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Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis – a review

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The colonization of airways by filamentous fungi and the development of respiratory infections require some predisposing factors as encountered in patients with cystic fibrosis (CF). Indeed, the defective mucociliary clearance which characterizes the disease is associated with local immunological disorders. In addition, the prolonged therapy with antibiotics and the use of corticosteroid treatments also facilitate fungal growth. An important fungal biota has been described in respiratory secretions of patients suffering from CF. *Aspergillus fumigatus*, *Scedosporium apiospermum* and *Aspergillus terreus* for filamentous fungi and *Candida albicans* for yeasts are the main fungal species associated with CF. Although less common, several fungal species including *Aspergillus flavus* and *Aspergillus nidulans* may be isolated transiently from CF respiratory secretions, while others such as *Exophiala dermatitidis* and *Scedosporium prolificans* may chronically colonize the airways. Moreover, some of them like *Penicillium emersonii* and *Acrophialophora fusispora* are encountered in humans almost exclusively in the context of CF. As fungal complications in CF patients are essentially caused by filamentous fungi the present review will not include works related to yeasts. In CF patients, fungi may sometimes be responsible for deterioration of lung function, as occurs in allergic broncho-pulmonary aspergillosis (ABPA) which is the most common fungal disease in this context. Additionally, although the clinical relevance of the fungal airway colonization is still a matter of debate, filamentous fungi may contribute to the local inflammatory response, and therefore to the progressive deterioration of the lung function.

Keywords Cystic fibrosis, airway colonization, respiratory infections, filamentous fungi, *Aspergillus fumigatus, Scedosporium apiospermum*

Introduction

Cystic fibrosis (CF) is the major genetic disease in the European Caucasian population and, by the number of patients (about 6,500), the third orphan disease in

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frequency in France [1]. It results from mutations in the gene *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator), which is located on chromosome 7 and encodes a chloride channel involved in electrolytic exchanges through the plasma membrane of numerous epithelial cell types. While several organs are involved, morbidity and mortality in CF patients is mainly dependent on the severity of lesions of the respiratory tract [2]. Mutations in *CFTR* gene result in a defective mucociliary clearance and, as a consequence, production of thick and sticky bronchial mucus facilitates the entrapment of the airborne bacteria and fungal spores and provides a suitable environment for the growth of these microorganisms.

Bacteria, particularly *Staphylococcus aureus*, *Pseudomonas aeruginosa, Burkholderia cepacia* and *Stenotrophomonas maltophilia*, are typical CF pathogens that cause recurrent exacerbations of the pulmonary disease and often determine the vital prognosis of patients [3]. However, the respiratory tract of the patients may also be colonized by filamentous fungi. Most of the epidemiological studies that have been conducted in CF populations deal with *Aspergillus fumigatus*, but the fungal biota is obviously more complex. Among clinically significant fungi, *Aspergillus* spp*., Scedosporium apiospermum* and *Exophiala dermatitidis* are the most frequent molds recovered from respiratory secretions of CF patients, but their isolation rate greatly varies according to the studies [4,5]. Moreover, Nagano *et al*. [4] reported an increasing prevalence of fungi, from 6% in 1995 to 13% in 2005, being isolated from sputum samples of CF patients in the USA. In addition, over the past decade, some cases of infection or chronic colonization have been associated with species that have only rarely or never been previously reported in human cases, such as *Penicillium emersonii* and *Acrophialophora fusispora*. Such changes in the epidemiology of fungal species involved in CF are probably related to the improvement in global disease management, which has led to a marked increase in life expectancy of the patients [1]. Nevertheless, they may also result from the improvement of the procedures used for examination of sputum samples in clinical laboratories. In this review, the various filamentous fungi associated with CF will be presented, with a particular emphasis on *Scedosporium* species.

Aspergillus spp.

Aspergillus fumigatus

Aspergillus fumigatus is by far the major agent involved in the colonization of the airways of CF patients. As illustrated in Table 1, mycological examination of respiratory secretions from CF patients in previous studies revealed a prevalence rate ranging from 16 to 56.7% [6–21].

Interestingly, colonization of the airways by *A. fumigatus* is uncommon in young children and usually follows bacterial respiratory infections. In an epidemiological study conducted in collaboration between our two CF centers (Angers and Giens), mean age of the patients at date of first isolation of *A. fumigatus* and *Scedosporium apiospermum* from respiratory secretions was about 12.3 and 14.1 years, respectively. In contrast, the first isolation occurred at 5.4 and 8.1 years for *S. aureus* and *P. aeruginosa*, respectively [15]. Bronchopulmonary epithelial tissue damage caused by the

Table 1 Prevalence rates for *Aspergillus fumigatus* and ABPA in cystic fibrosis

References	Country (total no. of patients)	*A. fumigatus* positive patients (%)	ABPA positive patients (%)
Nelson *et al*. [6]	USA (46)	21/37 (56.7)	5 (10.8)
Laufer *et al*. [7]	USA (100)	5/55 (9)	10 (10)
Bauernfeind *et al*. [8]	Germany (102)	6 (5.9)	Not specified
Simmonds *et al*. [9]	UK (137)	Not specified	8 (5.8)
Marchant *et al*. [10]	UK (160)	Not specified	11 (6.8)
Mroueh and Spock [11]	USA (236)	60 (25.4)	15 (6.4)
Becker *et al*. [12]	USA (49)	8 (16)	1 (1.9)
Milla *et al*. [13]	USA (212)	45 (21.2)	Not specified
Hutcheson *et al*. [14]	USA (118)	Not specified	6 (5.1)
Cimon *et al*. [15]	France (210)	45 (21.4)	2 (0.95)
Cimon *et al*. [16]	France (128)	59 (46.1)	5 (3.9)
Skov *et al*. [17]	Denmark (238)	61 (25.6)	26 (10.9)
Bakare *et al*. [18]	Germany (94)	43 (45.7)	Not specified
Taccetti *et al*. [19]	Italy (3089)	Not specified	191 (6.18)
Skov *et al*. [20]	Australia (270)	22 in 1998 (7.4) 52 in 2002 (18.8)	13 (4.7)
Valenza *et al*. [21]	Germany (60)	35 (58.3)	Not specified

bacterial proteases or the leukocyte elastase released during the inflammatory reaction were therefore suggested to be required for adherence of the airborne fungal spores to the host tissues. The resulting exposition of the subepithelial extracellular matrix proteins laminin and fibronectin or the fibrinogen/fibrin deposits formed at the surface of wounded epithelia would mediate the adherence process [22]. In addition to laminin and fibronectin, receptors have been identified at the surface of *A. fumigatus* conidia [23,24]. Furthermore, several studies showed that filamentous fungi may also be responsible for direct damage of the respiratory mucosa that is similar to those produced by bacteria [15]. Indeed, *A. fumigatus* produces some secondary metabolites and proteolytic enzymes that are able to impair the mucociliary clearance or to inhibit phagocytosis. For example, it has been shown that *A. fumigatus* alkaline protease may cause the detachment of A549 cells, a cell line with characteristics of pneumocytes type II [25].

