ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2017, 0, 1–8
doi: 10.1093/mmy/myx108
Advance Access Publication Date: 0 2017
Original Article

Original Article

Direct detection of *Exophiala* and *Scedosporium* species in sputa of patients with cystic fibrosis

Min Chen[1,2,3,*], Nahid Kondori[4], Shuwen Deng[7], A. H. G. Gerrits van den Ende[2], M. Lackner[5], Wanqing Liao[1] and G. S. de Hoog[1,2,3,6,*]

[1]Department of Dermatology, Shanghai Key Laboratory of Molecular Medical Mycology, Shanghai Institute of Medical Mycology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, 200003, China, [2]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, [3]Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands, [4]Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, Sweden, [5]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria, [6]Peking University First Hospital, Research Center for Medical Mycology, Beijing, China; Department of Basic Biology, University of Paraná, Curitiba, Brazil and [7]Department of Medical Microbiology, People's Hospital of Suzhou National New & Hi-Tech Industrial Development Zone, Jiangsu, China

*To whom correspondence should be addressed. Min Chen. E-mail: chenmin9611233@163.com
G. S. de Hoog. E-mail: s.hoog@westerdijkinstitute.nl

Received 26 June 2017; Revised 31 August 2017; Accepted 5 October 2017; Editorial Decision 17 September 2017

Abstract

Detection of species of *Exophiala* and *Scedosporium* in the respiratory tracts of cystic fibrosis (CF) patients remains controversial because of highly variable results. The results of our study suggested a significantly higher prevalence and more complex colonization than previously estimated. Approximately 17% (27/162) of clinical sputum samples were found to be positive for *Exophiala dermatitidis* and 30% (49/162) were positive for *Scedosporium apiospermum* / *S. boydii* species complex determined by reverse line blot (RLB) hybridization. In contrast, only 14.2% (23/162) and 1.2% (2/162) of clinical sputa were positive for *E. dermatitidis* and *S. apiospermum* / *S. boydii* species complex when tested by culture, respectively. Molecular detection methods, such as loop-mediated isothermal amplification (LAMP) or reverse line blot (RLB) hybridization, have the potential to become powerful alternatives to selective culture, providing a more realistic understanding on the prevalence of *E. dermatitidis* and *S. apiospermum* / *S. boydii* species complex in the respiratory tract of CF patients.

Key words: *Exophiala dermatitidis*, *Scedosporium*, cystic fibrosis, sputum.

Introduction

Affecting over 70,000 individuals worldwide, cystic fibrosis (CF) is the most common genetically inherited disease among Caucasian populations.[1] CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) protein, which leads to the production of thick and sticky

bronchial mucus that may facilitate microbial accumulation and colonization.[2,3] In addition to bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, several fungi colonize the respiratory tracts of CF patients,[3,4] though their prevalence and pathogenicity remain controversial.[5,6] *Aspergillus fumigatus* is the filamentous fungus most commonly isolated from CF patients and is capable of precipitating a chronic allergic inflammatory response or invasive infection after lung transplantation.[4] However, the fungal biota colonizing the lungs of CF patients are more complex and include various non-*Aspergillus* filamentous fungi such as *Scedosporium apiospermum*[7–9] and *Exophiala dermatitidis*.[4,10–12] In the genus *Scedosporium*, *S. apiospermum*, and *S. boydii* have recently been aggregated as the '*S. apiospermum* complex' because of their genetic similarity and the lack of clinical differences observed between sibling species.[13,14]

The black yeast *E. dermatitidis* is another filamentous fungus regularly involved in colonizing the respiratory tract of CF patients.[7–12] Persistent colonization and repeated infection with *E. dermatitidis* or *S. apiospermum* / *S. boydii* may occur in CF patients over decades.[10–12] *Scedosporium* species are resistant to many antifungal agents including amphotericin B.[15] In addition to *Aspergillus* and *Candida*, patients with CF were observed to be colonized by *E. dermatitidis* or *Scedosporium*; however, this may be an artifact because published studies that detected them in combination are scant. In addition, culture only has a limited sensitivity, these species usually remain undetected because they are easily outcompeted by *Aspergillus* and *Candida* species.[15,17] Possible correlations with underlying diseases remain uncertain because of limited amount of data available. An exceptionally high recovery rate of approximately 20% of *E. dermatitidis* was recently reported by incubating sputa on erythritol-chloramphenicol agar (ECA) medium from Sweden, but conventional culture-based diagnosis strategies are hampered by the mucoid consistency of CF sputum or bronchoalveolar lavage (BAL) specimens.[18] Thus, frequencies of accompanying fungi may be underestimated,[12,18] though recent applications of semi-selective media, which inhibit rapidly growing *Aspergillus* and *Candida*, enable fungi with delayed growth to be revealed.[19,20] Detection by culture still requires viable cells in the samples and highly skilled laboratory technicians for successful cultivation.

