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Indoor fungal contamination of moisture-damaged and allergic patient housing analysed using real-time PCR

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Abstract

Aims: The aim of our study was to compare, using real-time (Rt) PCR, quantitative levels of five fungal species in three kinds of dwellings.

Methods and Results: Three groups of homes were recruited: moisture-damaged homes (MDH, $n = 30$), allergic patient homes (APH, $n = 25$) and paired control homes (CH, $n = 55$). Five moulds with allergenic compounds or mycotoxin production characteristics (*Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Aspergillus versicolor*, *Alternaria alternata* and *Stachybotrys chartarum*) were quantified using Rt-PCR. Cycle threshold results were expressed in spore equivalent per volume or surface unit using a direct calculation based on a spore standard curve. MDH presented significantly higher amounts of DNA from *C. sphaerospermum* in both air and surface samples than CH ($P < 0.001$). APH presented slightly elevated amounts of DNA from *A. versicolor* in both air and surface samples, compared to CH ($P < 0.05$).

Conclusion: Rt-PCR quantification of targeted fungal species is a rapid, reliable tool that could be included in a global indoor mould evaluation.

Significance and Impact of the Study: Quantification of *C. sphaerospermum* using Rt-PCR can help to better target social service intervention in MDH. Quantification of *A. versicolor* DNA could be informative for characterization of APH.

Introduction

Presence of moulds within homes has been associated with human health problems. Mould-related injuries are caused by infection, toxic reactions and, more frequently, allergic reactions such as asthma or rhinitis. In industrialized countries, it has been estimated that approx. 5% of the population suffered from fungal allergies (Hardin *et al.* 2003). Although some recent reports suggest that a much more diverse range of fungi represent sources of allergen (Green *et al.* 2005, 2006), species of *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* are the most common fungi found indoor (Beguín and Nørdal 1994; Yang and Johanning 2007). Furthermore, some fungi such as *Aspergillus versicolor* and *Stachybotrys chartarum* are able to produce

mycotoxins and have been associated to sick building syndrome (Kuhn and Ghannoum 2003).

Many studies have reported the ill effects related to indoor mould growth. However, most of them failed to provide clear aetiological links. One of the reasons for this is the difficulty in identifying the symptoms. In addition, the absence of standardized sampling and analysis method impair data comparison (Grinshpun *et al.* 2007). In some reports, a subjective assessment of mould contamination by moisture indices, mould odour or visible mould were used as indices of exposure to fungi (Mahooti-Brooks *et al.* 2004; Park *et al.* 2004). Other reports have aimed at measuring the specific influence of each species using direct examination of spores or culture-based methods (Beguín and Nørdal 1994). At last, some authors suggest that future

advance in understanding the health risks posed by fungi requires accurate, quantitative estimates of the occurrence of individual species with common allergenic and/or biologically active compound production characteristics (Haugland *et al.* 2004). Real time-PCR (Rt-PCR) using specific systems of primers and probes for mould has been proposed for epidemiological studies performed in mouldy houses (Meklin *et al.* 2004; Vesper *et al.* 2004). Rt-PCR allows rapid results with increased sensitivity and is particularly suited for the detection of slowly growing moulds or moulds which rapidly lose their viability (Cruz and Buttner 2007).

The aim of our study was to compare, using Rt-PCR, the quantitative levels of five fungal species in three kinds of dwellings: (i) moisture-damaged homes (MDH), defined as homes for which inhabitants lodged a complaint to social services for the presence of moulds; (ii) allergic patient homes (APH), defined as homes in which inhabitants presented allergic symptoms and positive cutaneous tests to moulds; and (iii) control homes (CH), defined as paired homes proposed by inhabitants of the first two groups. We targeted five moulds with allergenic compounds or mycotoxin-producing properties: *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *A. versicolor*, *Alternaria alternata* and *S. chartarum*. The second aim of our study was to meet the request for public health and social services to provide thresholds that would enable objective identification of building mould problems.

Materials and methods

Selection of homes

Three groups of homes were recruited in Franche-Comté, a region in the East of France.

Moisture-damaged homes

MDH ($n = 30$) were recruited by social services (Service d'Hygiène et Santé de Besançon et Direction Départementale des Affaires Sanitaires et Sociales du Doubs). Inhabitants of these homes lodged a complaint to social services for indoor presence of visible moulds or mould odour.