In the context of CF, *A. fumigatus* may be responsible for various diseases including asthma, bronchitis and aspergilloma. Moreover, this fungus may also cause invasive pulmonary infections after lung transplantation [26,27]. However, the main clinical form caused by *A. fumigatus* in patients with CF remains allergic broncho-pulmonary aspergillosis (ABPA) [28], for which the prevalence rate varies from 0.95 to 10.9% depending on the studies (Table 1). These variations are due in large part to differences in the definition of ABPA since some of the criteria initially proposed for its diagnosis were not always considered in these studies. Therefore, it may be useful to consider revisions of the definitions which have remained unchanged since an international conference held in Bethesda, USA, in June 2001 [29]. The established diagnosis included acute or subacute clinical deterioration not attributable to another etiology, associated with (i) an elevated total serum IgE titer, (ii) an immediate cutaneous reactivity to *A. fumigatus* or the presence of specific serum IgE, and (iii) the presence of specific serum IgG, or recent signs on chest radiography or tomodensitometry of infiltrates or bronchiectasis that are not cured by antibiotics.

Additionally, recent genetic or immunological studies of the host provided new insights into the pathogenesis of ABPA. It has been demonstrated that some HLA-DR molecules. i.e., DR2, DR5, and possibly DR4 or DR7 contribute to susceptibility, while HLA-DQ2 contributes to resistance, and that a combination of these genetic events determines the outcome of ABPA in patients with CF [30]. Moreover, the presence of single-strand polymorphisms in the interleukin 4 receptor α chain, in particular the substitution I75V, appears to be a genetic risk for the development of ABPA [31]. Likewise, it has been suggested that polymorphisms in the promoter region of the interleukin 10 gene may influence the CF host response to *A. fumigatus* [32]. In this study of a large number of CF patients, a significant relationship was seen between the GG genotype at position –1082 in the promoter region of this gene and both ABPA and colonization of the airways by *A. fumigatus*.

But genotype studies of the isolates collected from patients with CF have also provided interesting information about the colonization of the airways. Indeed, genotype study of multiple and sequential isolates recovered from patients recently colonized by *A. fumigatus*, performed by sequence-specific DNA primer and by random amplification of polymorphic DNA (RAPD) using the primers NS3 and NS7, revealed a huge diversity of genotypes even in the same sputum sample [33]. However, as colonization continues, a genotype common to the chronically colonized patients tended to become dominant. Moreover, this genotype was associated with the presence of anti-catalase antibodies which have been suggested as markers of an altered lung function [34]. Interestingly, similar observations were reported by Neuvéglise *et al*. [35] using restriction fragment length polymorphism (RFLP) followed by hybridization with the repetitive probe *Afut1*, a retrotransposon-like element from *A. fumigatus*. A great variety of genotypes were detected in sputum samples collected at the beginning of the colonization of the airways, whereas a dominant genotype was observed in later samples. More recently, 256 isolates recovered from 89 respiratory samples, obtained from eight CF patients from Belgium and France, were genotyped using a highly discriminant microsatellite-based typing method [36]. Although different genotypes were identified in 88.3% of the patients, recurrent genotypes for seven out of the eight CF patients were isolated on several occasions more than five months apart. These results indicate that some genotypes are capable of chronically colonizing the airways. Thus, it seems that some particular genotypes are selected with prolonged colonization, but the mechanisms of this genotype selection have not been investigated.

Other *Aspergillus* species

Other *Aspergillus* species may be encountered in the context of CF. For instance, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* are commonly reported, but are usually found transiently. Conversely,

Aspergillus terreus is usually responsible for the chronic colonization of the airways of CF patients. In our experience, this fungus ranked third among filamentous fungi colonizing the airways of CF patients, with a prevalence rate ranging from 1.9 to 6.2% [15,16]. Moreover, *A. terreus* was responsible in a few cases for clinical signs, including ABPA and IA which are similar to those reported for *A. fumigatus.*

Little is known about the epidemiology of this fungus. *Aspergillus terreus* is seldom reported from environmental sources, contrasting with its occurrence in CF. Therefore, additional studies should be conducted to improve our knowledge on the habitat of the fungus and on the influence of human activities which might, in turn, contribute to prophylactic measures. In the framework of an epidemiological study performed at the CF clinic of the Hospital Renée Sabran (Giens, France), potential sources of fungal contamination were investigated by analysis of air and surface samples from patient rooms, and soil samples from the park adjacent to the hospital. *Aspergillus terreus* was detected exclusively from soil samples [37]. Moreover, environmental isolates were typed by random amplification of polymorphic DNA (RAPD), using the two-primer set NS3 and NS7 according to Symoens *et al.* [38], and compared to clinical isolates from CF patients followed in this hospital during the study period [37]. As expected, the three patients studied were colonized by a unique genotype, different from one patient to another, that persisted during the study period. The clonality of the colonization pattern suggests real colonization of CF patients by *A. terreus,* rather than a transient occurrence of airborne spores entrapped in the viscous mucus. However, no genotype was found to be common to clinical and environmental isolates. Considering the usual chronicity of the colonization of the airways by this fungus, a hospital-acquired infection seems to be unlikely and additional environmental studies should be conducted, particularly at the homes of the patients.

Scedosporium apiospermum

Usually a saprophyte, *S. apiospermum* (formerly proposed as the anamorph state of the Ascomycete *Pseudallescheria boydii)*, may cause various mycoses as a result of traumatic or iatrogenic inoculation of its telluric conidia (for a review see reference 39). Respiratory diseases, likely due to the inhalation of airborne conidia, may also occur in receptive hosts. The diseases associated with this fungus are sinusitis, lung fungus-ball and necrotizing pneumonia, as well as disseminated infections in immunocompromised patients [39]. However, all these clinical pictures are rare, contrasting with the relatively high occurrence of the fungus in CF patients.