Molecular techniques have improved detection and thereby our understanding of fungal colonization of the respiratory tracts of CF patients.[21,22] Among these methods, loop-mediated isothermal amplification (LAMP)[21] and reverse line blot (RLB) hybridization[7,21] have shown added value to elucidation of the epidemiology of less common colonizers in the CF patient population.[7,21,22] Molecular detection of *E. dermatitidis* in clinical samples was not conducted frequently to date. Therefore, we compared the detection of *E. dermatitidis*, *S. apiospermum*, and *S. boydii* from CF sputa by two molecular approaches (polymerase chain reaction [PCR]-RLB and LAMP) and culture.[20] The aim of this study is to improve our understanding of the prevalence of *E. dermatitidis*, *S. apiospermum*, and *S. boydii* in the respiratory tracts of CF patients.

Methods

Clinical specimens

Between September and December 2012, a total of 162 sputum specimens from 103 CF patients were collected by the Department of Clinical Microbiology, Sahlgrenska University Hospital, Sweden. Each sample was divided into two portions: one part for the isolation of conventional or semi-selective culture, the other part for molecular detection by PCR-RLB and LAMP assays.

Isolation of fungi from sputum samples by culture

Approximately 1 ml of sputum specimens from each CF patients was routinely cultured on Sabouraud glucose agar (SGA), CHROMagar Candida, maltose agar, and ECA plates. Sputum was liquefied by the addition of pancreatin (10 mg/ml) in a volume ratio of 1/1, vortexed and incubated at 20°C for 5 min prior to culture. Cultures were then incubated at 30°C and examined for fungal growth for up to 20 days. Isolated fungi were identified to the species level using morphological criteria, the ability to grow at present at 42°C and on mycobiotic agar plates supplemented with cycloheximide. A set of reference strains included 46 strains (Table 1) of *E. dermatitidis* and another 22 *Exophiala* species that are taxonomically close to *E. dermatitidis* were obtained from Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, for evaluation of sensitivity and specificity of designed *E. dermatitidis* species-specific primer sets of RLB and LAMP assays according to the previous analysis.[23,24] All reference strains were grown on potato dextrose agar (PDA) at 30°C for 7 days prior to use.

Molecular detection

Genomic DNA from reference strains was extracted using cetyltrimethylammonium bromide (CTAB) as described previously.[14] To extract fungal DNA directly from clinical sputum, a High Pure PCR Template Preparation Kit (Roche Inc., Mannheim, Germany) was used according to

Table 1. Fungal isolates (n = 46) analyzed with PCR-RLB and LAMP assays for detection of *E. dermatitidis.*

