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CLINICAL LAB ISSUES (K LAGROU, SECTION EDITOR)

The Airway Colonization by Opportunistic Filamentous Fungi in Patients with Cystic Fibrosis: Recent Updates

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Abstract There has been a remarkable increase recently in the isolation of fungi such as *Aspergillus fumigatus*, species of the *Pseudallescheria boydii*/*Scedosporium apiospermum* complex, and *Exophiala* sp. from the respiratory tract of patients with cystic fibrosis (CF). This review describes the recent insights into the epidemiology, ecology, and physiopathology of the filamentous fungi able to colonize and/or to infect the airways of CF patients, and that may be responsible for accelerated lung function decline. We summarize salient features not only on highly prevalent species such as *Aspergillus* and *Scedosporium*, but also on more recently described fungi such as *Rasamsonia argillacea*. In addition, we discuss the challenges inherent in tracking and interpreting rates of fungal colonization/infection in the CF patient population, taking into consideration the polymicrobial composition of the CF lung environment, the corresponding cross-kingdom interactions, and the new concept of mycobiota. Further research is warranted to clarify the role of fungi in CF lung disease and its therapeutic management.

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Introduction

Cystic fibrosis (CF) is the most common genetic inherited disease in the European white population, and based on the number of patients, is the third most common orphan disease. CF is caused by mutations in the CFTR gene that encodes a chloride channel involved in electrolytic exchanges through the plasma membrane of epithelial cells.

In the respiratory tract, mutations in CFTR result in a defective mucociliary clearance and a thickening of the bronchial mucus. These abnormal airway conditions facilitate the entrapment of the inhaled bacterial and fungal conidia, and provide a suitable environment for growth of microorganisms. This, in turn, leads to respiratory infections and inflammatory reactions which, together with the microorganisms, contribute to the progressive deterioration of lung function. Using conventional microbiological culture methods, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* complex have been identified as the principle pathogenic bacteria in adult CF pulmonary infection that cause recurrent exacerbations of the pulmonary disease and often determine the vital prognosis of patients [1, 2]. As such, over recent decades considerable attention has been paid to their prevention and treatment by antibiotics, which has improved patient management and resulted in a significant increase in the life expectancy of patients [3, 4]. As a consequence, the respiratory tracts of CF patients are at increasingly high risk of colonization and/or infection by several fungi [4, 5]. In this context, more and more attention has been drawn toward

filamentous fungi, as well as certain yeasts reaching the status of emerging or re-emerging microorganisms [6, 7].

While recent studies have shown an association between *Aspergillus fumigatus* colonization and poorer lung function, as well as negative prognostic value of an allergic bronchopulmonary aspergillosis (ABPA) status in CF [8, 9, 10•], fungal isolation in respiratory secretions remains poorly studied in the context of CF, and continues to present a dilemma for the clinician in terms of therapeutic management [11].

In this review, we provide a summary and discussion of recent insights into the epidemiology, ecology, and physiopathology of the filamentous fungi that are able to colonize and/or infect the airways of patients with CF, and that which may be responsible for accelerated lung function decline [8, 9, 10•, 12, 13]. We focus not only on highly prevalent species such as *Aspergillus*, *Scedosporium*, and *Exophiala*, but also on more recently described fungi such as *Rasamsonia argillacea* [7, 12–15]. With this insight into the fungal community, we propose to ascertain the worldwide occurrence of fungal colonization in the respiratory tract (including estimated ABPA rate) in CF. Our focus is on the role of the fungus in the context of improving survival (especially with regard to lung transplantation), taking into account the new concept of lung mycobiota. Further studies are warranted to evaluate the role of fungi in CF lung disease and to determine guidelines for therapeutic management.

CF: An Old Disease, with Survival Improved by New Therapies Targeted at Fungal Colonization/Infection

CF is the most common fatal genetic disease (autosomal recessive transmission without sex ratio) in white populations. It has likely been noted in various definitions in the literature since the days of antiquity, described in Northern Europe in the 19th century as "misery to the child who leaves a salty taste when he kisses: he will die soon." In 2012, the estimated number of worldwide cases of CF reached 70,000, including 30,000 in the United States, according to recent Cystic Fibrosis Foundation (CFF) estimates [16]. In Europe, the number of individuals with CF was estimated at over 32,000 in 2010 [17], and in Australia at 3,156 people [18] (Fig. 1). The overall incidence of CF in white populations is thought to be between 1/2,500 and 1/4,000; it is lower for Asian and African populations (Fig. 1) [19–24, 25••, 29].

In recent years, the life expectancy of patients with CF has increased significantly [3, 16, 17, 19], for example, from 31 years in 2002 to 41 years in 2012 in the U.S. [16]. This improvement is seen as a result of the creation of CF treatment centers staffed with multidisciplinary teams, offering of a more effective treatment paradigm for the disease [3, 4]. Nevertheless, CF remains virtually incurable, a disease in which abnormal mucociliary function and local immunogenic injury promote fungal development in the lungs and promote bacterial colonization and infection. Patients are at risk of death from respiratory failure related to acute pulmonary exacerbations, and for which prompt aggressive treatment with antibiotics is recommended [3]. Antibiotic therapy for airway infection in CF is now well-documented, with consensus guidelines in North America and Europe [3]. Although long-term antibiotic therapy using inhaled and/or oral broad-spectrum antibiotics has been associated with fungal colonization in patients with CF [26, 27, 30], a recent study has shown a significant reduction in the presence of *A. fumigatus* after a short-term intravenous antibiotic regimen against *P. aeruginosa* [31]. Approximately 10 % to 20 % of patients who present with pulmonary exacerbation do not respond or respond only partially to intravenous antibiotic treatment, which usually targets the dominant bacteria identified by cultures [2, 3, 28, 32]. In these populations, the risk of failure to recover lung function has been associated with CF-related diabetes, pancreatic insufficiency, malnourishment, liver disease, *P. aeruginosa* multi- or pan-drug-resistant isolates, chronic *P. aeruginosa* infection, methicillin-resistant *S. aureus*, *B. cepacia* complex infection, and/or ABPA [28, 32, 33]. An increase in the prevalence of fungal infections has been noted with respect to rising numbers of hospital admissions and/or with a decreased lung function [10•, 12, 30]. As such, assessment for the presence of fungi in respiratory secretions, in addition to ABPA criteria, is now considered as essential in the follow-up of patients with CF [3].