Allergic patient homes

APH ($n = 25$) were recruited by an association of allergologists (Réseau d'Allergologie de Franche-Comté). Inhabitants of these homes had allergic symptoms (asthma, allergic rhinitis or conjunctivitis). The link between allergic symptoms and the indoor environment was assessed by a physician, using a medical questionnaire and cutaneous test including *A. alternata* and *P. chrysogenum*.

Control homes

Inhabitants of homes of the previous two groups were asked to propose paired housing. CH ($n = 55$) were recruited and paired with respect to the surface of the housing ($\pm 10\%$), the number of inhabitants per housing (± 1), type of housing (flat or house) and location (urban or rural, within a 15-km area). Inhabitants of CH had no allergic problems.

Sampling

Sampling was carried out from October to May over two consecutive years (2004–2006). In each of the 110 recruited homes, one air sample and one surface sample were carried out in two rooms: (i) one room with plumbing (bathroom) and (ii) one room without plumbing (bedroom). We analysed 440 samples (220 air samples and 220 surface samples).

Air sampling

Filtration sampling was carried out using a Gilian[®] pump (Sensidyne, Clearwater, FL, USA) with a cassette containing a $0.45\text{-}\mu\text{mol l}^{-1}$ pore size Teflon filter (TJ Environmental, Amsterdam, the Netherlands). Sampling was carried out at 3 l min^{-1} for 33 min for a total volume of 100 l of air. After sampling, 4 ml of Tween 0.1% (Tween 80; Merck[®], Darmstadt, Germany) was poured into the cassettes that was vortexed for 1 min. Then, the filter and the washing liquid were transferred to a Stomacher bag and shaken for 10 min in the Stomacher apparatus (AES Laboratory, Combourg, France). The suspension was used for culture and Rt-PCR analysis.

Surface sampling

Surface sampling was performed by swabbing a 25-cm^2 area. Swabbing was carried out on visible mouldy areas. If no mouldy areas were present, swabbing was performed under the windows and near the sink in windowless bathrooms. Swabs were washed using 2 ml of sterile distilled water and vortex shaken for 30 s. The suspension was used for culture and Rt-PCR analysis.

Rt-PCR assays

Preparation of calibrated spore suspensions

Calibrated suspensions of reference strains *C. sphaerospermum* (BCCM/IHEM 18883), *P. chrysogenum* (BCCM/IHEM 22667), *A. versicolor* (BCCM/IHEM 22671), *A. alternata* (BCCM/IHEM 22669) and *S. chartarum* (BCCM/IHEM 22672) were prepared from a culture plate by counting on Malassez cells. Tenfold serial dilutions of the initial suspension were made, and each of the seven diluted suspensions (10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10 and 1 spore per ml) was submitted to DNA extraction.

DNA extraction

DNA extraction was carried out using High Pure PCR template kit (Roche Diagnostics, Meylan, France), with 400 μ l of spore suspensions from reference strains or 400 μ l of the washing liquid from filters or swabs. After shaking in a 2-ml screw-cap tube containing 0.3 g of 0.3 mm glass beads (Sigma-Aldrich®, St Louis, MO, USA) using a Magna Lyser™ (Roche Diagnostic®), the sample was submitted to the extraction protocol according to manufacturer's recommendations.

PCR amplification

DNA from *C. sphaerospermum*, *A. versicolor*, *P. chrysogenum*, *A. alternata* and *S. chartarum* was detected by Rt-PCR using primers and Taqman probes previously described by Haugland *et al.* (2004) (<http://www.epa.gov/nerlcwww/moldtech.htm#primers>). The PCR mix was prepared in a 20- μ l final volume using the LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics), and the PCR were run on a LightCycler 2.0 (Roche Diagnostics).

Sterile distilled water samples were used as negative controls in each series of extractions and in each series of amplification. Twenty air samples from MDH, positive when amplified by *A. versicolor* primers and probes, were tested before and after a tenfold dilution of DNA extracts to investigate for PCR inhibitors. PCR inhibition was also investigated using an internal control in 20 surface samples from MDH. The protocol used were: 2 μ l of calibrated spore suspension of *Geotrichum candidum* (BCCM/IHEM 5560) (10^5 spores per ml) was added to 400 μ l of the washing liquid from swabs, DNA extraction was carried out and followed by amplification with *G. candidum* primers and probe as previously described (Haugland *et al.* 2004).