Cystic fibrosis was first described as a risk factor for airway colonization by *S. apiospermum*, following the concomitant isolation of this fungus from three patients after the eradication of *A. fumigatus* by itraconazole treatment [40]. This risk factor was further demonstrated in an epidemiological survey, conducted in collaboration between Angers and Giens hospitals (France). First, 210 patients with CF were investigated by means of mycological examination of a single sputum sample and by serological analysis [15]. Then, a longitudinal study was performed on 128 patients who were followed for five years [41]. Mycological results from the two studies were compared to clinical data, and to other biological data (including results from bacteriological analysis of sputum samples, blood eosinophil count, total IgE level and presence of specific anti-*A. fumigatus* IgE). In both studies, sputum analyses were conducted using the same procedures. The latter included the inoculation of a defined volume of the sample on two yeast extract-peptone-dextrose-agar (YPDA) plates, one of which was supplemented with cycloheximide. Plates were incubated at 37°C, and cultures were examined daily for seven days, even if some rapidly growing fungi were recovered. As shown in Table 2, *S. apiospermum* ranked the second most common filamentous fungus associated with CF, with a prevalence rate of 8.6% in our longitudinal survey [41]. This high occurrence in CF cases was later confirmed in two distinct surveys performed in Australia and Austria, in which prevalence rates of 10% [42] and 6.5% [43], respectively were reported. The recovery of *S. apiospermum* from respiratory secretions was found to usually follow the colonization of the airways by *A. fumigatus. Scedosporium apiospermum* was found with *A. fumigatus* in ten out of the eleven positive patients, and emerged subsequently, after an average delay of 14 months in nine patients. Considering the frequency of this association, it is obvious that the use of a semi-selective culture medium

Table 2 The main filamentous fungi responsible for colonization of the airways in our experience

Fungal species	Prevalence rate (%)	
	Transversal study [15] (210 patients)	Longitudinal study [41] (128 patients)
Aspergillus fumigatus	21.4	46.1
Scedosporium apiospermum	3.3	8.6
Aspergillus terreus	1.9	6.2

such as YPDA+cycloheximide greatly facilitates the detection of *S. apiospermum*. However, this procedure might be improved, for example by the use of other semi-selective culture media such as dichloran-rose bengal-chloramphenicol agar supplemented with benomyl. Likewise, another semi-selective culture medium, called SceSel+, has been described recently [44]. It has been shown to facilitate the isolation of *S. apiospermum* from environmental sources, and its potential value in mycological analyses of sputum specimens should be evaluated.

Several questions are raised by the relatively high occurrence of *S. apiospermum* in CF patients. First, what is the origin of the fungus recovered from the patients. Indeed, *S. apiospermum* has been described mainly in highly polluted soils and water [45]. Several studies showed the capacity of this fungus to use various aromatic compounds as a carbon source [46,47]. However, apart from these substrates, *S. apiospermum* has rarely been encountered in the environment. When Beguin and Nolard [48] investigated the air and surfaces in 130 dwellings in Brussels (Belgium) over a 10-year period, 52 fungal genera were recovered and among them, the genus *Scedosporium* ranked the 49th most common. Additionally, the fungus was not found on surfaces, and it was recovered from the air only once, representing only 1 colony forming unit (CFU) out of a total number of about 20,000 CFUs [48]. An environmental study was therefore performed at the homes of six CF patients colonized by *S. apiospermum*, and followed in Angers University Hospital [49]. Air and surfaces were sampled in the patient's bedroom, the bathroom and living room. In addition, soil from the garden, as well as from all potted plants present at home of the patients, were sampled. Thus, a total number of 164 samples were analyzed. Among the air and surface samples, only one from a radiator in the bedroom of one patient revealed the presence of *S. apiospermum*. In contrast, out of 55 potted plants that were sampled, *S. apiospermum* was isolated from 36 of these samples. The fungus was recovered at homes of each patient, and almost all positive samples were highly contaminated. These results, which are in agreement with those from Summerbell *et al*. [50] who reported several years ago the presence of the fungus in the soil of potted plants, clearly indicate that potted plants constitute a risk factor of airway colonization by *S. apiospermum*. Therefore, the presence of potted plants at home of CF patients should be avoided.

In addition, the discrepancy between the relatively high occurrence of *S. apiospermum* in CF and its paucity in the environment raises questions about the mechanisms by which it is acquired by patients. Obviously, this fungus expresses specific virulence factors which distinguish it from other more common airborne moulds. However, we have only limited knowledge about the pathogenic mechanisms of *S. apiospermum*. Regarding the adherence step, it was demonstrated that conidia of *S. apiospermum* attached to, and were internalized by HEp 2 cells through a lectin-mediated process involving a peptido-rhamnomannan of the fungal wall [51]. Likewise, several extracellular peptidases have been identified in *S. apiospermum*, including a 28-kDa pepdidase which is fully inhibited by 1,10-phenanthroline (a potent zinc-metallopeptidase inhibitor). The pepdidase may cleave different protein substrates, such as extracellular matrix proteins and disialylated proteins [52,53]. Similarly, the release of siderophores, which are essential for iron uptake and therefore for growth of all microorganisms, has been suggested by the growth of the fungus in culture on casamino acids agar [54]. However, none of these compounds has been characterized as yet, and only two putative virulence factors have been currently purified and fully characterized in *S. apiospermum*. These factors include an extracellular proteinase belonging to the subtilisin subfamily of serine-proteinase, and very close to the alkaline proteinase produced by *A. fumigatus* [55], and a copper-zinc cytosolic superoxide dismutase [56].

One of the most important questions for physicians involved in the follow-up of CF patients remains the clinical significance of the isolation of this fungus from respiratory secretions. As demonstrated in a genotype study performed on multiple and sequential isolates recovered from long-term colonized patients, *S. apiospermum* is usually responsible for a chronic colonization of the airways. Each patient was colonized by a unique genotype different from one patient to another, and conserved over time despite antifungal treatments [57]. Usually, this chronic colonization of the airways is not associated with clinical signs. However, Cimon *et al.* [41] showed that *S. apiospermum* may cause allergic broncho-pulmonary mycoses similar to the well known ABPA. Indeed, an allergic broncho-pulmonary mycosis was suspected for 6 out of the 128 patients followed up in our longitudinal study, and one of them presented episodes of bronchospasm associated with high counts of blood eosinophils and with high titers of total serum IgE. Specific anti-*A. fumigatus* IgE were not detected, and mycological examination of successive sputum samples revealed exclusive growth of *S. apiospermum*, suggesting its involvement in the clinical pattern. Moreover, *S. apiospermum* may be also responsible for cerebral or disseminated mycoses in

severely immunocompromised patients, such as lung transplant recipients [39].

