Isolation of the Fungus *Geosmithia argillacea* in Sputum of People with Cystic Fibrosis

R. C. Barton, 1, * A. M. Borman, 4 E. M. Johnson, 4 J. Houbraken, 5 R. P. Hobson, 1 M. Denton, 2 S. P. Conway, 3 K. G. Brownlee, 3 D. Peckham, 3 and T. W. R. Lee 5

Mycology Reference Centre1 and Department of Microbiology; 2 Leeds General Infirmary, Leeds LS1 3EX, United Kingdom; Leeds Regional Cystic Fibrosis Centre, St. James’s University Hospital, Leeds LS9 7LF, United Kingdom; 3 HPA Mycology Reference Laboratory, HPA Southwest, Bristol, United Kingdom; 4 and CBS—Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, Netherlands

Received 29 January 2010/Returned for modification 8 March 2010/Accepted 20 April 2010

We report the repeated isolation of the fungus *Geosmithia argillacea* from sputum samples of people with cystic fibrosis. Identification was based on morphology and DNA sequence analysis. Isolation of *G. argillacea* did not appear to be associated with clinical deterioration. The pathogenic potential of *G. argillacea* is discussed.

People with cystic fibrosis (CF) are at risk of colonization by and, in some cases, subsequent allergic reaction to or infection with a number of fungi. Most notable among these are *Aspergillus* species, *Scedosporium apiospermum*, and *Exophiala dermatitidis* (7). Recently, the fungus *Geosmithia argillacea* has been repeatedly isolated from sputum of several people with CF attending clinics at the Leeds Regional Cystic Fibrosis Centre. This report describes these findings and associated clinical features and discusses their possible clinical significance.

Approximately 500 subjects (150 pediatric and 330 adult) with a confirmed diagnosis of CF attending clinics at the Leeds Teaching Hospitals Trust were examined between 1 January 2005 and 30 June 2007. Sputum culture was carried out following treatment with 0.1% (wt/vol) solution of dithiothreitol inoculated onto Sabouraud’s agar plates with 20 mg/liter colistin and gentamicin (E&O Laboratories) and incubated at 29, 36, and 45°C for 7 days. A relatively slow-growing, pale-brown, powdery mold was isolated from sputum samples of eight CF subjects (Fig. 1a), in many cases from several sputum samples from the same person (Table 1). Growth was seen on plates incubated at all temperatures, in some cases with other fungi (Table 1). Microscopic examination of cultures revealed an extensively sporing, *Penicillium*-like fungus with phialides produced in a terverticillate mode of branching (Fig. 1b). Phialides were cylindrical with tapering apices and produced chains of columnar or “box-shaped” conidia that later became ovoid to globose (Fig. 1c). This fungus also grew on Columbia blood agar at a slightly lower rate than it did on Sabouraud’s agar. Molecular identification was carried out as described previously (1, 2). Briefly, DNA was extracted from fungal cultures and subjected to PCR using primers designed to amplify large subunit (LSU) or internal transcribed spacer 1 (ITS1) regions of the nuclear rRNA gene cassette (1). For certain isolates, regions of the β-tubulin gene were also analyzed. Sequences of the PCR amplicons were used to search the GenEMBL database and a database generated by the HPA Mycology Reference Laboratory, Bristol, United Kingdom. The sequences from all three strains shared very high homologies with *Geosmithia argillacea* sequences in the public databases. A representative isolate of *G. argillacea* from the present study has been stored in the National Collection of Pathogenic Fungi (NCPF) as NCPF 7710. Antifungal susceptibility testing of representative *G. argillacea* isolates was performed according to the CLSI M38-A methodologies (4). *G. argillacea* exhibited low MICs to amphotericin B, itraconazole, posaconazole, and caspofungin but high MICs to voriconazole (16 mg/liter) (Table 2).