Serial number	Strains	Species	Source	PCR-RLB detection	LAMP detection
1	CBS 207.35	*Exophiala dermatitidis*	Clinical strain	+	+
2	CBS 424.67	*Exophiala dermatitidis*	Clinical strain	+	+
3	CBS 632.69	*Exophiala dermatitidis*	Environmental strain	+	+
5	CBS 314.90	*Exophiala dermatitidis*	Environmental strain	+	+
7	CBS 292.49	*Exophiala dermatitidis*	Environmental strain	+	+
8	CBS 109140	*Exophiala dermatitidis*	Environmental strain	+	+
9	CBS 109146	*Exophiala phaeomuriformis*	Environmental strain	-	-
11	CBS 134012	*Exophiala phaeomuriformis*	Environmental strain	-	-
12	CBS 124194	*Exophiala phaeomuriformis*	Environmental strain	-	-
13	CBS 121744	*Exophiala phaeomuriformis*	Environmental strain	-	-
14	CBS 120563	*Exophiala phaeomuriformis*	Clinical strain	-	-
15	CBS 120555	*Exophiala phaeomuriformis*	Environmental strain	-	-
16	CBS 899.68	*Exophiala spinifera*	Clinical strain	-	-
17	CBS 425.92	*Exophiala spinifera*	Environmental strain	-	-
18	CBS 269.28	*Exophiala spinifera*	ND	-	-
19	CBS 668.76	*Exophiala exophialae*	Environmental strain	-	-
20	CBS 507.90	*Exophiala jeanselmei*	Clinical strain	-	-
22	CBS 482.92	*Exophiala angulospora*	Environmental strain	-	-
23	CBS 352.52	*Exophiala bergeri*	Clinical strain	-	-
24	CBS 353.52	*Exophiala bergeri*	Clinical strain	-	-
25	CBS 526.76	*Exophiala bergeri*	Clinical strain	-	-
26	CBS 402.95	*Exophiala mesophila*	Environmental strain	-	-
27	CBS 836.95	*Exophiala mesophila*	Environmental strain	-	-
28	CBS 158.58	*Exophiala castellanii*	Clinical strain	-	-
29	CBS 102400	*Exophiala lecanii-corni*	Environmental strain	-	-
30	CBS 232.39	*Exophiala lecanii-corni*	Clinical strain	-	-
31	CBS 256.92	*Exophiala psychrophila*	ND	-	-
32	CBS 191.87	*Exophiala psychrophila*	ND	-	-
33	CBS 521.82	*Exophiala alcaophila*	Environmental strain	-	-
34	CBS 157.67	*Exophiala salmonis*	ND	-	-
35	CBS 537.73	*Exophiala pisciphila*	Clinical strain	-	-
36	CBS 101610	*Exophiala pisciphila*	Environmental strain	-	-
37	CBS 520.76	*Exophiala moniliae*	ND	-	-
38	CBS 109807	*Exophiala oligosperma*	Clinical strain	-	-
39	CBS 109811	*Exophiala opportunistica*	Environmental strain	-	-
40	CBS 109812	*Exophiala castellanii*	Environmental strain	-	-
41	CBS 115142	*Exophiala cancerae*	Environmental strain	-	-
42	CBS 115831	*Exophiala xenobiotica*	ND	-	-
43	CBS 117641	*Exophiala xenobiotica*	Clinical strain	-	-
44	CBS 118722	*Exophiala alcalophila*	Environmental strain	-	-
45	CBS 119638	*Exophiala heteromorpha*	Environmental strain	-	-
46	CBS 119912	*Exophiala aquamarina*	Clinical strain	-	-

ND, no data; +, positive result; -, negative result.

the manufacturer instructions with two modifications: eight acid-washed glass beads (diameter 10 mm) were separately added into 1 ml of each clinical sample and thoroughly shaken using a MoBio vortex for 10 min, after which proteinase K (40 μl) digestion was performed at 70°C for 1 hour instead of 10 min. The quality of extraction of fungal DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). DNA Samples were stored at −20°C until use. During the period of the molecular detection, we simultaneously collected 1 ml human sputum from one single healthy donor in Utrecht, The Netherlands, which was tested as a negative control. Fungal cells of *S. apiospermum* type strain CBS 116899^T (ranging from 8600 cells/ml to 8.6 cells/ml) and *E. dermatitidis* strain CBS 207.35^T (ranging from 4,800 cells/ml to 4.8 cells/ml) were separately added into sputa from the

Table 2. Primers and probes used in this study.

Primer or probe	Oligonucleotides (5′–3′)	Specificity	Reference
Probes used for PCR-RLB:			
Apio_P	amino-GAGGTAAGTTTTTGGCTAAAGC	*S. apiospermum*	18
Boy_P	amino-CGAGGTAAGTTTTTGGTTCAAA	*S. boydii*	18
PE_P	amino-CTGCTGTCGCTGGGACTAACAAA	*E. dermatitidis*	This study
Primers used for LAMP:			
FIP_SA (F1c+F2)	CAACCGGCCCGTGGCTTTAGCTTGAGCGCATGAGCGTC	*S. apiospermum*	18
BIP_SA (B1c+B2	GTGTCATCCGGCCTCCGTTGTTTGTTGCCCGAAGCCTAT	*S. apiospermum*	18
F3_SA	ATGGCACTTCTGAACTCCAG	*S. apiospermum*	18
B3_SA	GGGCTCGAGATCTACAAGGA	*S. apiospermum*	18
FIP_SB (F1c+F2)	AACCAGCCCGTGGTTTGAACCCTTGAGCGCATGAGCGTT	*S. boydii*	18
BIP_SB (B1c+B2)	GTGTGGTGTCATCCAGCCTCCTTTGTTGCCCGAAGCCTAT	*S. boydii*	18
F3_SB	ATGGCACTTCTGAACTCCAG	*S. boydii*	18
B3_SB	CGAGATCTACAAGGACAGCG	*S. boydii*	18
FIP_ED (F1c+F2)	TCCGCAACCTACTACAGTGAATGGGTCTTCAGCTTGGGTCATTGA	*E. dermatitidis*	This study
BIP_ED (B1c+B2)	TGCAATTGAGACACTTACCAGGCCACGACCATAAAATCAGTAGCGT	*E. dermatitidis*	This study
F3_ED	GGACTTGTACACAGTCTCATCA	*E. dermatitidis*	This study
B3_ED	ATGCGACACAAGCACCAG	*E. dermatitidis*	This study

healthy donor for sensitivity testing in spiked specimens and as positive controls, with 10 times dilution of fungal cells.