Additionally, even in the absence of true respiratory infection, we can't disregard the chronic colonization of the airways by this fungus. Indeed, *S. apiospermum* undoubtedly contributes to the inflammatory reaction and the progressive deterioration of the lung function by secreting proteases and releasing polysaccharide and protein antigens. In addition, since the fungus is able to cause disseminated human infections and responds poorly to antifungal treatment, it would be advisable to critically evaluate in CF patients, especially those that have undergone lung transplantation [58,59]. This is highlighted by the clinical case recently reported by Symoens *et al*. [60], which occurred in 1999 in a 26-year old female CF patient followed in Brussels. In April of that year, the individual underwent a double lung transplant due to the major alteration of the lung function of the patient. The lungs had been colonized for several years by *S. apiospermum*. She was prophylactically treated with antibacterial and antifungal agents, the latter consisting of oral itraconazole associated with aerosols of amphotericin B. However, four weeks later, the patient presented a bilateral chorioretinitis and subcutaneous nodules. Direct examination of the clinical samples (vitreal fluid and biopsies of the subcutaneous lesions) revealed the presence of septate hyphae, and cultures yielded numerous colonies of *S. apiospermum*. An antifungal treatment was therefore initiated which consisted of daily doses of voriconazole, associated with intra-ocular injections of miconazole for two months which lead to a cure of the lesions. Miconazole was therefore stopped, but voriconazole was maintained for an additional four months. However, two days after total cessation of therapy, the patient developed a subacute meningitis and *S. apiospermum* was recovered from the cerebro-spinal fluid. Voriconazole therapy was started again, but the patient died three weeks later. Unfortunately, isolates from the cerebro-spinal fluid were not preserved. Available isolates were analysed by RAPD using the three-primer set described by Zouhair *et al*. [61]. Genotyping revealed that the isolates from vitreal fluid were identical to those from sputum samples collected a few days after transplantation, as well as to isolates obtained from two sputum samples collected 18 months and the day before the transplantation, respectively.

Furthermore, probably as a result of local immunological disorders which characterize CF, dissemination of the fungus from the respiratory tract may also occur in those with severe immunodepression. For instance, a multifocal spondylitis was reported recently in a 28-year-old male CF patient. This patient was colonized by *S. apiospermum* since 1993 and presented with diabetes (a common complication of CF) diagnosed in 2003 as the sole risk factor for invasive fungal infection [62]. In 2002, a left pleurisy was diagnosed, which was attributed to the fungus because of its isolation from sputum, and an antifungal therapy was started. However, in December 2004, the patient who was still on voriconazole treatment, began to complain of inflammatory back pain. Four months later, a spondylitis was diagnosed by X-ray of the back and discal magnetic resonance imaging. Three vertebral biopsies were carried out, but bacteriological and mycological examination was negative. An empiric antibiotic treatment was therefore started, but it was stopped one month later because of the progression of the disease. A surgical biopsy was then carried out, which permitted the recovery of *S. apiospermum*. Additionally, serum samples from the patient were analyzed retrospectively by counter-immunoelectrophoresis (CIE) which revealed 9–11 precipitin lines using a somatic extract (personal data).

Finally, recent studies performed by the group of Josep Guarro in Reus, Spain, have demonstrated that *P. boydii* is a complex of species. Sixty clinical or environmental isolates from different countries, including 16 isolates from CF patients from France, were studied by Gilgado *et al*. [63]. On the basis of morphological and sequencing data (partial sequence of beta-tubulin and calmodulin genes, and sequence of the internal transcribed spacer regions), five clades were identified. The study resulted in the proposal of two of the clades as new species, i.e., *Pseudallescheria minutispora* (exclusively for environmental samples) and *Scedosporium aurantiacum*. More recently, on the basis of physiological tests such as resistance to cycloheximide and assimilation of various carbohydrate sources, the three other clades were proposed as new species, namely *Scedosporium dehoogii*, *Scedosporium apiospermum sensu stricto*, and *Scedosporium boydii* which corresponds to the anamorph of *P. boydii* [64,65]. Interestingly, most of the isolates from CF patients were identified as *S. apiospermum sensu stricto* or as the new combination *S. boydii*, but two isolates from French patients were found to be *S. aurantiacum*. Further studies are therefore needed to define the respective prevalence of these new species in CF, and their clinical significance.

Other filamentous fungi

The respiratory tract of patients with CF may also be colonized transiently by other filamentous fungi such as *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp.

or *Paecilomyces variotii* [4,15]. *Scedosporium prolificans* (formerly *S. inflatum*) and *Exophiala dermatitidis* (formerly *Wangiella dermatitidis*) have also been reported in the context of CF (Fig. 1), sometimes with a high frequency, but strikingly these species seem to be restricted to some geographic areas. Additionally, two thermophilic species, *Penicillium emersonii* (the anamorph state of *Talaromyces emersonii*) and *Acrophialophora fusispora*, have been isolated in human, almost exclusively in the context of CF.

Scedosporium prolificans

Conversely to *S. apiospermum*, *S. prolificans* is known principally as a causative agent of disseminated infections in patients with hematological malignancies [66]. Only a few reports, mainly from Australia, Spain and the USA, deal with its isolation from respiratory secretions of CF patients [42,67–71].

Moreover, its pathogenic role in this context remains questionable, since only one case of endophtalmitis after lung transplantation has been reported today in CF [72].

Exophiala dermatitidis

Although distributed worldwide, *E. dermatitidis* which grows as a black yeast at 37°C, and as a filamentous fungus at room temperature, has been described in the context of CF mainly from German patients [73–77]. Its prevalence rate in Germany and Belgium, which varies from 4.8–15.7% [73–78], contrasts with its very low occurrence in other countries like France or North America. As it has been suggested for the colonization of the airways by *P. aeruginosa* [79], genetic factors may account for this discrepancy. However, it may also result from differences in the way of life between the two countries, for example the use of sauna facilities since this fungus can survive for many months in hot and moist conditions [80]. Nevertheless, this discrepancy may also reflect the lack of standardization of mycological examination of respiratory secretions from CF patients, since detection of this fungus may be difficult with routine isolation procedures. A prolonged incubation time (up to 4 weeks) seems to be needed for isolation of this usually slow growing fungus [73–75]. Moreover, due to its frequent association with yeasts and other filamentous fungi as illustrated in Fig. 1, as well as with bacteria such as *P. aeruginosa*, the use of a selective medium such as erythritol-chloramphenicol agar (ECA) is highly recommended for its isolation [75]. Besides, *P. aeruginosa* which produces toxic compounds, such as pyocyanin and 1-hydroxyphenazine capable of inhibiting the fungal growth and therefore to hamper the recovery of filamentous fungi [81,82], was not detected on ECA [75].