New therapies that target the global CFTR protein deficiency – i.e., organ transplantation, and more recently CFTR potentiators such as ivacaftor (Kalydeco®, Vertex Pharmaceuticals) – are expected to have an impact on rates of morbidity and mortality with regard to fungal-related pulmonary disorders in patients with CF. Treatment with CFTR potentiators requires further study to provide data on its long-term effectiveness [34]. Transplantation, and particularly lung transplantation, is increasingly common (Fig. 1), and is associated with the risk of developing invasive mycosis [16–18, 35–37]. The incidence of invasive aspergillosis (IA) among lung transplant patients has been reported between 10 % and 22.5 % [35, 37, 38], and is also associated with higher mortality rates in CF [35, 37].

Overall, there is a growing body of evidence that the fungi, and filamentous fungi in particular (including *Exophiala* species, which are dimorphic fungi, able to grow as black yeast at body temperature and as filamentous fungi at room temperature) present in the lungs of patients with CF are pathogens rather than spectator microorganisms. As they able to confer a large spectrum of disease (sensitization, ABPA, and/or invasive mycosis), in the following two sections we will review

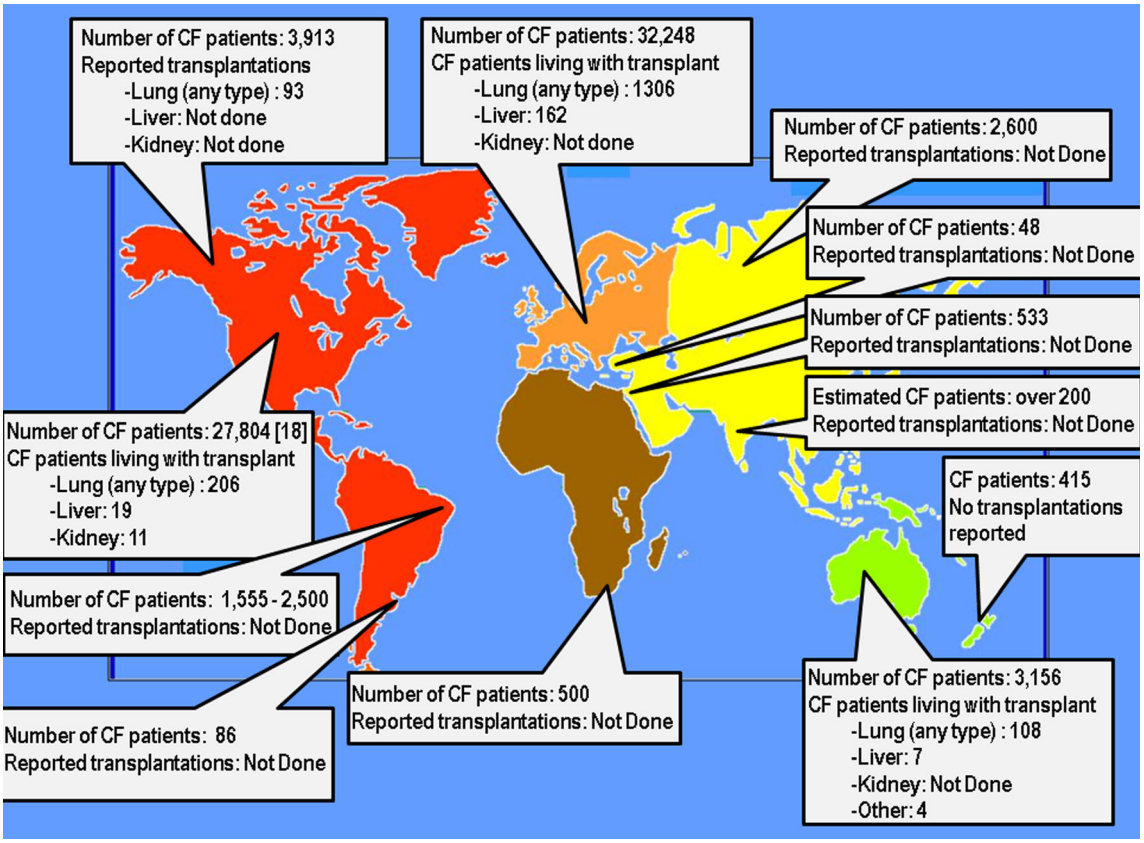

Fig. 1 Worldwide distribution of patients diagnosed with CF and estimated number of CF patients living with transplant (data collected from [18–24, 25••, 26–28]

the fungal species recently reported in CF and discuss their potential pathogenicity.

Aspergillus Fumigatus: The Tree That Hides the Forest

Aspergillus species are ubiquitous molds, present everywhere in soil, air, vegetation, food, and indoor and outdoor human environments. While over 200 species have been described, only about 20 are pathogenic to humans [39]. The *Aspergillus* species, particularly *A. fumigatus*, are those most frequently isolated in the respiratory tracts of patients with inflammatory chronic pulmonary disease such as asthma or CF [reviewed in 40, 41]. The prevalence of *A. fumigatus* ranges from 5 % to almost 90 % [10•, 15, 23, 27, 30, 32, 42–55] (Table 1). *Aspergillus* species are associated with significant morbidity, and are associated with diseases that likely remain underdiagnosed [25••]. They manifest clinically in various ways, ranging from infection to ABPA (reviewed in [39]). While ABPA is the most common clinical manifestation in CF, IA and aspergilloma have also been reported [39, 40]. Aspergilloma, which is observed rarely in CF, requires a pre-existing lung cavity to develop, is difficult to treat, and may require surgical resection or long-term antifungal treatment [39]. Two entities of invasive disease have been differentiated and proposed in CF: invasive aspergillosis (IA), occurring in the setting of immunosuppressive states during transplantation, and invasive pulmonary aspergillosis (IPA) in non-transplant CF patients with lung impairments. IPA is the most frequently observed pathology in CF, although invasive diseases as a whole remain highly rare in non-transplant CF patients. IA has been recently associated with a fourfold higher risk of occurrence in the case of colonization with *A. fumigatus* [38], and its management remains a diagnostic and therapeutic challenge.