PCR-RLB

A primer set for *E. dermatitidis* targeting the *BT2* region of the β-tubulin gene was designed (Table 2). Additionally, the primers developed by Lu et al. were used for *Scedosporium*.[7,21] Two species-specific probes and a species complex specific probe PE_P specific for *Exophiala* species were labeled with C6-amino linker at the 5′ end. All primers and probes were BLAST searched against nucleotide databases available at GenBank and the CBS in-home database, to verify specificity. PCR amplification of *BT2* was performed consisting of 1 × GoTaq Green Master Mix (Promega, Fitchburg, WI, USA), 400 nmol of primers and 2 μl template DNA (100 ng/μl) to 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 56°C for 45 s, 72°C for 90 s and post-elongation at 72°C for 7 min. Sensitivity tests were repeated twice using 10-fold serial dilutions (ranging from 200 ng/ml to 2 pg/ml) of template DNA extracted from reference strains and spiked sputum specimens (ranging from 4800 fungal cells/ml to 4.8 fungal cells/ml).

LAMP

Assays for detection of *Scedosporium* species were performed as previously described.[7,21] LAMP used in the present study detects *E. dermatitidis* with a combination of the F3, B3, FIP, and BIP primers designed using Primer Explorer V4 software (http://primerexplorer.jp/e/) based on the partial sequence (*BT2*) in the β-tubulin region (GenBank KF928572) of type strain of *E. dermatitidis*, CBS 207.35^T because fragment *BT2* of the β-tubulin gene provided more information than ITS as a target for the identification of *E. dermatitidis* and *Scedosporium* species.[21] The sequences of the primer sets used in this study are listed in Table 2. All LAMP reagents were bought from New England Biolabs (Ipswitch, MA, USA). LAMP reactions were conducted in 25 μl reaction volumes using a PCR machine containing the following reagents: 4 μl each FIP and BIP (10 pmol), 0.5 μl each F3 and B3 (10 pmol), 0.5 μl $MgSO_4$ (80 mM), 2 μl dNTP (5 mM), 4 μl of 5 M betaine (Sigma, Zwijndrecht, The Netherlands), 2.5 μl of 10 × Thermo buffer [20 mM Tris-HCl, pH 8.8, 10 mM KCl, 2 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100], 1 μl *Bst* DNA polymerase (New England BioLabs), 5 μl double-distilled water (ddH_2O), and 500 pg template DNA (1 μl). The reaction was performed by heating samples to 65°C for 90 min, followed by 85°C for 2 min to terminate the reaction. Similarly, the sensitivity tests were repeated twice using template DNA extracted from reference strains and spiked sputum specimens, respectively.

Statistical analysis

The statistical package for SAS version 9.3 (SAS Institute, Cary, NC, USA) was used to analyze the data. The obtained data were evaluated by percentage ratios. Comparison of percentage ratios yielded by molecular assays was accomplished using the χ^2 test. A P value of less than .05 was considered to be statistically significant.

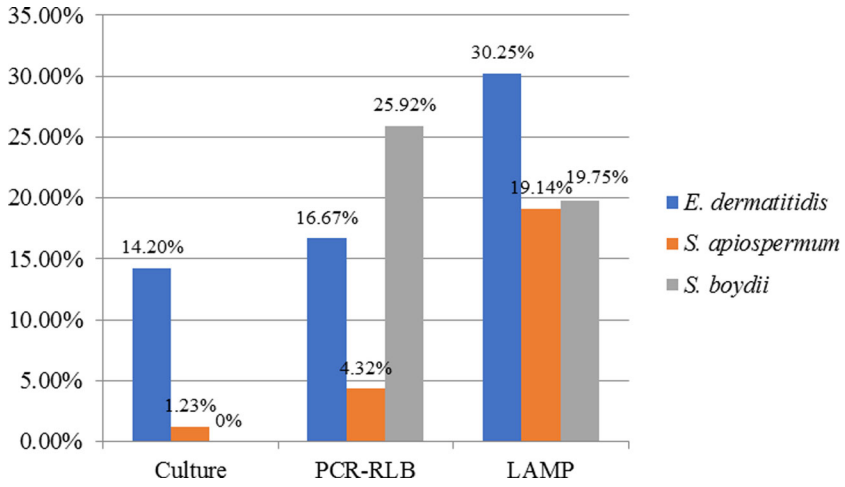

Figure 1. Comparison of results yielded from culture, PCR-RLB and LAMP.