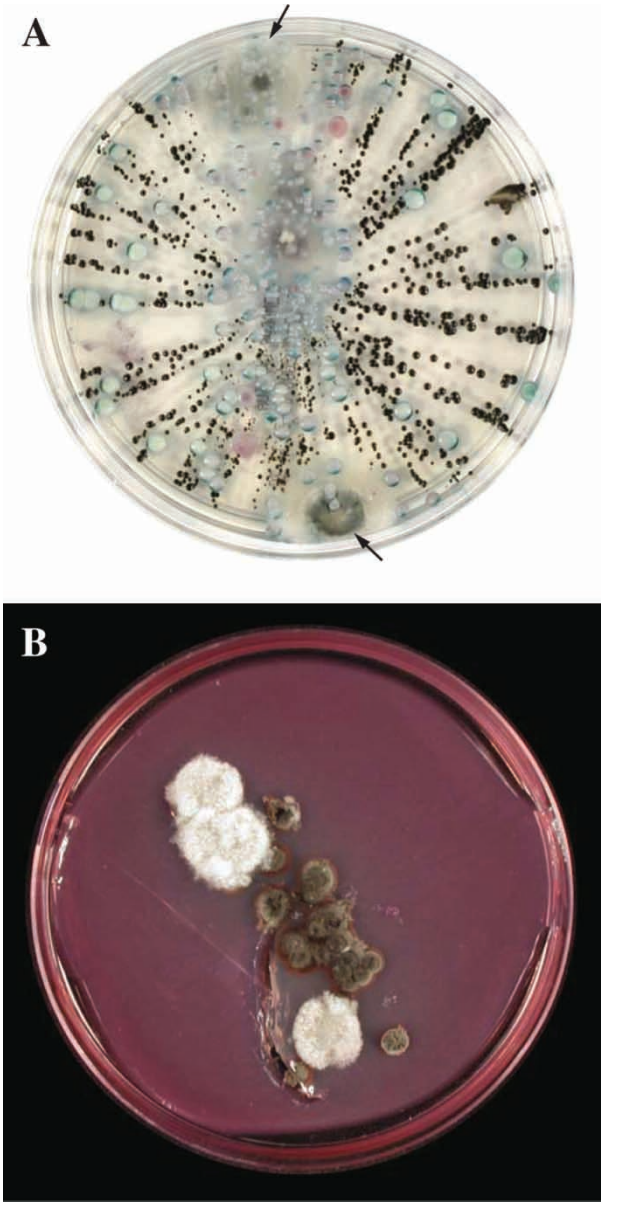

Fig. 1 Two examples of mixed cultures from sputum samples of CF patients incubated at 37°C, showing the simultaneous growth of *Candida albicans* (green colonies), *Candida glabrata* (purple colonies), *Aspergillus fumigatus* (arrows) and *Exophiala dermatitidis* (black colonies) on CHROMagar Candida from Becton-Dickinson (A) and of *Scedosporium apiospermum* (pale grey velvety colonies) and *Scedosporium prolificans* (dark brown colonies) on in-house-prepared dichloran-rose bengal-chloramphenicol agar supplemented with benomyl (B). This figure is reproduced in colour in *Medical Mycology* online.

In the context of CF, *E. dermatitidis* may be responsible for a transient or chronic colonization of the airways, but usually without any clinical signs.

However, this fungus may also cause true respiratory infections, or invasive fungal infections [73,76]. Additionally, *E. dermatitidis*, as well as species of the genus *Aspergillus* and *Scedosporium*, isolated from respiratory secretions of patients with bronchiectasis, should be considered as a biomarker of a *CFTR*-related disease. For example, the regular isolation of *E. dermatitidis* from sputum samples of a 60-year-old female patient, who presented since several years bronchiectasis with recurrent episodes of bronchial infections, led us to search for an atypical form of CF. This was confirmed by sequencing of the gene *CFTR*, which revealed minor point mutations for both alleles. Moreover, CIE performed with a somatic antigenic extract of the fungus showed a serological response, with several precipitin lines (Bouchara *et al*., unpublished data).

Penicillium emersonii

Apart from *Penicillium marneffei*, species of the genus *Penicillium* are usually considered as contaminating moulds. However, some like *Penicillium emersonii* may chronically colonize the airways of CF patients. This thermophilic telluric fungus, which is characterized by its biverticillate asymmetrical penicilli and its cylindrical to ellipsoidal conidia, was first reported associated with humans in 1999. A case involving this fungus in a CF patient has been reported since this date [83]. *P. emersonii* was isolated repeatedly for nearly 5 years, suggesting that it was continuously present in the airways during this period. In addition, a progressive sensitization of the patient to the fungus was revealed by Western-blotting. However, since colonization does not appear to contribute to the development of disease, it seems difficult to assign a pathogenic role to this fungus.

Acrophialophora fusispora

Acrophialophora fusispora is also a thermotolerant soil fungus which is rarely encountered in clinical samples. Only eight cases have been reported to date involving three cases of keratitis [84,85], three pulmonary infections [86] in non-immunocompromised adults and one brain abscess in a child with acute lymphoblastic leukemia [87]. In CF, a unique case was described, corresponding to a transient colonization in a Spanish patient aged 11 [84]. However, during the last few years, the fungus was detected in a 26-year-old patient followed in the South of France, as well as in three patients investigated at Necker University Hospital in Paris [88]. In these observations, the detection of the fungus in respiratory secretions always followed the colonization of the airways by *A. fumigatus*. Two of the patients were chronically colonized by *A. fusispora*. However, for all patients, the clinical status remained unchanged, and particularly no clinical signs of bronchopulmonary infections were observed during colonization by this fungus.

The prevalence of *A. fusispora* in CF is therefore very low, but it may be underestimated due to the currently poor knowledge of this fungus in most of laboratories of medical mycology. For instance, misidentifications have been reported with other fungi, such as *Scopulariopsis chartarum* [89] or *Paecilomyces* species [87], but also with *S. prolificans* [90,91]. However, the morphological features of *A. fusispora* are different since it produces on YPDA plates buff colonies of 5–6 cm in diameter at day 7. Microscopically, the fungus presents basally inflated phialides with an elongated neck, arising mostly singly on pale brown vegetative hyphae. These conidiogenous cells produce long chains of pale brown limoniform or fusiform single-celled conidia with ornamentations arranged in spiral bands which may be visualized by interferential phase-contrast microscopy.

Conclusion

As illustrated in Fig. 2, a large variety of filamentous fungi may be encountered in CF patients. However, apart from *A. fumigatus*, the real prevalence of the different species associated with CF is certainly underestimated and their pathogenic role needs to be defined.