Recent studies of *Aspergillus* sensitization, ABPA, and *Aspergillus* bronchitis (an entity described in CF patients with decreased FEV1 [25••, 56]) have indicated that they are underdiagnosed in the worldwide CF adult population [25••]. The estimated numbers of adult CF patients with *Aspergillus* sensitization, ABPA, and *Aspergillus* bronchitis have been estimated at 5,506, 6,675, and 11,314, respectively [25••]. ABPA represents a severe complication in patients suffering from CF. It is difficult to diagnose due to its overlapping clinical and radiological features with those of CF exacerbations. Since early treatment of ABPA has been found to reduce symptoms, improve lung function, and prevent long-term damage such as bronchiectasis and fibrosis, the prompt diagnosis

Table 1 Worldwide frequency of isolation of *Aspergillus* spp., *Scedosporium* sp., and *Exophiala dermatitidis* in CF respiratory tract obtained from bibliography from 2010 to date

Continents and Countries (Number of CF patients) [Bibliography]	Frequency of fungal Isolation (%)							
	Aspergillus species					Non-*Aspergillus* species		
	A.fumigatus	*A. flavus*	*A.terreus*	*A. niger*	Other *species*	Species of the complex *P. boydii* / *S. apiospermum*	*S.prolificans*	*E.dermatitidis*
Europe								
Austria (113) [15]	34	3	2	ND*		2	ND	3
Belgium (154) [45]	7.8	0.6				ND	ND	5.9
Denmark (148) [46]	28.8	2.7	0.7	0.7	0.0	*S. apiospermum* 0.7 *S. aurantiacum* 0.7	0.7	ND
Denmark (287) [47]	13.6	4.1–4.4**	1.9–2.6	4.7–0.8	0–1.4	< 1	ND	ND
France (251) [10•]	27.1	ND				ND	ND	ND
France (249) [48]	52.6	ND				ND	ND	ND
France (201) [49]	56.7	10.4				3.4	ND	ND
France (291)*** [42]	45.4	3.4	0.0	1.7	2.1	3.8	0.0	0.0
Germany (81) [44]	*Aspergillus* sp.: 47.8					4.7	0.7	1.6
Ireland (77) [43]	5.2	3.9				3.9	ND	3.9
Italia (1837) [50]	11.8	3.0	2.4	0.6	ND	1.9	ND	0.05
The Netherlands (259) [27]	23.5	ND				ND	ND	ND
Sweden (97) [51]	10.4	ND				ND	ND	19
UK (51) [32]	37.0	ND				ND	ND	ND
UK (69) [42]	43.5	2.9	5.8	1.4	1.4	1.4	0.0	0.0
UK (36) [42]	88.9	5.6	11.1	0.0	0.0	8.3	0.0	1.4
Middle East								
Turkey (48) [23]	10.4	8.3				ND	ND	ND
Israel (468) [52]£	*Aspergillus* sp.: 35.5					ND	ND	ND
North America								
USA (614) [30]	36.3	26.1				2.3££		5.0£££
South America								
Brazil (74) [53]£	*Aspergillus* sp.: 5.6					ND	ND	ND
Asia								
India (41) [54]	18.2	ND				ND	ND	ND
Oceania								
Australia (72) [55]	66.7	10.1	ND	ND	8.7	*S. aurantiacum* 5.8 Other complex species: 5.8	5.8	0.0

*ND: Not done; **Data analysis was performed during two separate periods (2007 and 2009); ***French data from Angers, Lille, and Rouen reported in 2006 (Table 2 in [41]) have been included together; £ Only data published before 2010 were available; ££ Reported as Scedosporium spp; £££ Reported as other filamentous species including *Alternaria*, *Curvularia*, *Paecilomyces*, *Penicillium*, and *Exophiala* species

of ABPA is imperative. New criteria based on established serological tests (IgG and IgE serum levels) combined with real-time PCR and levels of galactomannan in sputum have recently been proposed in order to classify patients with CF into three groups of aspergillosis, which may be helpful for the management and follow-up of *Aspergillus* disease in CF [56, 57]. In addition to sporadic use of voriconazole, the recommended protocols to treat ABPA in CF are based on itraconazole to target *A. fumigatus*, which then raises the more general question of developing azole resistance [48, 58–60]. An ongoing prospective randomized study, ATCF (Azole Therapy in Cystic Fibrosis [EudraCT: 2011-005799-41]) coordinated by Gangneux and Denning is addressing (i) the efficacy of itraconazole and voriconazole in CF patients, (ii) the relationship between plasma azole concentrations and

the clinical response, and (iii) the relationship between fungal molecular typing and in vitro chemosensitivity to different antifungal agents. This study may pave the way for new therapeutic guidelines.

Other species – *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus Niger*, or *Aspergillus nidulans* – are capable of colonizing the respiratory tract in CF patients, in frequencies ranging from 11 % to less than 0.1 % (Table 1). They have been reported in ABPA cases as well as invasive aspergillosis [15, 39, 58, 61]. Unusual *Aspergillus* species such as *Aspergillus lentulus* or *Neosartorya pseudofischeri*, which are known to exhibit low susceptibility to amphotericin B and/or voriconazole, have also been described in CF [58, 62]. The frequency of isolation of *Aspergillus* species in CF, and particularly *A. fumigatus*, has varied among recent published data (Table 1), increasing significantly with patient age, use of antibiotics, and decreased lung function [10•, 27, 30]. In addition, *Aspergillus*-persistent carriage and sensitization have been shown to exhibit an independent effect on lung function in CF [10•], and *Aspergillus* sensitization has also been associated with decreased lung function [63].

Genotypic studies of *Aspergillus* species (largely *A. fumigatus*) have been conducted in order to clarify the epidemiology of aspergillosis and to identify potential sources, transmission routes, and colonization patterns of *Aspergillus* isolates [64]. As was recently shown with the *A. terreus* isolate [61], different profiles of genotypes can be observed among the species: (i) transient colonization by a single genotype, (ii) chronic colonization by several genotypes, and (iii) chronic colonization by one or a few dominant genotype(s). In fact, the respiratory tract of CF patients is more frequently colonized by multiple *Aspergillus* strains, whereas only a single genotype is identified from deep organs of patients with invasive disease. Molecular typing methods and results have been exhaustively reviewed elsewhere [64].

Recent findings have underscored the complex interplay between the respiratory epithelium and the host response in the presence of inhaled spores. A recent review looked at the classical major components of the innate immune system as well as the cytokine host response involved in recognition and removal of *A. fumigatus* [39]. In addition to its thermotolerance and its propensity to disseminate in immunocompromised hosts (due to the small size of conidia, 2–3.5 μm, that may be inhaled into the alveoli), *A. fumigatus* is able to modulate the inflammatory response in epithelial cells [63, 65], due to the compounds of its fungal surface cell (reviewed in [66]). It has also been associated with significant local inflammatory response in young CF patients [67], a fact that is highly supportive of the use of specific surveillance for such filamentous fungus.