Results

A total of 162 clinical sputum samples were verified for growth of *E. dermatitidis* and *Scedosporium* species. *E. dermatitidis* was detected in 23 sputum samples (14.2%) using selective ECA culture, while *S. apiospermum* / *S. boydii* was detected in two samples (1.2%) using nonselective routine media.

Detection by PCR-RLB

The specificity and sensitivity of the PCR-RLB assay for *S. apiospermum* and *S. boydii* has previously been reported to be 100% and approximately 5.0×10^3 copies of genomic DNA for specificity and sensitivity.[21] Genomic DNA from 46 reference and clinical strains covering each of the main genotypes of *E. dermatitidis* were analyzed to determine the specificity of our newly-designed PCR-RLB assay for *E. dermatitidis* (Table 1). No cross-reaction was observed, including DNA of a sibling species, *E. phaeomuriformis*, CBS 109146. Thus, specificity of the PCR-RLB assay was 100% in our study. The detection limit of PCR-RLB for genomic DNA of *E. dermatitidis* was approximately 1.1×10^3 genomic DNA copies (20 pg) per run. Sensitivity of the PCR-RLB assay was determined using spiked samples, in which concentrations above 4.8×10^3 *E. dermatitidis* spores/ml were positive.

The PCR-RLB assay detected *E. dermatitidis* in approximately 17% of clinical samples (Fig. 1). Among the 23 *E. dermatitidis* culture -positive samples, 21 samples were positive by the PCR-RLB assay. The PCR-RLB assay yielded approximately 30% positive *S. apiospermum* / *S. boydii*, of which 4% were *S. apiospermum* and 26% were *S. boydii*, respectively (Fig. 1). Only one of the two *Scedosporium* culture-positive samples was confirmed to be positive by PCR-RLB assay.

Detection by LAMP

The specificity and sensitivity of the LAMP assay for *S. apiospermum* / *S. boydii* were previously shown to be 100% and approximately 5.0×10^2 copies of genomic DNA, respectively.[21] In this study, no cross-reaction with any of the tested *Exophiala* species was observed after 90 minutes of the LAMP reaction (Table 1). The sensitivity of the LAMP assay was then further evaluated with spiked sputum samples, in which concentrations above 4.8×10^2 *E. dermatitidis* cells/ml were positive.

A total of 162 sputum samples, including two *Scedosporium*-culture positive samples and 23 *E. dermatitidis*-culture positive samples, were analyzed by our LAMP assay. LAMP assay of the DNA extracted from sputa yielded 32% of positive *S. apiospermum* / *S. boydii* samples. Both *Scedosporium* culture-positive samples were confirmed to be positive by LAMP. Additionally, the LAMP assay detected *E. dermatitidis* in 30% of clinical samples, and 49 samples were *E. dermatitidis* positive by LAMP, including the *E. dermatitidis*-culture positive samples.

Comparison of results yielded from culture, PCR-RLB, and LAMP

Of the 162 sputum samples from CF patients, 12 clinical samples were positive by both PCR-RLB and LAMP, whereas five LAMP-negative samples were PCR-RLB positive, 36 PCR-RLB-negative samples were positive with LAMP, and 109 of the total samples were negative by both PCR-RLB and LAMP. For *E. dermatitidis*, 14% of the samples were positive by culture, 17% by PCR-RLB, 30% by LAMP and 14% were positive by both PCR-RLB and LAMP. For *Scedosporium*, LAMP had a higher detection rate (27%) than PCR-RLB ($P < .0001$). Similarly, the

LAMP assay also provided a higher detection rate (30%) for *E. dermatitidis* ($P = .0039$).

Discussion

Previous studies revealed a highly variable prevalence of *Exophiala* and *Scedosporium* species in the respiratory tracts of CF patients (Table 3).[8,12,16,17,25–31] Lack of standardization of the procedures for detection on filamentous fungi in sputum samples from CF patients may be a possible cause for the variable reported data.[31] Moreover, routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence.[18] Notably, LAMP technology can rapidly and accurately amplify genomic DNA in an isothermal step from partially processed and/or nonprocessed samples in approximately 1 hour without the need for sophisticated equipment and the products can be assessed by the naked eye.[32] The PCR-RLB assay has been used to detect *Scedosporium* species in the respiratory samples of CF patients.[7] Thus, *E. dermatitidis*-specific and *Scedosporium*-specific molecular assays, PCR-RLB and LAMP, and culture assays were used to detect the prevalence of *E. dermatitidis* and *Scedosporium* species in 162 clinical sputa in a double blind experiment.