Large multicenter studies are therefore needed in order to determine the prevalence rates of the different filamentous fungi which may colonize the respiratory tract of these patients, the possible variations in their geographic distribution and their clinical significance.

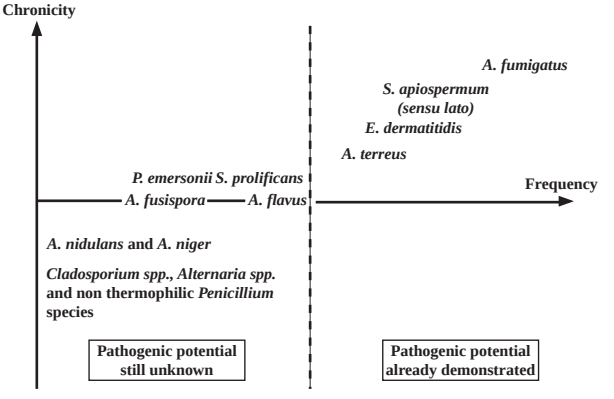

Fig. 2 Filamentous fungi associated with CF according to their frequency in this clinical context and to their capacity to chronically colonize the airways.

This implies the improvement of the biological diagnosis. Particularly, guidelines for mycological examination of sputum samples from patients with CF should be defined, including the number and type of culture media to be used, as well as the temperature and duration of incubation. Alternatively, molecular tools allowing both detection and direct identification of these fungi should be developed. Finally, environmental studies designed to identify the reservoirs of these fungi, and thus the origin of contamination of the patients, should be promoted. To reach these objectives, a working group on Fungal respiratory infections in cystic fibrosis was approved by the ISHAM council on October 2006.

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References

1 Bellis G, Cazes MH, Parant A, *et al*. Cystic fibrosis mortality trends in France. *J Cyst Fibros* 2007; **6**: 179–186.
2 Koch C, Høiby N. Diagnosis and treatment of cystic fibrosis. *Respiration* 2000; **67**: 239–247.
3 Mariani-Kurkdjian P, Bingen E. Pathogenic bacteria in cystic fibrosis. *Arch Pediatr* 2003; **10** (Suppl. 2): 342s–346s.
4 Nagano Y, Millar BC, Johnson E, *et al*. Fungal infections in patients with cystic fibrosis. *Rev Med Microbiol* 2007; **18**: 11–16.
5 Burns JL, Emerson J, Stapp JR, *et al*. Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998; **27**: 158–163.
6 Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis* 1979; **120**: 863–873.
7 Laufer P, Fink JN, Bruns WT, *et al*. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol* 1984; **73**: 44–48.
8 Bauernfeind A, Bertele RM, Harms K, *et al*. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection* 1987; **15**: 270–277.
9 Simmonds EJ, Littlewood JM, Evans EG. Cystic fibrosis and allergic bronchopulmonary aspergillosis. *Arch Dis Child* 1990; **65**: 507–511.
10 Marchant JL, Warner JO, Bush A. Rise in total IgE as an indicator of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Thorax* 1994; **49**: 1002–1005.
11 Mroueh S, Spock A. Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Chest* 1994; **105**: 32–36.
12 Becker JW, Burke W, McDonald G, *et al*. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest* 1996; **109**: 1536–1540.
13 Milla CE, Wielinski CL, Regelmann WE. Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996; **21**: 6–10.
14 Hutcheson PS, Knutsen AP, Rejent AJ, Slavin RG. A 12-year longitudinal study of *Aspergillus* sensitivity in patients with cystic fibrosis. *Chest* 1996; **110**: 363–366.
15 Cimon B, Carrère J, Chazalette JP, *et al*. Fungal colonization and immune response to fungi in cystic fibrosis. *J Mycol Méd* 1995; **5**: 211–216.
16 Cimon B, Carrère J, Chazalette JP, *et al*. [Bronchopulmonary mycoses in cystic fibrosis. Results of a five-year longitudinal study]. *J Mycol Méd* 2000; **10**: 128–135. [In French.]
17 Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. *Allergy* 2000; **55**: 50–58.
18 Bakare N, Rickerts V, Bargon J, Just-Nubling G. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. *Mycoses* 2003; **46**: 19–23.
19 Taccetti G, Procopio E, Marianelli L, *et al*. Allergic bronchopulmonary aspergillosis in Italian cystic fibrosis patients: prevalence and percentage of positive tests in the employed diagnostic criteria. *Eur J Epidemiol* 2000; **16**: 837–842.
20 Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. *Respir Med* 2005; **99**: 887–893.
21 Valenza G, Tappe D, Turnwald D, *et al*. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. *J Cyst Fibros* 2008; **7**: 123–127.
22 Bouchara JP, Sanchez M, Esnault K, Tronchin G. Interactions between *Aspergillus fumigatus* and host matrix proteins. *Contrib Microbiol* 1999; **2**: 167–181.
23 Tronchin G, Esnault K, Renier G, *et al*. Expression and identification of a laminin-binding protein in *Aspergillus fumigatus* conidia. *Infect Immun* 1997; **65**: 9–15.
24 Gil ML, Peñalver MC, Lopez-Ribot JL, O'Connor JE, Martinez JP. Binding of extracellular matrix proteins to *Aspergillus fumigatus* conidia. *Infect Immun* 1996; **64**: 5239–5247.
25 Tomee JF, Wierenga AT, Hiemstra PS, Kauffman HK. Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. *J Infect Dis* 1997; **176**: 300–303.
26 Sole A, Vicente R, Morant P, *et al*. Lung transplantation for cystic fibrosis: infectious events. *Med Clin (Barc)* 2006; **126**: 255–258.
27 Iversen M, Burton CM, Vand S, *et al*. *Aspergillus* infection in lung transplant patients: incidence and prognosis. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 879–886.
28 Mastella G, Rainisio M, Harms HK, *et al*. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000; **16**: 464–471.
29 Stevens DA, Moss RB, Kurup VP, *et al*. Allergic bronchopulmonary aspergillosis in cystic fibrosis--state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; **37** (Suppl. 3): S225–264.
30 Chauhan B, Santiago L, Hutcheson PS, *et al*. Evidence for the involvement of two different MHC class II regions in susceptibility or protection in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2000; **106**: 723–729.
31 Knutsen AP, Kariuki B, Consolino JD, Warrier MR. IL-4 alpha chain receptor (IL-4Rα) polymorphisms in allergic bronchopulmonary aspergillosis. *Clin Mol Allergy* 2006; **4**: 3.
32 Brouard J, Knauer N, Boelle PY, *et al*. Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. *J Infect Dis* 2005; **191**: 1988–1991.
33 Cimon B, Symoens F, Zouhair R, *et al*. Molecular epidemiology of airway colonisation by *Aspergillus fumigatus* in cystic fibrosis patients. *J Med Microbiol* 2001; **50**: 367–374.