Fungal Risk in CF and Recent Findings on Non-*Aspergillus* Species

Candida is the most commonly isolated yeast in the respiratory tracts of patients with CF. It is considered as a commensal microorganism belonging to the oral microbial community regardless of the fact that *Candida albicans* has been associated with a decline in FEV1 [12, 15, 23, 30, 42–44, 68]. In addition to *A. fumigatus*, other moulds such as *Scedosporium* sp. have been reported, at times with higher frequency than those of non-fumigatus *Aspergillus* (Table 1) [42, 43, 45, 50, 69]. These wide ranges are likely as a result of one or more of the following points: (i) the non-standardized protocol used to isolate fungi from samples (protocols are different according to addition or absence of pretreatment step, number and choice of media, incubation temperature, culture duration, and methods used to identify isolated fungi), (ii) the types of sampling used (nasal swab, sputum, bronchoalveolar lavage), (iii) the age of CF patients studied (adults or children), and (iv) the characteristics of local climatic conditions [5, 11, 15, 25••, 42, 70–72]. Although mycological culture methods vary from one center to another and suffer from poor sensitivity [38, 57, 71], isolation of fungi such as species of the *Pseudallescheria boydii*/*Scedosporium apiospermum* complex or *Exophiala* sp. is remarkably more common, and seems to be associated with older age [27, 30, 42], decreased lung function [10•, 30, 69], and/or use of antibiotics [27, 30, 53]. In this context, filamentous fungi are by far the most pathogenic micromycetes frequently isolated, but black yeasts (*Exophiala* sp.) are also reported.

Scedosporium species are ranked as the second most common genus associated with CF after *Aspergillus* (Table 1). It formerly comprised two species, *S. prolificans* and *S. apiospermum*, but the latter has recently emerged as the *P. boydii*/*S. apiospermum* complex initially described by Gilgado et al. [73–75]. The species from the *P. boydii*/*S. apiospermum* complex are the more prevalent in CF (Table 1) [74], but *S. prolificans* is the more lethal due to its ability to disseminate and its antifungal resistance [6]. Chronic colonization and fatal outcome with *S. apiospermum* have also been reported [74, 76]. In a retrospective single-center study, it appeared to be responsible for the development of ABPA-like symptoms, or ABPM (allergic bronchopulmonary mycosis) [49]. Among the newly described species of the *P. boydii*/*S. apiospermum* complex, *Scedosporium aurantiacum* has been more frequently isolated from sputum samples of CF patients in France and in Australia [55, 56, 77] (Table 1). These differences in rates of recovery are likely due to methodology discrepancies, since species of the *P. boydii*/*S. apiospermum* complex are known to be optimally detected with selective medium and a long period (up to 7–11 days) of medium incubation [44, 55, 71, 77, 78]. The risk factors for *Scedosporium* acquisition in CF remain poorly identified. Co-

colonization of *Pseudallescheria*/*Scedosporium* species with other moulds, primarily *A. fumigatus*, has been described [49, 55, 71]. Univariate analysis has shown that bacterial colonization and antimicrobial exposure likely influence *Scedosporium* colonization [55]. In Australia and France, soil sampling revealed an abundance of *Pseudallescheria*/*Scedosporium* species, mostly in areas associated with human activity [77–79], which is consistent with the high prevalence of *Scedosporium* infection and colonization previously described in CF patients [80]. In Australia, *S. aurantiacum* was the most frequently isolated species, at 54.6 %, followed by *S. prolificans* (43 %), *P. boydii* (2.1 %), and *S. dehoogii* (0.3 %) [80]. Recent molecular studies have suggested that most patients were chronically infected with a single strain, with no sharing of similar strains [77, 81].

Among the various *Exophiala* species isolated from respiratory tracts of CF patients, *Exophiala dermatitidis* is most frequently observed [82], and appears to be more frequent in the north of Europe (Table 1). In a Swedish study [51], this black yeast was recovered more frequently than *A. fumigatus* from the sputa of patients with CF (Table 1). With regard to associated risk factors, *E. dermatitidis* culture-positive patients tended to be significantly older than 12 years, pancreatic-insufficient, homozygous for the F508del mutation, and colonized with *A. fumigatus* [45, 51, 69]. In a recent study, antibody production (IgG) against *E. dermatitidis* was significantly associated with an inflammatory response (higher white blood cell count) and decreased respiratory function (lower value of FEV1% predicted) [69]. In terms of molecular analysis, most of the *Exophiala* isolates were grouped according to the patient origin, with no association with respect to the geographical origin of the isolates, the isolation date, or antifungal susceptibility [82].

Rasamsonia argillacea, previously known as *Geosmithia argillacea*, is a filamentous fungus similar to the *Penicillium* species and genus, which can lead to misidentification, and certainly may contribute to its underestimation [14]. Recent molecular analyses have led to the identification of a species complex that requires further studies [7, 13–15, 83].

Chronic colonization with *R. argillacea* has recently been reported in France, Italy, and the UK [7, 13–15, 50, 83]. While the number of cases is low, predisposing factors have been identified and include homozygosis for the F508del mutation and previous use of azole to manage mould colonization [83]. *R. argillacea* showed a significant sensitivity to echinocandin during in vitro susceptibility tests [13, 14]. Notwithstanding the increasingly common isolation of *R. argillacea* in the respiratory tract, its pathogenicity remains poorly demonstrated [14].

Isolation of *Acrophialophora fusispora* in the context of CF has not been reported since 2005. Since this mould is as difficult to identify as *R. argillacea*, even in expert mycology departments, its incidence is likely underreported, and hence its clinical relevance poorly documented. The fungus has been reported to be responsible for keratitis and pulmonary infection in non-immunocompromised adults, and was noted to be associated with a brain abscess in a child with acute lymphoblastic leukaemia. In patients with CF, chronic colonization with *A. fusispora* may contribute to progressive pulmonary impairment by promoting local inflammation [84].

Microbial Diversity in CF Airways: From in Vitro Interactions Among Microorganisms to Clinical Insights in CF Lung Microbiota

A number of studies have shown that the CF airways are colonized by diverse polymicrobial communities, recently referred to as "lung microbiota," with bacteria, fungi, and viruses all present and potentially contributing to infection and inflammation [85–98]. While microbiological cultures are useful for the diagnosis of bacterial and/or fungal infections, they are less suitable for identifying co-infections and dynamics of microbial populations, as viral microorganisms are usually not diagnosed. Yet such organisms have become increasingly recognized as important agents, especially in CF pulmonary exacerbations [91–94]. Therefore, determining the microbial composition of the upper airways that characterize each patient becomes important, as well as determining its microbial evolution during CF pulmonary disease. In addition, significant associations (co-colonization or exclusion) between fungi or between fungus and bacteria have been described in the context of CF [44, 51, 55, 99], and highlight the aptitude of the fungus to interact with other members of the microbial community colonizing the airways of patients with CF, and consequently contributing to the alteration of lung function. Bronchoalveolar lavages of children with CF that grew more than one microorganism have also been associated with greater inflammatory levels [67]. Such interactions, especially between *A. fumigatus* and *P. aeruginosa*, have also been demonstrated in vitro. These two organisms are able to produce biofilm, and involve quorum-sensing molecules.