The clinical characteristics of CF subjects from whom *G. argillacea* had been isolated were examined for any apparent associated decline in clinical status and to establish any other relevant clinical associations (Table 1). The first recognized isolation of *G. argillacea* in CF sputum culture at this center coincided with results determined by a different unit within the Department of Microbiology carrying out identification of filamentous fungi. It is likely that *G. argillacea* was present prior to the study period but not recognized or reported as such. No association between isolation of *G. argillacea* and either age or lung function was apparent. There was no subjective evidence of clinical decline in these subjects that could be attributed to *G. argillacea* upon detailed review of the case notes. No obvious epidemiological links could be identified between people with CF with positive sputum culture for *G. argillacea*. The subjects were distributed widely throughout the catchment area for the CF clinics involved; they included both pediatric and adult patients with CF, and as such, they would have been seen at different clinics and by different clinicians; and there was no evidence that acquisition of *G. argillacea* correlated with bronchoscopy. A full analysis of risk factors will be required in order to determine why we were able to grow *G. argillacea* from samples from some people with CF and not from those of others.

---

* Corresponding author. Mailing address: Mycology Reference Centre, Department of Microbiology, Leeds General Infirmary, Leeds LS1 3EX, United Kingdom. Phone: 44 113 3923390. Fax: 44 113 3435649. E-mail: Richard.Barton@leedsth.nhs.uk.

† Published ahead of print on 26 April 2010.
Previously, *G. argillacea*, which is related to *Penicillium*, has been described as a causal agent of food spoilage, where its heat resistance was also noted (10). We assume that the subjects in the current report acquired *G. argillacea* through inhalation of airborne spores.

![FIG. 1. Morphology of *G. argillacea*. (a) Photograph of plate of *G. argillacea* growing on Sabouraud dextrose agar after 7 days at 37°C. (b) Microscopic appearance of the conidiophore and conidia of *G. argillacea*. Bar = 5 μm. (c) Microscopic appearance of conidia of *G. argillacea*.](image-url)
TABLE 2. Antifungal susceptibility of Geosmithia argillacea isolates to amphotericin B, itraconazole, voriconazole, posaconazole, and caspofungin obtained using the NCCLS/CLSI broth microdilution method.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>MIC (mg/liter) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amphotericin</td>
</tr>
<tr>
<td>NCPF 2801</td>
<td>Sputum, CF patient, UK, 1991</td>
<td>1</td>
</tr>
<tr>
<td>NCPF 7594</td>
<td>Isolated from blood culture, patient with peritonitis, UK, 2002</td>
<td>2</td>
</tr>
<tr>
<td>NCPF 7596</td>
<td>Isolated from peritoneal dialysis fluid of same patient as NCPF 7594</td>
<td>2</td>
</tr>
<tr>
<td>CF isolate 1</td>
<td>Leeds Teaching Hospitals Trust, CF subject 4</td>
<td>0.5</td>
</tr>
<tr>
<td>CF isolate 2</td>
<td>Leeds Teaching Hospitals Trust, CF subject 3</td>
<td>2</td>
</tr>
</tbody>
</table>

* UK, United Kingdom; ND, not determined.

It is possible that Geosmithia argillacea may be isolated more commonly than has been realized up to now and overlooked given its morphological similarities to Penicillium and Paecilomyces spp. A molecular characterization of fungal isolates stored in the NCPF that might represent misidentified Geosmithia argillacea isolates supports this contention. Isolate NCPF 2801, from sputum of a CF patient from the United Kingdom in 1991, was indistinguishable genetically from Geosmithia argillacea (data not shown), and Geosmithia argillacea was recently isolated from a bronchoalveolar lavage specimen from a 51-year-old patient in Leeds without CF being treated for a persistent cough following smoking inhalation. Furthermore, two more isolates, NCPF 7594 and 7596, isolated from blood culture and peritoneal dialysis fluid from a patient from the United Kingdom with peritonitis in 2001 were also revealed to be Geosmithia argillacea by β-tubulin gene sequencing (data not shown).

While it appears that colonization of people with CF by Geosmithia argillacea does not present a significant clinical problem, a concern remains where people with CF become immunocompromised, for example, if they undergo lung transplantation. Aspergillus fumigatus and Scedosporium apiospermum have been documented to cause invasive disease that is frequently fatal in this clinical setting (8, 9). It is possible that Geosmithia argillacea might cause systemic disease when colonized patients become immunocompromised following lung transplantation. It is pleasing to note, however, that subsequent to the study period, two of the CF patients have undergone heart lung transplantation at another center, and to date, there was no evidence of systemic fungal infection by Geosmithia argillacea posttransplantation in either patient.

Nucleotide sequence accession numbers. Sequences from the Geosmithia argillacea isolates described here have been deposited into GenBank under accession numbers AM744972 to AM744975.

REFERENCES