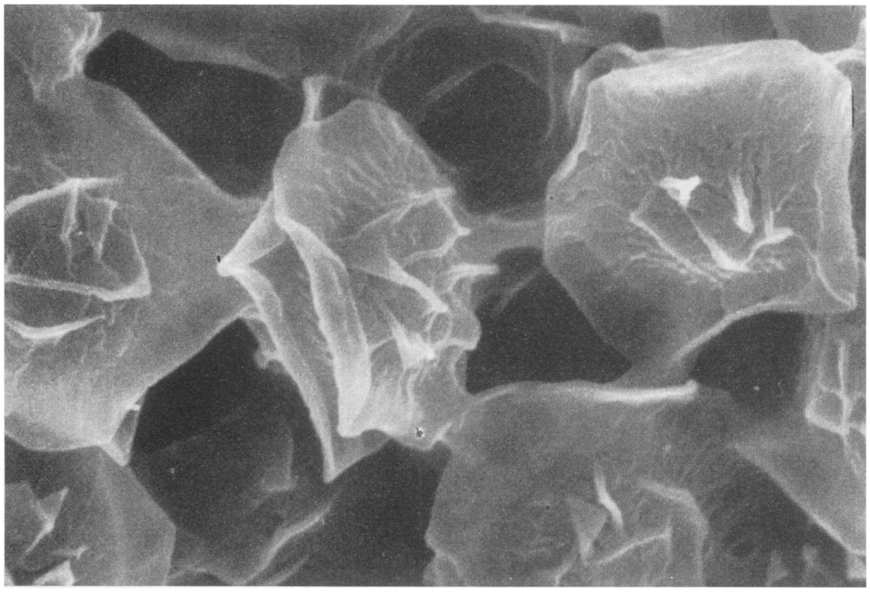

FIG. 5. SEM view of ascospores of *N. pseudofischeri* (B-4812) showing surface ornamentation and the presence of smooth area near the flanges. Magnification, ×9,000.

gyrophilic and easily demonstrated when stained with Gomori's methenamine silver (Fig. 2 and 3). Conidiophores and conidia were not observed, and there was no histologic evidence of a coexisting mycosis or other infection.

The isolate CDC B-4812 was recognized as a member of the *Aspergillus* section *Fumigati* by its conidiophores, which were smooth, hyaline, and faintly yellowish and terminated apically in subglobose vesicles bearing a single series of ampulliform phialides (Fig. 4). The whitish fast-growing colonies and abundant development of ascomata on malt agar and to a lesser extent on Czapek's agar confirmed the isolate to be a species of *Neosartorya*. The isolate was further studied and compared with other species of *Neosartorya* by one of us (S.W.P.) by light and scanning electron microscopy (SEM) and DNA hybridization procedures (9). On the basis of several significant features, the teleomorph was described by Peterson (9) as a new species, *N. pseudofischeri*, and the isolate from this case was selected as ex-type culture. Features of the teleomorph (ascospore ornamentation) and anamorph (vesicle and phialide morphology) distinguish *N. pseudofischeri* from other species in *Aspergillus* section *Fumigati*. The convex walls of *N. pseudofischeri* ascospores are ornamented with variably shaped triangular flaps of tissue (Fig. 5) that do not anastomose into regular patterns. By contrast, *N. spinosa* ascospores are ornamented with echines, and *Neosartorya glabra* ascospores are only finely roughened, giving a nearly smooth appearance. The vesicles and phialides

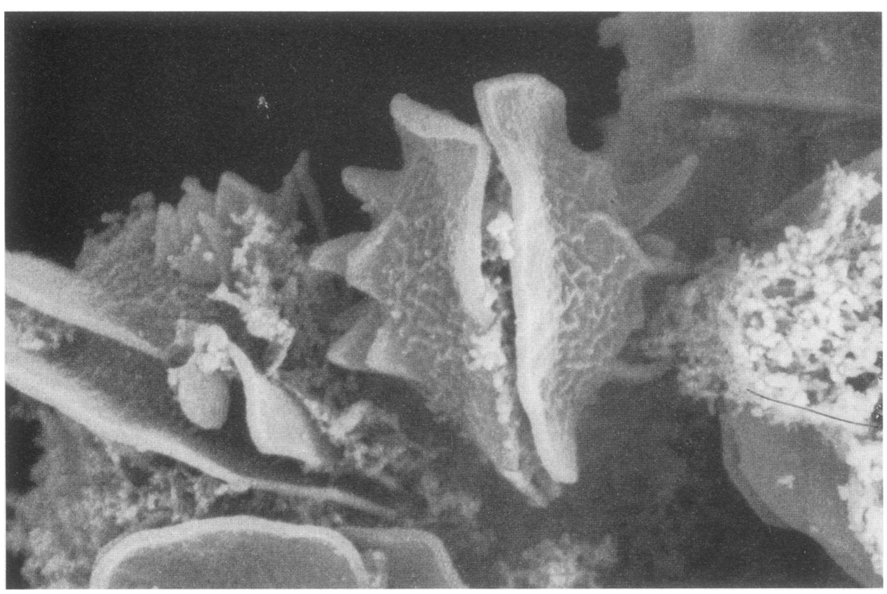

FIG. 6. SEM view of ascospores of Coriglione et al. (2) isolate (NRRL 22808 = CDC B-5491) of *N. pseudofischeri* showing variably shaped triangular flaps of tissue. Magnification, ×10,000.