Although the development of numerous molecular techniques has provided for accurate fungal detection and classification, few of these techniques have addressed the polymicrobial composition of the fungal community in CF [85–90, 92–98]. Advanced techniques such as deep-sequencing methods, which are able to massively identify microbial sequences (thousands of sequences in a few hours), have provided new insights into the depth and breadth of lung microbiota, particularly in CF. The vast majority of the published data have explored the diversity of bacterial communities [93–97], with only a few studies focusing on viruses and phages [92] or fungi [86, 87, 89, 96]. On the whole, published results are

promising: they uncover the presence of a bacterial community present in both healthy and pathologic lungs [88, 95], which may represent a subpopulation of the microbiota. The whole flora is able to evolve according to the primary pulmonary disease (COPD, asthma, bronchiectasis, or CF) and based on the presence of absence of acute exacerbation [92–94]. In fact, each microbiota has its own composition and evolution that is unique and specific to the patient, and which may play a role in the deterioration of lung function. High-throughput technologies provide the opportunity to simultaneously analyze the whole (bacteria, viruses, and fungi) microbial community without a priori knowledge of existing microorganisms, and consequently represent the most promising investigational strategy in the context of pulmonary chronic diseases such as CF.

With respect to fungal diversity or mycobiota, these new approaches have recently allowed us to identify a higher number of fungal species versus conventional culture-based methods, including species previously undescribed in the context of CF such as *Malassezia* species [87, 89, 90]. These are lipophilic yeasts, difficult to grow in standard culture media, and commensally found in the human skin. Their reduced abundance in respiratory samples was recently correlated to a decline in CF pulmonary function [88]. This result is consistent with the complex bacterial and fungal diversity recently reported using the pyrosequencing method, in which more than 60 % of the species or genera had not been identified in cultures [87]. Strikingly, the diversity and species richness of fungal and bacterial communities observed was significantly lower in patients with decreased lung function and poor clinical status/outcome [86, 87]. Whether all members of this fungal community play a direct or indirect role in pulmonary decline has yet to be fully elucidated, especially regarding the cooperative, competitive, and adaptive interactions of microorganisms isolated in the CF lung microbiome, as recently proposed [98]. Further, larger studies based on deep-sequencing approaches are now warranted to address mycobiota and microbiome in the context of CF. These studies should take into account newly proposed criteria that could improve clinical classification of aspergillosis in CF [31, 57].

Conclusions: Implications in the Microbiological Diagnosis of Fungi as non-Bystanders in the CF Lung

With the exception of *A. fumigatus*, for which the role is well-documented, the pathogenic role of fungi colonizing the CF respiratory tract is still a matter of debate. However, all represent true opportunist pathogens. They share biological features that are essential to colonize and infect lungs: thermotolerance, a capacity to produce biofilm, and an ability to disseminate and/or to resist to various antifungals, especially the azole drugs [14, 48, 59, 60, 71]. As such, greater insight into their clinical relevance (such as an increasing prevalence associated with older age of CF patients and/or a decreased lung function) has emerged within the past decade [10•, 27, 30, 42, 63, 69].

From a practical point of view, greater attention should be given to any chronic colonization by filamentous fungi, especially through the use of specific procedures for mycological analysis of CF respiratory samples. A recent international initiative, the MucoFong International Project (MFIP) [100], designed to compare the performance of different media used for fungal cultures and develop a standardized approach for the mycological examination of sputum samples from CF patients, will facilitate the establishment of unique, or at least comparable, mycological procedures. Briefly, MFIP study was organized within the framework of our ECMM/ISHAM Working Group on Filamentous Fungi and Chronic Respiratory Infections in Cystic Fibrosis. It is based on a unique protocol shared by all participating centers [100]. This protocol has been collectively validated according to the MFIP questionnaire that we organized as a preliminary step to design the MFIP study (i.e., each center was allowed during the same three-month period of time to include 25–30 sputa from CF patients, used the same procedure and the same mycological media to isolate fungi, and summarized its results onto a prepared Excel table; all media were prepared in Lille and dispatched to the participating centers; results were synthesized and analyzed in Lille). MFIP protocol includes pretreatment (to homogenize) of sputum samples that improved the isolation of non-*Aspergillus* species [15].

Given the emergence of resistant species (primarily azole-resistant *Aspergillus* isolates), their contribution to ABPM disease, and the importance of these fungi as potential sources of infection after lung transplantation, azole-susceptibility testing should also be performed on all fungal isolates chronically colonizing the airways of patients with CF (at least all *Aspergillus* spp.) for patients requiring antifungal treatment [14, 48, 59, 60, 71].

Finally, shortly thereafter, deep-sequencing and metagenomic analysis should be performed for the re-examination of samples that do not yield the usual CF pathogens and belong to patients with non-efficient clinical outcome.

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Compliance with Ethics Guidelines

Conflict of Interest K Touati and LN Do Ngoc both declare no conflicts of interest.