We noticed that only two samples were positive for *S. apiospermum* / *S. boydii* and 23 samples were positive for *E. dermatitidis* upon culture assay in our study. This may due to cultivation of *Exophiala* by selective medium, and *Scedosporium* culture was nonselective. Some filamentous fungi such as *A. fumigatus* can overlap other fungi such as *Scedosporium* species on non-selective medium because they grow faster than other non-*Aspergillus* fungi that colonize the respiratory tract of CF patients.[4] Generally, our results are similar to the recovery rates reported using comparable media for *Scedosporium* species and *E. dermatitidis*.[10–12,33,34] In contrast, positive recovery rates for *Scedosporium* tend to increase at approximately 15% when the sputum samples processed by benomyl-based media.[33]

In addition to *A. fumigatus*, *E. dermatitidis* and species of the *S. apiospermum* complex are the most frequent molds recovered from respiratory secretions of CF patients, but their recovery rates vary greatly.[18,34,35] With its ability to grow at 37°C, *E. dermatitidis* has a worldwide distribution in hot environments, not only in CF respiratory tracts[10,26,36,37] but also warm indoor environments such as steambaths[38] and dishwashers.[39] Although the significance of *E. dermatitidis* in causing disease in patients with CF remains unclear, this fungus is known to cause severe invasive fungal infections.[40] With selective ECA medium, the prevalence of *E. dermatitidis* ranges from 5% in Germany to 19% in Sweden.[11,12,18] Published recovery rates in Sweden were exceptionally high, around 17–19% by selective culture.[12,37] Our data, which were also from Sweden, showed a high positive rate of 14%, confirming that this detection rate is rather consistent. PCR-RLB and LAMP assays yielded 15 and 30% of *E. dermatitis* detection, respectively, which was significantly higher than culture. The prevalence in Sweden is 2 to almost 20 times higher than in European countries, which is possibly because of differences in indoor conditions. For a better understanding of *E. dermatitidis* prevalence among CF patients, further multi-center epidemiological studies are needed.

Regarding the positive rate for *Scedosporium* species, the results of our molecular assays showed that approximately 10.5% of the CF airway samples were positive for the *S. apiospermum* complex using the PCR-RLB assay, and the positive rate yielded by the LAMP assay was approximately 31.5%. This positive rate for *S. apiospermum* / *S. boydii* was lower than the maximum reported rate of 61.5% that was obtained using PCR-RLB.[7] The high detection rate yielded by LAMP is likely closest to actual value, and LAMP technology would be the most efficient for amplification of target DNA from partially processed and/or non-processed CF specimens.[32] The selective ECA medium yielded a comparatively high positive recovery rate of *E. dermatitidis*, and this figure might be tripled to approach the real prevalence generated by molecular methods. However, LAMP may have a higher risk of false positive results than PCR-RLB, which might be caused by pre-PCR contamination of samples, as the method is highly sensitive. Careful precautions to prevent cross-contamination need to be taken during sample collection, and the methods should be executed using separated rooms and filtered tips. In general, analysis of larger numbers of samples is required to determine their prevalence in clinical samples, and the method should be repeated several times to enable for reliable determination of the presence in a single patient. The recent application of next-generation sequencing (NGS) technology has allowed us to conduct meta-genomic studies,[41] which may provide more comprehensive insight into chronic fungi colonization in airways of CF patients. Finally, we noticed two samples with positive culture results for *E. dermatitidis* were negative upon both PCR-RLB and LAMP assays. The quality of the fungi DNA extracted from respiratory sputum samples is likely poor, and sputa from CF patients might contain PCR inhibiting substances, both of which could influence the performance of the molecular assays.

In summary, current data on the prevalence of *Exophiala* and *Scedosporium* in the respiratory tract of CF patients remains variable and controversial. The results of our comparative detection study suggested a significantly higher prevalence and more complex colonization than previously

Table 3. Overview of *E. dermatitidis* and *Scedosporium* detection in sputum of CF patients.