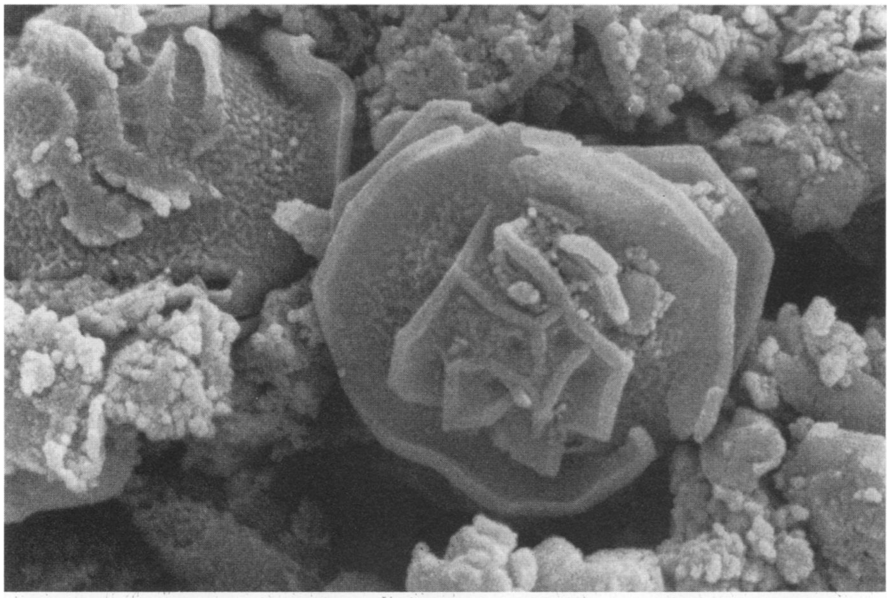

FIG. 7. SEM view of convex walls of ascospores of Summerbell et al. (15) isolate (NRRL A-28710 = CDC B-5472) of *N. pseudofischeri* showing triangular flaps of tissue. Magnification, ×10,000.

of the anamorphic states of *Neosartorya* species are similar to each other and resemble those of *A. fumigatus*. However, the vesicles of the *Neosartorya* species are smaller and less darkly pigmented than the vesicles of *A. fumigatus*, and the conidia are loosely columnar in *Neosartorya* versus compactly columnar in *A. fumigatus*. The most obvious difference is that *Neosartorya* species almost always produce ascoma on malt agar or Czapek's agar, while *A. fumigatus* does not have the capacity to form ascoma.

Peterson (9) showed that sexually compatible strains of *Aspergillus fennelliae* that anastomose to give fully fertile *Neosartorya fennelliae* are between 92 and 100% related by nuclear DNA complementarity. The 13 strains identified morphologically as *N. pseudofischeri* also showed roughly 90 to 100% intraspecific DNA complementarity. Interspecific DNA complementarity between species of *Neosartorya* and *Aspergillus* species from section *Fumigati* ranged from ca. 20% to ca. 65% (9). A total of 4 of the 39 *Neosartorya* isolates examined by Peterson (9) were isolated from humans with mycoses (NRRL 182, ex sputum from a lung disease patient, from Sayer, Washington, D.C., to Charles Thom, 1929; NRRL 20748 = CDC B-4812 from A. A. Padhye, 1989, ex L_2 and L_3 human vertebrae with lesions [the present case]; NRRL 20752 = C37 = UCLA B-421 from G. F. Orr to C. R. Benjamin to NRRL, 1961; NRRL 20753 = 652 RTB, a case of aspergillosis, from J. W. Brandsburg, Kansas City, Kans., to D. I. Fennell, NRRL, 1971 [7]). All four of the isolates from humans were identified as strains of *N. pseudofischeri* on the basis of SEM morphology of the ascospores and on the basis of DNA complementarity. Because of these findings, two additional *Neosartorya* strains, *N. fischeri* var. *fischeri*, reported by Coriglione et al. (2) (CDC B-5491 = NRRL 22808), and *N. fischeri* var. *spinosa*, reported by Summerbell et al. (15) (UAMH 7109 = CDC B-5472 = NRRL A-28710), from clinical cases of aspergillosis were obtained for taxonomic reexamination.

The SEM figures of these two latest isolates (Fig. 6 and 7) show the ascospore ornamentation typical of *N. pseudofischeri*. On the basis of this information, the strains are reidentified as *N. pseudofischeri*. The six clinical isolates of *Neosartorya* examined to date have all been strains of the single species *N. pseudofischeri*.

Human pathogenic species *N. pseudofischeri* is thermotolerant and occurs in soil worldwide (4). *Neosartorya* species are most commonly isolated from cellulosic material in contact with soil (8), and their selective isolation is facilitated by soil steaming (4). The present isolate CDC B-4812 grew well up to 50°C, similar to *A. fumigatus* isolates. In spite of their close relationship with *A. fumigatus*, species of *Neosartorya* are rarely pathogenic for humans and lower animals. Media containing cycloheximide often inhibit growth of *Neosartorya* species. On Sabouraud dextrose agar at 25°C, the growth is often devoid of sporulation. When tested by the exoantigen test, B-4812 produced two precipitin bands of identity with the reference system of *A. fumigatus*, one band of identity with *A. flavus* reference system, and two weak precipitin bands of identity with *A. nidulellus*. It showed no reaction against *A. niger* and *A. terreus* reference antigen-antibody reference systems. On the other hand, Summerbell et al. (15), using Fung and Tilton's (5) technique of counterimmunoelectrophoresis and antigens from Diagnostic Pasteur, Marnes-la-Coquette, France, against their *N. pseudofischeri* (*N. fischeri* var. *spinosa*), found the presence of precipitins against antigens of *A. niger*, but not against antigens of *A. flavus*, *A. fumigatus*, *A. nidulellus*, and *A. terreus*. Serologic testing using agar gel diffusion for anti-*Aspergillus* antibodies performed in two cases (3, 14) at selected intervals gave negative results for *Aspergillus* precipitins. In the present case, serum specimens from the patient were not available for serologic studies.

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