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References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Zemanick ET, Wagner BD, Harris JK, et al. Pulmonary exacerbations in cystic fibrosis with negative bacterial cultures. Pediatr Pulmonol. 2010;45:569–77.
2. Aaron SD, Vandemheen KL, Ferris W, et al. Combination antibiotic susceptibility testing to treat exacerbations of cystic fibrosis associated with multiresistant bacteria: a randomised, double-blind, controlled clinical trial. Lancet. 2005;366:463–71.
3. Smyth AR, Bell SC, Bojcin S, et al. European Cystic Fibrosis Society Standards of Care: Best Practice guidelines. J Cyst Fibros. 2014;13S1:S23–42.
4. Conway S, Balfour-Lynn IM, De Rijcke K, et al. European Cystic Fibrosis Society Standards of Care: Framework for the Cystic Fibrosis Centre. J Cyst Fibros. 2014;13S1:S3–22.
5. Horré R, Symoens F, Delhaes L, et al. Fungal respiratory infections in cystic fibrosis: a growing problem. Med Mycol. 2010;48(O1):S1–3.
6. Grenouillet F, Botterel F, Crouzet J, et al. *Scedosporium prolificans*: an emerging pathogen in France? Med Mycol. 2009;47:343–50.
7. Giraud S, Pihet M, Razafimandimby B, et al. *Geosmithia argillacea*: an emerging pathogen in patients with cystic fibrosis. J Clin Microbiol. 2010;48:2381–6.
8. Amin R, Dupuis A, Aaron SD, et al. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. Chest. 2010;137:171–6.
9. Speirs JJ, van der Ent CK, Beekman JM. Effects of *Aspergillus fumigatus* colonization on lung function in cystic fibrosis. Curr Opin Pulm Med. 2012;18:632–8.
10. • Fillaux J, Brémont F, Murris M, et al. Assessment of *Aspergillus* sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. Scand J Infect Dis. 2012;44:842–7. *In a large cohort (251 patients) of well-documented patients with CF, a retrospective study demonstrated for the first time that ABPA, Aspergillus sensitization, or persistent carriage was significantly associated with a larger decline in FEV1 compared with a control group. This association was independent of other associated factors (P. aeruginosa transient detection, age, being underweight, and low FEV1 at baseline).*
11. Liu JC, Modha DE, Gaillard EA. What is the clinical significance of filamentous fungi positive sputum cultures in patients with cystic fibrosis? J Cyst Fibros. 2013;12:187–93.
12. Chotirmall SH, O'Donoghue E, Bennett K, et al. Sputum *Candida albicans* presages FEV1 decline and hospital-treated exacerbations in cystic fibrosis. Chest. 2010;138:1186–95.
13. Marguet C, Favennec L, Matray O, et al. Clinical and microbiological efficacy of micafungin on *Geosmithia argillacea* infection in a cystic fibrosis patient. Med Mycol Case Rep. 2012;1:79–81.
14. Giraud S, Favennec L, Bougnoux ME, et al. *Rasamsonia argillacea* species complex: taxonomy, pathogenesis and clinical relevance. Future Microbiol. 2013;8:967–78.
15. Masoud-Landgraf L, Badura A, Eber E, et al. Modified culture method detects a high diversity of fungal species in cystic fibrosis patients. Med Mycol. 2014;52:179–86.
16. Cystic Fibrosis Patient Registry 2012 Annual Data Report Bethesda, Maryland: http://www.cff.org/UploadedFiles/research/ClinicalResearch/PatientRegistryReport/2012-CFF-Patient-Registry.pdf
17. ECFS Patient Registry Annual Data Report 2010: https://www.ecfs.eu/files/webfm/webfiles/File/ecfs_registry/ECFSPR_Report10_v12014_final_020614.pdf
18. Cystic fibrosis Australia, 15th Annual Report from the Australian Cystic Fibrosis Data Registry, Baulkham Hills NSW 2153. https://www.google.fr/search?q=20.+Cystic+fibrosis+Australia%2C+15th+Annual+Report+from+the+Australian+Cystic+Fibrosis+Data+Registry%2C+Baulkham+Hills+NSW+2153.&rlz=1C1TEUA_enFR575FR575&oq=20.+Cystic+fibrosis+Australia%2C+15th+Annual+Report+from+the+Australian+Cystic+Fibrosis+Data+Registry%2C+Baulkham+Hills+NSW+2153.&aqs=chrome..69i57.673j0j8&sourceid=chrome&es_sm=122&ie=UTF-8
19. Cystic Fibrosis Canada, The Canadian Cystic Fibrosis Registry 2011, Annual Report. Toronto, Canada http://www.cysticfibrosis.ca/wp-content/uploads/2013/10/Registry2011FINALOnlineEN.pdf
20. Noke C, Kumar Kabra S. Cystic Fibrosis in India 2006, Available at http://www.cfww.org/docs/pub/edition07/10_india.pdf
21. de Abreu e Silva, FA. Brazilian Cystic Fibrosis Study Group 2007, Available at http://www.cfww.org/pub/english/cfwnl/9/391/Brazilian_Cystic_Fibrosis_Study_Group
22. Kashirskaya NY, Kapranov NI. Management of cystic fibrosis in Russia. Available on http://www.cf-rf.ru/content/en/home.html
23. Güngör O, Tamay Z, Güler N, Erturan Z. Frequency of fungi in respiratory samples from Turkish cystic fibrosis patients. Mycoses. 2013;56(2):123–9.
24. Monaghan KG, Feldman GL. The risk of cystic fibrosis with prenatally detected echogenic bowel in an ethnically and racially diverse North American population. Prenat Diagn. 1999;19:604–9.
25. •• Armstead J, Morris J, Denning DW. Multi-country estimate of different manifestations of aspergillosis in cystic fibrosis. PLoS One. 2014;9:e98502. *This is representative of the unique studies that address ABPA disease in CF at the worldwide level. The authors estimate the burden of aspergillosis and ABPA in adult patients with CF in 30 countries reporting CF, using national and international CF registry data. Only about one-third of the cases of ABPA in CF are currently recorded, which indicates that it is significantly underdiagnosed. The authors propose recently published diagnostic criteria and methods to facilitate the identification of aspergillosis in CF, and thus to improve therapeutic management.*
26. Bargon J, Dauletbaev N, Köhler B, et al. Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients. Respir Med. 1999;93:835–8.
27. de Vrankrijker AM, van der Ent CK, van Berkhout FT, et al. *Aspergillus fumigatus* colonization in cystic fibrosis: implications for lung function? Clin Microbiol Infect. 2011;17:1381–6.