Time	Geography	Samples (n)	*E. dermatitidis*	*Scedosporium* species	Reference
1991	Germany	121	SGA + CDA: 9%	NA	25
1998	Australia	52	NA	IGS-PCR: 10% (*S. apiospermum* complex)	26
2000	France	128	NA	Culture using filtrate antigens:8.6% (*S. apiospermum* complex)	16
2003	Germany	94	SGA + ECA: 1%	NA	27
2004	Germany	81	ECA: 6.2%	NA	32
2009	Germany	42	NA	SceSel: 14% (*S. apiospermum* complex)	28
2009	Australia, Germany, France, Spain, USA	162	NA	SGA: 14% (*S. prolificans*)	17
2010	Belgium	154	ECA: 5.8%	NA	11
2011	Sweden	97	ECA: 19%	NA	12
2011	France	59	NA	SceSel + PCR-RLB: 61.5% (*S. apiospermum* complex)	7
2013	France	50	NA	SGA + YPGA: 24% (*S. apiospermum* complex)	8
2015	Germany	2346	NA	SceSel: 3.1% (*S. apiospermum* complex)	20
2016	Germany	3186	ECA: 2.8%	SceSel: 2.4% (*S. apiospermum* complex)	30
2017	Sweden	162	SGA, ECA, LAMP, RLB: 17–30%	SGA, LAMP, RLB: 30–39% (*S. apiospermum* complex)	This study

Abbreviations used: CDA, Czapeck Dox Agar; ECA, Erythritol Chloramphenicol Agar; NA, not applied; SGA, Sabouraud's Glucose Agar; SceSel, Scedosporium Select Agar; YPGA, Yeast Peptone Glucose Agar.

estimated. Molecular detection methods such as the LAMP or NGS technology have the potential to become powerful tools that can be used in place of selective culture for to provide a more realistic description of the prevalence of *Exophiala* and *Scedosporium* in the respiratory tracts of CF patients, upon the improvement of the protocols for the extraction of filamentous fungi DNA from clinical specimens.

Acknowledgments

This study was funded in part with the grants from Major National R&D Projects of the National Health Department (2014ZX09J14106-02A), and the National Natural Science Foundation of China (81201269).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary material

Supplementary data are available at *MMYCOL* online.

References

1. Bellis G, Cazes MH, Parant A et al. Cystic fibrosis mortality trends in France. *J Cyst Fibros*. 2007; 6: 179–186.
2. Girodon E, Cazeneuve C, Lebargy F et al. CFTR gene mutations in adults with disseminated bronchiectasis. *Eur J Hum Genet*. 1997; 5: 149–155.
3. O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet*. 2009; 373: 1891–1904.
4. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev*. 2010; 23: 299–323.
5. de Vrankrijker AM, Wolfs TF, van der Ent CK. Challenging and emerging pathogens in cystic fibrosis. *Paediatr Respir Rev*. 2010; 11: 246–254.
6. Chotirmall SH, McElvaney NG. Fungi in the cystic fibrosis lung: bystanders or pathogens? *Int J Biochem Cell Biol*. 2014; 52: 161–173.
7. 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: 5–11.
8. Zouhair R, Rougeron A, Razafimandimby B, Kobi A, Bouchara JP, Giraud S. Distribution of the different species of the *Pseudallescheria boydii/Scedosporium apiospermum* complex in French patients with cystic fibrosis. *Med Mycol*. 2013; 51: 603–613.
9. Harun A, Blyth CC, Gilgado F, Middleton P, Chen SC, Meyer W. Development and validation of a multiplex PCR for detection of Scedosporium spp. in respiratory tract specimens from patients with cystic fibrosis. *J Clin Microbiol*. 2011; 49: 1508–1512.
10. Haase G, Skopnik H, Kusenbach G. Exophiala dermatitidis infection in cystic fibrosis. *Lancet*. 1990; 336: 188–189.
11. Lebecque P, Leonard A, Huang D et al. *Exophiala* (*Wangiella*) *dermatitidis* and cystic fibrosis: prevalence and risk factors. *Med Mycol*. 2010; 48: S4–9.
12. Kondori N, Gilljam M, Lindblad A, Jonsson B, Moore ER, Wenneras C. High rate of *Exophiala dermatitidis* recovery in the airways of patients with cystic fibrosis is associated with pancreatic insufficiency. *J Clin Microbiol*. 2011; 49: 1004–1009.
13. Cortez KJ, Roilides E, Quiroz-Telles F et al. Infections caused by Scedosporium spp. *Clin Microbiol Rev*. 2008; 21: 157–197.
14. Chen M, Zeng J, De Hoog GS et al. The 'species complex' issue in clinically relevant fungi: a case study in *Scedosporium apiospermum*. *Fungal Biol*. 2016; 120: 137–146.
15. Guarro J, Kantarcioglu AS, Horre R et al. *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. *Med Mycol*. 2006; 44: 295–327.
16. Cimon B, Carrere J, Vinatier JF, Chazalette JP, Chabasse D, Bouchara JP. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2000; 19 53–56.

