

Nucleotide Sequencing for Diagnosis of Sinusal Infection by *Schizophyllum commune*, an Uncommon Pathogenic Fungus

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***Schizophyllum commune*, a basidiomycete fungus, is a rare cause of mycotic disease. We report here a case of sinusitis in a 35-year-old woman that underscores the value of molecular biology for the diagnosis of this fungal infection.**

CASE REPORT

A 35-year-old Caucasian woman presented to the Department of Otorhinolaryngology, Rangueil University Hospital, Toulouse, France. She complained of a predominantly right-sided nasal obstruction, accompanied by thick, right-sided mucopurulent rhinorrhea and headaches. There was no history of asthma or other allergic diseases, medical or surgical treatment, smoking, or drug abuse. No potential risk factor such as diabetes mellitus, immunodeficiency, or previous facial trauma was determined after questioning the patient. Laboratory investigations did not show neutrophil white cells in the nasal mucus, any blood eosinophilia, or any increase of the inflammation markers.

Endoscopic examination of the nasal fossae found a right-sided septal deviation, without any anomaly of the mucosa or any abnormal secretions from the middle meatus. A sinus computed tomographic scan showed an isolated heterogeneous opacity, filling the right ethmoid, with thinning bony walls (Fig. 1). The dental examination showed no infectious sites.

An endonasal endoscopic surgical treatment was undertaken that included both right ethmoidectomy and septoplasty. Gross examination of ethmoidal cells found them filled with thick, viscous, and brown tinted secretions evocative of a fungal infection. The immediate postoperative course was simple, with nasal packing removed on the first postoperative day.

Microscopy examination of the secretions showed the presence of thin, septate hyphae with a width ranging from 1.5 μ m to 3 μ m and without any particular features (Fig. 2). After treatment by Digest'EUR (Eurobio, France), a mucolytic agent, the specimen was inoculated in four tubes containing Sabouraud-chloramphenicol-gentamicin agar medium (Bio-Rad, France). After 6 days at 32°C, growth of a white, cottony mold with a pale brown reverse was observed. This mold produced a strong and disagreeable smell. Microscopic examination of the mycelium showed hyaline, septate, branched hy-

phae without clamp connections, spicules, or fruiting bodies. Subcultures on home-made malt (2% malt extract [Merck, Germany], 1.4% Pastagar [Bio-Rad, France], 0.05% Thio-phenicol [Sanofi-Synthelabo, France], 100 ml of distilled sterile water) and potato-carrot agar (PC) media yielded the same results, apart from the presence of swellings and chlamydo-spores on the malt agar medium. These elements were insufficient for an optical identification of the fungus that was therefore labeled as "sterile mycelium."

In order to reach a diagnosis by molecular biology, a fragment of the surgical specimen was disrupted by using the MagNA Lyser system based on the collision of ceramic beads (Roche Diagnostics, Germany). Genomic DNA was then extracted by using the High-Pure PCR template preparation kit (Roche Diagnostics, Germany). Cycle sequencing was performed on ABI Prism 3100 sequencer (Perkin-Elmer) with universal fungus-specific primers (5'-ATTGGAGGGCAAGTC TGGTG and 5'-CCGATCCCTAGTCGGCATAG) (9) binding to a highly conserved region of the 18S ribosomal DNA of most

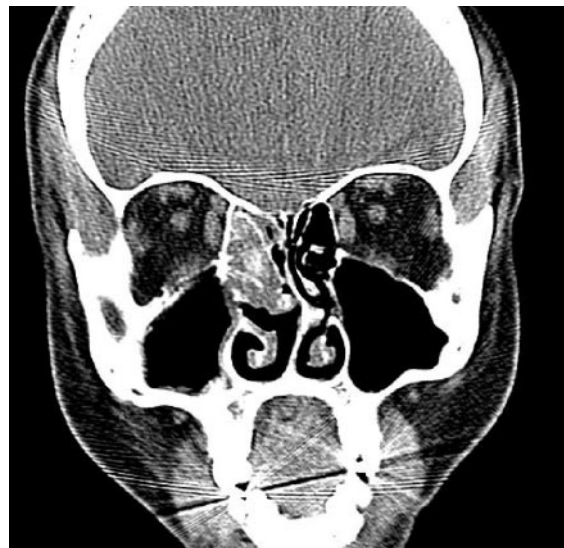


FIG. 1. A coronal computed tomographic scan shows heterogeneous opacity of the right ethmoid sinus.