28. Parkins MD, Rendall JC, Elborn JS. Incidence and risk factors for pulmonary exacerbation treatment failures in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa. Chest. 2012;141:485–93.
29. Pentz A, Becker P, Masekela R, et al. The impact of chronic pseudomonal infection on pulmonary function testing in individuals with cystic fibrosis in Pretoria, South Africa. S Afr Med J. 2014;104:191–4.
30. Sudfeld CR, Dasenbrook EC, Merz WG, et al. Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis. J Cyst Fibros. 2010;9:110–6.
31. Baxter CG, Rautemaa R, Jones AM, et al. Intravenous antibiotics reduce the presence of *Aspergillus* in adult cystic fibrosis sputum. Thorax. 2013;68:652–7.
32. Sanders DB, Bittner RC, Rosenfeld M, et al. Pulmonary exacerbations are associated with subsequent FEV1 decline in both adults and children with cystic fibrosis. Pediatr Pulmonol. 2011;46:393–400.
33. Sequeiros IM, Jarad N. Factors associated with a shorter time until the next pulmonary exacerbation in adult patients with cystic fibrosis. Chron Respir Dis. 2012;9:9–16.
34. Whiting P, Al M, Burgers L, et al. Ivacaftor for the treatment of patients with cystic fibrosis and the G551D mutation: a systematic review and cost-effectiveness analysis. Health Technol Assess. 2014;18:1–106.
35. Tofte N, Jensen C, Tvede M, et al. Use of prophylactic voriconazole for three months after lung transplantation does not reduce infection with *Aspergillus*: a retrospective study of 147 patients. Scand J Infect Dis. 2012;44:835–41.
36. Harring TR, Nguyen NT, Liu H, et al. Liver transplantation in cystic fibrosis: a report from Baylor College of Medicine and the Texas Children's Hospital. Pediatr Transplant. 2013;17:271–7.
37. Danziger-Isakov LA, Worley S, Arrigain S, et al. Increased mortality after pulmonary fungal infection within the first year after pediatric lung transplantation. J Heart Lung Transplant. 2008;27:655–61.
38. Luong ML, Chaparro C, Stephenson A, et al. Pretransplant *Aspergillus* colonization of cystic fibrosis patients and the incidence of post-lung transplant invasive aspergillosis. Transplantation. 2014;97:351–7.
39. Chotirmall SH, Al-Alawi M, Mirkovic B, et al. *Aspergillus*-Associated Airway Disease, Inflammation, and the Innate Immune Response. BioMed Res Int. 2013;2013:1–14.
40. Jones AM, Horsley A, Denning DW. What is the importance of classifying *Aspergillus* disease in cystic fibrosis patients? Expert Rev Respir Med. 2014;29:1–4.
41. Denning DW, Pashley C, Hartl D, et al. Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy. 2014;4:14.
42. Borman AM, Palmer MD, Delhaes L, et al. Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. Med Mycol. 2010;48 Suppl 1:S88–97.
43. Nagano Y, Elborn JS, Millar BC, et al. Comparison of techniques to examine the diversity of fungi in adult patients with cystic fibrosis. Med Mycol. 2010;48:166–76.
44. Horré R, Marklein G, Siekmeier R, Reiffert S-M. Detection of hyphomycetes in the upper respiratory tract of patients with cystic fibrosis: Hyphomycetes in the upper respiratory tract of CF patients. Mycoses. 2011;54:514–22.
45. Lebecque P, Leonard A, Huang D, et al. *Exophiala* (*Wangiella*) *dermatitidis* and cystic fibrosis - Prevalence and risk factors. Med Mycol. 2010;48 Suppl 1:S4–9.
46. Nielsen SM, Kristensen L, Søndergaard A, et al. Increased prevalence and altered species composition of filamentous fungi in respiratory specimens from cystic fibrosis patients. APMIS. 2014; Ahead of print.
47. Mortensen KL, Jensen RH, Johansen HK, et al. Aspergillus Species and Other Molds in Respiratory Samples from Patients with Cystic Fibrosis: a Laboratory-Based Study with Focus on *Aspergillus fumigatus* Azole Resistance. J Clin Microbiol. 2011;49:2243–51.
48. Burgel PR, Baixench MT, Amsellem M, et al. High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. Antimicrob Agents Chemother. 2012;56:869–74.
49. Paugam A, Baixench MT, Demazes-Dufeu N, et al. Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. Med Mycol. 2010;48 Suppl 1:S32–6.
50. Manso E, Cariani L, Fiscarelli E, et al. Isolation of filamentous fungi in the sputum of cystic fibrosis patients in Italy: State of Art and epidemiology. 2nd Meeting of the ECMM/ISHAM Working Group "Fungal respiratory infections in Cystic Fibrosis" (Fri-CF) Angers (France), 2011, September 1st - 2nd.
51. Kondori N, Gilljam M, Lindblad A, et al. High Rate of *Exophiala dermatitidis* Recovery in the Airways of Patients with Cystic Fibrosis Is Associated with Pancreatic Insufficiency. J Clin Microbiol. 2011;49:1004–9.
52. Levy I, Grisaru-Soen G, Lener-Geva L, et al. Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients. Isr Emerg Infect Dis. 2008;14:378–84.
53. Costa Caneiro AC, Moreira Lemos AC, Arruda SM, et al. Prevalence of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis in the state of Bahia. Braz J Bras Pneumol. 2008;34:900–6.
54. Sharma VK, Raj D, Xess I, et al. Prevalence and risk factors for allergic bronchopulmonary aspergillosis in Indian Children with cystic fibrosis. Indian Pediatr. 2014;51:295–7.
55. Blyth CC, Middleton PG, Harun A, et al. Clinical associations and prevalence of *Scedosporium* spp. in Australian cystic fibrosis patients: identification of novel risk factors? Med Mycol. 2010;48 Suppl 1:S37–44.
56. Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. Chest. 2006;130:222–6.
57. Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. J Allergy Clin Immunol. 2013;132:560–6.
58. Mortensen KL, Johansen HK, Fuursted K, et al. A prospective survey of *Aspergillus* spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. Eur J Clin Microbiol Infect Dis. 2011;30:1355–63.
59. Fischer J, van Koningsbruggen-Rietschel S, Rietschel E, et al. Prevalence and molecular characterization of azole resistance in Aspergillus spp. isolates from German cystic fibrosis patients. J Antimicrob Chemother. 2014;69:1533–6.
60. Astvad KM, Jensen RH, Hassan TM, et al. First detection of TR46/Y121F/T289A and of TR34/L98H in azole naïve patients in Denmark despite negative findings in the environment. Antimicrob Agents Chemother. 2014; Ahead of print.
61. Rougeron A, Giraud S, Razafimandimby B, et al. Different colonization patterns of *Aspergillus terreus* in patients with cystic fibrosis. Clin Microbiol Infect. 2014;20:327–33.
62. Symoens F, Haase G, Pihet M, et al. Unusual *Aspergillus* species in patients with cystic fibrosis. Med Mycol. 2010;48 Suppl 1:S10–6.
63. Baxter CG, Moore CB, Jones AM, et al. IgE-mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. Chest. 2013;143:1351–7.
64. Vanhee LM, Nelis HJ, Coenye T. What can be learned from genotyping of fungi? Med Mycol. 2010;48 Suppl 1:S60–9.