17. Rodriguez-Tudela JL, Berenguer J, Guarro J et al. Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. *Med Mycol*. 2009; 47: 359–370.
18. 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: 433–438.
19. Pham T, Giraud S, Schuliar G, Rougeron A, Bouchara JP. Scedo-Select III: a new semi-selective culture medium for detection of the *Scedosporium apiospermum* species complex. *Med Mycol*. 2015; 53: 512–519.
20. Sedlacek L, Graf B, Schwarz C et al. Prevalence of *Scedosporium* species and Lomentospora prolificans in patients with cystic fibrosis in a multicenter trial by use of a selective medium. *J Cyst Fibros*. 2015; 14 237–241.
21. Lu Q, Gerrits van den Ende AH, Bakkers JM et al. Identification of *Pseudallescheria* and *Scedosporium* species by three molecular methods. *J Clin Microbiol*. 2011; 49: 960–967.
22. Lackner M, Klaassen CH, Meis JF, van den Ende AH, de Hoog GS. Molecular identification tools for sibling species of Scedosporium and Pseudallescheria. *Med Mycol*. 2012; 50: 497–508.
23. Hoog GS, Guarro J, Gene J, Figueras MJ. *Atlas of Clinical Fungi, CD-rom* 3rd ed. Utrecht / Reus: CBS-KNAW Fungal Biodiversity Centre/ Universitat Rovirai Virgili, 2009.
24. Najafzadeh MJ, Dolatabadi S, Saradeghi Keisari M, Naseri A, Feng P, de Hoog GS. Detection and identification of opportunistic *Exophiala* species using the rolling circle amplification of ribosomal internal transcribed spacers. *J Microbiol Methods*. 2013; 94: 338–342.
25. 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.
26. Williamson EC, Speers D, Arthur IH, Harnett G, Ryan G, Inglis TJ. 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.
27. 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.
28. Horre R, Marklein G, Siekmeier R, Nidermajer S, Reiffert SM. Selective isolation of *Pseudallescheria* and *Scedosporium* species from respiratory tract specimens of cystic fibrosis patients. *Respiration*. 2009; 77: 320–324.
29. Lackner M, Rezusta A, Villuendas MC, Palacian MP, Meis JF, Klaassen CH. Infection and colonisation due to *Scedosporium* in Northern Spain: an in vitro antifungal susceptibility and molecular epidemiology study of 60 isolates. *Mycoses*. 2011; 54: 12–21.
30. Ziesing S, Suerbaum S, Sedlacek L. Fungal epidemiology and diversity in cystic fibrosis patients over a 5-year period in a national reference center. *Med Mycol*. 2016; 54: 781–786.
31. 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: S88–97.
32. Kaneko H, Kawana T, Fukushima E, Suzutani T. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *Journal of biochemical and biophysical methods*. 2007; 70: 499–501.
33. Horre R, Schaal KP, Siekmeier R, Sterzik B, de Hoog GS, Schnitzler N. Isolation of fungi, especially Exophiala dermatitidis, in patients suffering from cystic fibrosis: a prospective study. *Respiration*. 2004; 71: 360–366.
34. 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.
35. Nagano Yuriko, Millar B Cherie et al. Fungal infections in patients with cystic fibrosis. *Rev Med Microbiol*. 2007; 18: 11–16.
36. Kusenbach G, Skopnik H, Haase G, Friedrichs F, Dohmen H. Exophiala dermatitidis pneumonia in cystic fibrosis. *Eur J Pediatr*. 1992; 151: 344–346.
37. Kondori N, Lindblad A, Welinder-Olsson C, Wenneras C, Gilljam M. Development of IgG antibodies to Exophiala dermatitidis is associated with inflammatory responses in patients with cystic fibrosis. *J Cyst Fibros*. 2014; 13: 391–399.
38. Sudhadham M, Prakitsin S, Sivichai S et al. The neurotropic black yeast Exophiala dermatitidis has a possible origin in the tropical rain forest. *Stud Mycol*. 2008; 61: 145–155.
39. Zalar P, Novak M, de Hoog GS, Gunde-Cimerman N. Dishwashers–a man-made ecological niche accommodating human opportunistic fungal pathogens. *Fungal Biol*. 2011; 115: 997–1007.
40. Sood S, Vaid VK, Sharma M, Bhartiya H. Cerebral phaeohyphomycosis by Exophiala dermatitidis. *Indian J Med Microbiol*. 2014; 32: 188–190.
41. Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. *Cell*. 2013; 155: 27–38.