34 Schønheyder H, Jensen T, Høiby N, Koch C. Serum antibodies to *Aspergillus fumigatus* catalase in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 40–44.

35 Neuvéglise C, Sarfati J, Debeaupuis JP, *et al*. Longitudinal study of *Aspergillus fumigatus* strains isolated from cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 1997; **16**: 747–750.

36 Vanhee LM, Symoens F, Bouchara JP, Nelis HJ, Coenye T. High-resolution genotyping of *Aspergillus fumigatus* isolates recovered from chronically colonised patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2008; **27**: 1005–1007.

37 Cimon B, Zouhair R, Symoens F, *et al*. *Aspergillus terreus* in a cystic fibrosis clinic: environmental distribution and patient colonization pattern. *J Hosp Infect* 2003; **53**: 81–82.

38 Symoens F, Bouchara JP, Heinemann S, Nolard N. Molecular typing of *Aspergillus terreus* isolates by random amplification of polymorphic DNA. *J Hosp Infect* 2000; **44**: 273–280.

39 Guarro J, Kantarcioglu AS, Horré R, *et al*. *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. *Med Mycol* 2006; **44**: 295–327.

40 Chabasse D, Bouchara JP, Chazalette JP, *et al*. [Cystic fibrosis and fungal colonization by *Scedosporium apiospermum*. About three cases]. *J Mycol Méd* 1991; **1**: 152–155. [In French.]

41 Cimon B, Carrere J, Vinatier JF, *et al*. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 53–56.

42 Williamson EC, Speers D, Arthur IH, *et al*. Molecular epidemiology of *Scedosporium apiospermum* infection determined by PCR amplification of ribosomal intergenic spacer sequences in patients with chronic lung disease. *J Clin Microbiol* 2001; **39**: 47–50.

43 Rainer J, Mayr A, Lass-Flörl C, Dierich MP. Abundance of *P. boydii* in CF-patients, detected with a semi-selective isolation method. Presented at the 2nd Meeting ECMM/ISHAM Working Group on *Pseudallescheria*/*Scedosporium* infections, Angers (France), 7–8 June 2007.

44 Rainer J, Kaltseis J, de Hoog SG, Summerbell RC. Efficacy of a selective isolation procedure for members of the *Pseudallescheria boydii* complex. *Antonie Van Leeuwenhoek* 2008; **93**: 315–322.

45 de Hoog GS, Marvin-Sikkema FD, Lahpoor GA, *et al*. Ecology and physiology of the emerging opportunistic fungi *Pseudallescheria boydii* and *Scedosporium prolificans*. *Mycoses* 1994; **37**: 71–78.

46 Claußen M, Schmidt S. Biodegradation of phenol and *p*-cresol by the hyphomycete *Scedosporium apiospermum*. *Res Microbiol* 1998; **149**: 399–406.

47 Claußen M, Schmidt S. Biodegradation of phenylbenzoate and some of its derivatives by *Scedosporium apiospermum*. *Res Microbiol* 199: **150**: 413–420.

48 Beguin H, Nolard N. Mould diversity in homes. Air and surface analysis of 130 dwellings. *Aerobiologica* 1994; **10**: 157–166.

49 Cimon B, Zouhair R, Licznar-Fajardo P, *et al*. *Scedosporium apiospermum*: environmental study at home of patients with cystic fibrosis. Presented at the 2nd Meeting ECMM/ISHAM Working Group on *Pseudallescheria*/*Scedosporium* infections, Angers (France), 7–8 June 2007.

50 Summerbell RC, Krajden S, Kane J. Potted plants in hospitals as reservoirs of pathogenic fungi. *Mycopathologia* 1989; **106**: 13–22.

51 Pinto MR, de Sá AC, Limongi CL, *et al*. Involvement of peptidorhamnomannan in the interaction of *Pseudallescheria boydii* and HEp2 cells. *Microbes Infect* 2004; **6**: 1259–1267.

52 da Silva BA, dos Santos AL, Barreto-Bergter E, Pinto MR. Extracellular peptidase in the fungal pathogen *Pseudallescheria boydii*. *Curr Microbiol* 2006; **53**: 18–22.

53 Silva BA, Pinto MR, Soares RM, Barreto-Bergter E, Santos AL. *Pseudallescheria boydii* releases metallopeptidases capable of cleaving several proteinaceous compounds. *Res Microbiol* 2006; **157**: 425–432.

54 de Hoog GS, Marvin-Sikkema FD, Lahpoor GA, *et al*. Ecology and physiology of the emerging opportunistic fungi *Pseudallescheria boydii* and *Scedosporium prolificans*. *Mycoses* 1994; **37**: 71–78.

55 Larcher G, Cimon B, Symoens F, *et al*. A 33 kDa serine proteinase from *Scedosporium apiospermum*. *Biochem J* 1996; **315**: 119–126.

56 Lima OC, Larcher G, Vandeputte P, *et al*. Molecular cloning and biochemical characterization of a Cu, Zn-superoxide dismutase from *Scedosporium apiospermum*. *Microbes Infect* 2007; **9**: 558–565.

57 Defontaine A, Zouhair R, Cimon B, *et al*. Genotyping study of *Scedosporium apiospermum* isolates from patients with cystic fibrosis. *J Clin Microbiol* 2002; **40**: 2108–2114.

58 Hennequin C, Benailly N, Silly C, *et al*. *In vitro* susceptibilities to amphotericin B, itraconazole, and miconazole of filamentous fungi isolated from patients with cystic fibrosis. *Antimicrob Agents Chemother* 1997; **41**: 2064–2066.

59 Gilgado F, Serena C, Cano J, Gené J, Guarro J. Antifungal susceptibilities of the species of the *Pseudallescheria boydii* complex. *Antimicrob Agents Chemother* 2006; **50**: 4211–4213.

60 Symoens F, Knoop C, Schrooyen M, *et al*. Disseminated *Scedosporium apiospermum* infection in a cystic fibrosis patient after double-lung transplantation. *J Heart Lung Transplant* 2006; **25**: 603–607.

61 Zouhair R, Defontaine A, Ollivier C, *et al*. Typing of *Scedosporium apiospermum* by multilocus enzyme electrophoresis and random amplification of polymorphic DNA. *J Med Microbiol* 2001; **50**: 925–932.

62 Guignard S, Hubert D, Dupont B, *et al*. Multifocal *Scedosporium apiospermum* spondylitis in a cystic fibrosis patient. *J Cyst Fibros* 2008; **7**: 89–91.