65. Reihill JA, Moore JE, Elborn JS, Ennis M. Effect of *Aspergillus fumigatus* and *Candida albicans* on pro-inflammatory response in cystic fibrosis epithelium. J Cyst Fibros. 2011;10:401–6.
66. Osherov N. Interaction of the pathogenic mold *Aspergillus fumigatus* with lung epithelial. Cells Front Microbiol. 2012;3:346.
67. Gangell C, Gard S, Douglas T, et al. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. Clin Infect Dis. 2011;53:425–32.
68. Gammelsrud KW, Sandven P, Høiby EA, et al. Colonization by *Candida* in children with cancer, children with cystic fibrosis, and healthy controls. Clin Microbiol Infect. 2011;17:1875–81.
69. Kondori N, Lindblad A, Welinder-Olsson C, et al. Development of IgG antibodies to *Exophiala dermatitidis* is associated with inflammatory responses in patients with cystic fibrosis. J Cyst Fibros. 2014;13:391–9.
70. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev. 2010;23:299–323.
71. Delhaes L, Borman A, Symoens F, et al. Towards standardization for mycological examination of sputum samples from Cystic Fibrosis patients: From the French multicenter experience to an international study. 2nd Meeting of the ECMM/ISHAM Working Group "Fungal respiratory infections in Cystic Fibrosis" (Fri-CF) Angers (France), 2011, September 1st - 2nd.
72. Pashley CH, Fairs A, Morley JP, et al. Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. Med Mycol. 2012;50:433–8.
73. Lackner M, Klaassen CH, Meis JF, et al. Molecular identification tools for sibling species of *Scedosporium* and *Pseudallescheria*. Med Mycol. 2012;50:497–508.
74. Kantarcioglu AS, de Hoog GS, Guarro J. Clinical characteristics and epidemiology of pulmonary pseudallescheriasis. Rev Iberoam Micol. 2012;29:1–13.
75. 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;43:4930–42.
76. Borghi E, Iatta R, Manca A, et al. Chronic airway colonization by *Scedosporium apiospermum* with a fatal outcome in a patient with cystic fibrosis. Med Mycol. 2010;48 Suppl 1:S108–13.
77. Zouhair R, Rougeron A, Razafimandimby B, et al. Distribution of the different species of the *Pseudallescheria boydii* /*Scedosporium apiospermum* complex in French patients with cystic fibrosis. Med Mycol. 2013;51:603–13.
78. Rougeron A, Schuliar G, Leto J, et al. Human-impacted areas of France are environmental reservoirs of the *Pseudallescheria boydii/Scedosporium apiospermum* species complex. Environ Microbiol. 2014. Epub ahead of print.
79. Harun A, Gilgado F, Chen SC, Meyer W. Abundance of *Pseudallescheria*/*Scedosporium* species in the Australian urban environment suggests a possible source for scedosporiosis including the colonization of airways in cystic fibrosis. Med Mycol. 2010;48 Suppl 1:S70–6.
80. 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;14:282–90.
81. Bernhardt A, Sedlacek L, Wagner S, et al. Multilocus sequence typing of *Scedosporium apiospermum* and *Pseudallescheria boydii* isolates from cystic fibrosis patients. J Cyst Fibros. 2013;12:592–8.
82. Packeu A, Lebecque P, Rodriguez-Villalobos H, et al. Molecular typing and antifungal susceptibility of *Exophiala* isolates from patients with cystic fibrosis. J Med Microbiol. 2012;61:1226–33.
83. Barton RC, Borman AM, Johnson EM, et al. Isolation of the fungus *Geosmithia argillacea* in sputum of people with cystic fibrosis. J Clin Microbiol. 2010;48:2615–7.
84. Cimon B, Challier S, Béguin H, et al. Airway colonization by *Acrophialophora fusispora* in patients with cystic fibrosis. J Clin Microbiol. 2005;43:1484–7.
85. Lu Q, van den Ende AH, de Hoog GS, et al. Reverse line blot hybridisation screening of *Pseudallescheria*/*Scedosporium* species in patients with cystic fibrosis. Mycoses. 2011;54 Suppl 3:5–11.
86. Charlson ES, Diamond JM, Bittinger K, et al. Lung-enriched organisms and aberrant bacterial and fungal respiratory microbiota after lung transplant. Am J Respir Crit Care Med. 2012;186:536–45.
87. Delhaes L, Monchy S, Fréalle E, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community–implications for therapeutic management. PLoS One. 2012;7(4):e36313.
88. Huang YJ, Charlson ES, Collman RG, et al. The role of the lung microbiome in health and disease. A National Heart, Lung, and Blood Institute workshop report. Am J Respir Crit Care Med. 2013;187:1382–7.
89. Harrison MJ, Twomey KB, McCarthy Y, et al. The role of second-generation sequencing in describing the fungal microbiota in the adult cystic fibrosis (CF) airway and its correlation with clinical phenotype. J Cyst Fibros. 2013;S16:WS8.1. *Official Journal of the European Cystic Fibrosis Society.*
90. Mounier J, Gouëllo A, Keravec M, et al. Use of denaturing high-performance liquid chromatography (DHPLC) to characterize the bacterial and fungal airway microbiota of cystic fibrosis patients. J Microbiol. 2014;52:307–14.
91. Goffard A, Lambert V, Salleron J, et al. Virus and cystic fibrosis: rhinoviruses are associated with exacerbations in adult patients. J Clin Virol. 2014;60:147–53.
92. Lim YW, Evangelista JS, Schmieder R, et al. Clinical insights from metagenomic analysis of sputum samples from patients with cystic fibrosis. J Clin Microbiol. 2014;52:425–37.
93. Carmody LA, Zhao J, Schloss PD, et al. Changes in cystic fibrosis airway microbiota at pulmonary exacerbation. Ann Am Thorac Soc. 2013;10:179–87.
94. Fodor AA, Klem ER, Gilpin DF, et al. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. PLoS One. 2012;7:e45001.
95. Cardenas PA, Cooper PJ, Cox MJ, et al. Upper airways microbiota in antibiotic-naïve wheezing and healthy infants from the tropics of rural Ecuador. PLoS One. 2012;7(10):e46803.
96. Charlson ES, Bittinger K, Chen J, et al. Assessing bacterial populations in the lung by replicate analysis of samples from the upper and lower respiratory tracts. PLoS One. 2012;7:e42786.
97. Filkins LM, Hampton TH, Gifford AH, et al. Prevalence of streptococci and increased polymicrobial diversity associated with cystic fibrosis patient stability. J Bacteriol. 2012;194:4709–17.
98. Conrad D, Haynes M, Salamon P, et al. Cystic fibrosis therapy: a community ecology perspective. Am J Respir Cell Mol Biol. 2013;48:150–6.
99. Hernández-Hernández F, Fréalle E, Caneiro P, et al. Prospective multicenter study of *Pneumocystis jirovecii* colonization among cystic fibrosis patients in France. J Clin Microbiol. 2012;50:4107–10.
100. Delhaes L, Bouchara JP, and ECMM/ISHAM Working Group on "Filamentous fungi and chronic respiratory infections in cystic fibrosis". 2011; https://www.ecfs.eu/special-projects/mucofong-international-project