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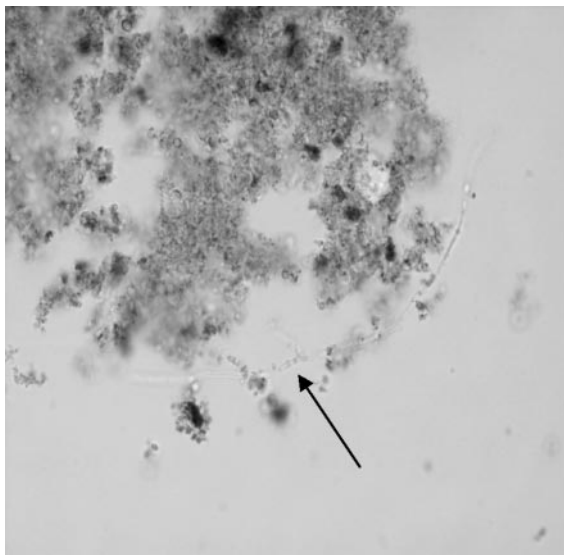


FIG. 2. *S. commune* in a surgical specimen. Septate hyaline hyphae can be seen (arrow). Magnification, $\times 400$.

fungi (filamentous species or yeasts). The amplicon was purified with the High-Pure PCR product purification kit (Roche Diagnostics) according to the manufacturer's instructions. The fungus was identified by using the BLASTn program (www.ncbi.nlm.nih.gov/BLAST) as *Schizophyllum commune* (accession number AF280758). The 504-nucleotide sequence showed 100% homology with the reference sequence. ITS1 and ITS2 sequencing confirmed this result. A review of the literature indicated that this identification was quite consistent with previous clinical and mycological reports (3, 4, 14).

Schizophyllum commune is a worldwide basidiomycete fungus growing on various trees and decaying wood (2) that was reported in only 37 cases of human infection over the last 50 years. The described clinical manifestations included chronic or allergic sinusitis (3, 4, 8, 13, 14), bronchopulmonary involvement (1, 5, 7, 10), fungus ball in the lung (15), ulcerative lesions of the hard palate (12), one atypical meningitis (2), one brain abscess (11), and one suspected onychomycosis (2). Sinusitis is the most reported feature in *S. commune* infection, representing half of the cases. In this case, the route of transmission probably occurs by inhalation of airborne basidiospores. In veterinary medicine, only one case has been reported in dogs, and it consisted of disseminated nodules (6).

Confusion of *S. commune* with *Aspergillus* sp. is possible during direct examination, since the hyphae of these fungi may look similar. The mycological diagnosis of *S. commune* is easy

to make if hyphae bearing spicules or clamp connections are present. Identification is especially difficult in the case of monokaryotic isolates, which, unlike dikaryotic ones, are devoid of clamp connections (15).

The present case report indicated that nucleotide sequencing is very helpful to accurately identify this fungus, especially if mycological features are absent or unknown in the laboratory. However, the use of universal primers targeting the 18S RNA gene or the ITS regions (2) appear to be more relevant than primers specific for *S. commune* since universal primers permit the identification of a wide range of fungi. Because of the ubiquitous repartition of *S. commune* and the limited number of reported cases, we think that the use of such a diagnostic tool would increase the frequency of identification of *S. commune* as an etiological agent of fungal infection.

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