63 Gilgado F, Cano J, Gené J, Guarro J. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *J Clin Microbiol* 2005; **43**: 4930–4942.

64 Cano J, Gilgado F, Gené J, Guarro J. Phenotypic characterization and new species in *Pseudallescheria*. Presented at the 2nd Meeting ECMM/ISHAM Working Group on *Pseudallescheria*/*Scedosporium* infections, Angers (France), 7–8 June 2007.

65 Gilgado F, Cano J, Gené J, Sutton DA, Guarro J. Molecular and phenotypic data supporting distinct species statuses for *Scedosporium apiospermum* and *Pseudallescheria boydii* and the proposed new species *Scedosporium dehoogii*. *J Clin Microbiol* 2008; **46**: 766–771.

66 Maertens J, Lagrou K, Deweerdt H, *et al*. Disseminated infection by *Scedosporium prolificans*: an emerging fatality among haematology patients. Case report and review. *Ann Hematol* 2000; **79**: 340–344.

67 García E, Rodríguez V, Gartner S, Gené J. [Isolation of *Scedosporium prolificans* in patients with cystic fibrosis]. *Enferm Infecc Microbiol Clin* 1998; **16**: 488–489. [In Spanish.]

68 del Palacio A, Garau M, Amor E, *et al*. Case reports. Transient colonization with *Scedosporium prolificans*. Report of four cases in Madrid. *Mycoses* 2001; **44**: 321–325.

69 Lopez L, Gaztelurrutia L, Cuenca-Estrella M, *et al*. [Infection and colonization by *Scedosporium prolificans*]. *Enferm Infecc Microbiol Clin* 2001; **19**: 308–313. [In Spanish.]

70 Sole M, Cano J, Rodriguez-Tudela JL, *et al*. Molecular typing of clinical and environmental isolates of *Scedosporium prolificans* by

inter-simple-sequence-repeat polymerase chain reaction. *Med Mycol* 2003; **41**: 293–300.
71 Delhaes L, Harun A, Chen SC, *et al*. Molecular typing of Australian *Scedosporium* isolates showing genetic variability and numerous *S. aurantiacum*. *Emerg Infect Dis* 2008; **14**: 282–290.
72 Vagefi MR, Kim ET, Alvarado RG, *et al*. Bilateral endogenous *Scedosporium prolificans* endophthalmitis after lung transplantation *Am J Ophthalmol* 2005; **139**: 370–373.
73 Haase G, Skopnik H, Kusenbach G. *Exophiala dermatitidis* infection in cystic fibrosis. *Lancet* 1990; **336**: 188–189.
74 Haase G, Skopnik H, Groten T, Kusenbach G, Posselt HG. Long-term fungal cultures from sputum of patients with cystic fibrosis. *Mycoses* 1991; **34**: 373–376.
75 Horré R, Schaal KP, Siekmeier R, *et al*. Isolation of fungi, especially *Exophiala dermatitidis*, in patients suffering from cystic fibrosis. A prospective study. *Respiration* 2004; **71**: 360–366.
76 Kusenbach G, Skopnik H, Haase G, Friedrichs F, Dohmen H. *Exophiala dermatitidis* pneumonia in cystic fibrosis. *Eur J Pediatr* 1992; **151**: 344–346.
77 Blaschke-Hellmessen R, Lauterbach I, Paul KD, Tintelnot K, Weissbach G. [Detection of *Exophiala dermatitidis* (Kano) De Hoog 1977 in septicemia of a child with acute lymphatic leukemia and in patients with cystic fibrosis]. *Mycoses* 1994; **37** (Suppl. 1): 89–96. [In German.]
78 Gigi J, Lebecque P, Reychler G, Symoens F. *Exophiala dermatitidis*: airway colonisation of the upper respiratory tract in Belgian cystic fibrosis patients. Presented at the Annual Congress of the Belgium Society of Mycology, Brussels (Belgium), 12 April 2008.
79 Koch C, Cuppens H, Rainisio M, *et al*. European Epidemiologic Registry of Cystic Fibrosis (ERCF): comparison of major disease manifestations between patients with different classes of mutations. *Pediatr Pulmonol* 2001; **31**: 1–12.
80 Matos T, De Hoog GS, De Boer AG, De Crom I, Haase G. High prevalence of the neurotropic *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses* 2002; **45**: 373–377.
81 Kerr J. Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from patients with cystic fibrosis. *J Infect* 1994; **28**: 305–310.
82 Kerr JR, Taylor GW, Rutman A, *et al*. *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. *J Clin Pathol* 1999; **52**: 385–387.
83 Cimon B, Carrère J, Chazalette JP, *et al*. Chronic airway colonization by *Penicillium emersonii* in a patient with cystic fibrosis. *Med Mycol* 1999; **37**: 291–293.
84 Gonzalez-Escalada A, Del Palacio A, Calvo MT, Gene J, Guarro J. Two cases of scalp wound colonization and respiratory tract by mycelial fungi. *Rev Iberoam Micol* 2000; **17**: 149–151.
85 Shukla PK, Khan ZA, Lal B, Agrawal PK, Srivastava OP. Clinical and experimental keratitis caused by the *Collectotrichum* state of *Glomerella cingulata* and *Acrophialophora fusispora*. *Sabouraudia* 1983; **21**: 137–147.
86 Sutton DA, Sigler L, Kalassian KG, *et al*. Pulmonary *Acrophialophora fusispora*: case history, literature review and mycology. Program and abstracts of 13th Congress of the International Society for Human and Animal Mycology, Salsomaggiore Terme, Parma (Italy), 8–13 June 1997: abstract 363.
87 Al-Mohsen IZ, Sutton DA, Sigler L, *et al*. *Acrophialophora fusispora* brain abscess in a child with acute lymphoblastic leukemia: review of cases and taxonomy. *J Clin Microbiol* 2000; **38**: 4569–4576.
88 Cimon B, Challier S, Beguin H, *et al*. Airway colonization by *Acrophialophora fusispora* in patients with cystic fibrosis. *J Clin Microbiol* 2005; **43**: 1484–1487.
89 Welsh RD, Ely RW. *Scopulariopsis chartarum* systemic mycosis in a dog. *J Clin Microbiol* 1999; **37**: 2102–2103.
90 Guarro J, Gene J. *Acrophialophora fusispora* misidentified as *Scedosporium prolificans*. *J Clin Microbiol* 2002; **40**: 3544; author reply 3545.
91 Sigler L, Sutton DA. *Acrophialophora fusispora* misidentified as *Scedosporium prolificans*. *J Clin Microbiol* 2002; **40**: 3544–3545; author reply 3545.

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