Quantification of fungal DNA

For each of the five targeted species, the spore standard curve was plotted using a mean Ct (cycle threshold) of two extracts from each calibrated suspension (10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10 and 1 spore per ml). The spore standard curves showed a linear relationship between log (spore) and the Ct. For *C. sphaerospermum*, DNA extracts from the suspensions containing 1 spore per ml and 10 spores per ml were PCR negative. The standard curve was established using the five other calibrated suspensions (10^6 , 10^5 , 10^4 , 10^3 and 10^2 spores per ml). Thus, concentrations of <100 spores per ml had to be extrapolated from the equation curve. For the other species, the suspension containing 1 spore per ml gave a Ct between 40 and 44 cycles for one of two replicates.

Two independent DNA extractions were performed for each environmental sample. The final result was the mean Ct of the two replicates. A Ct value of 45 cycles was assigned

to the PCR-negative samples. Results obtained in spore equivalents (sp-eq) per millilitre using the spore standard curve were then subsequently expressed in sp-eq per cubic metre for air sampling and in sp-eq per 25 cm² for surface sampling. Calculations were carried out using the volume (100 l) or the surface (25 cm²) sampled, the volume of washing liquid (4 ml for air samples, 2 ml for surface samples) and the volume used for DNA extraction (400 μ l).

Mycological cultures

For each air sample, two aliquots of 250 μ l each of the liquid obtained after filter washing were used for culture analysis: one aliquot was spread on malt agar extract (3%) medium plates (Difco, Detroit, MI, USA), and incubated at 20°C; the other was spread on dichloran 18% glycerol (DG18) (Oxoid, Basingstoke, Hampshire, UK) medium plates and incubated at 30°C. For each surface sample, two 250 μ l aliquots of the washing liquid from swabs underwent the same protocol as for air samples. Fungal species were identified by macroscopic and microscopic examination and counted. Results were expressed in colony-forming units per cubic metre of air (CFU per m³) for air samples and in colony-forming units per 25 square centimetres (CFU per 25 cm²) for surface samples.

Statistical analysis

STATA statistical software release nine (StatCorp LP 2005; Statacorp, College Station, TX) was used for statistical analysis. A Student's *t*-test was performed to compare the Ct of MDH and APH with their respective CH groups. A logarithmic transformation was carried out on swabbed and filtrated sample's values for statistical analysis by a Student's *t*-test. Statistical significance was set at the standard 5% level.

Results

Characteristics of Rt-PCR assays

No PCR-positive results were observed for the negative controls. No PCR inhibition was detected when the 20 diluted DNA extracts from air samples were amplified with *A. versicolor* primers and probes. The absence of PCR inhibition was also shown by obtaining the same Ct (Ct = 28 ± 0.4 cycles) when samples with *G. candidum* internal control were amplified using *G. candidum* Rt-PCR assay. Cross reactivity was checked by testing every primers/probes system with suspensions of the different species targeted. No cross-reactivity was observed. Frequency of PCR-positive samples and fungal DNA load in positive samples, air and surface are indicated in Table 1.

Table 1 Frequency of PCR-positive samples and fungal DNA load (air and surface, bathroom and bedroom) from moisture-damaged homes (MDH, *n* = 30) and allergic patients homes (APH, *n* = 25)

	MDH			CH-MDH		
	Number of positive (%)	Mean Ct $\pm$ SEM	Mean spore equivalent (sp-eq) per m ³ $\pm$ SEM	Number of positive (%)	Mean Ct $\pm$ SEM	Mean sp-eq per m ³ $\pm$ SEM
Air sample						
<i>Cladosporium sphaerospermum</i>	23/60 (38)	36.7 $\pm$ 0.9	2.5 $\times$ 10 ⁴ $\pm$ 2 $\times$ 10 ⁴	7/60 (12)	37 $\pm$ 0.9	2 $\times$ 10 ⁴ $\pm$ 3 $\times$ 10 ⁴
<i>Aspergillus versicolor</i>	17/60 (28)	38.4 $\pm$ 2	1.6 $\times$ 10 ² $\pm$ 2 $\times$ 10 ²	13/60 (22)	39.5 $\pm$ 1.7	57 $\pm$ 53
<i>Penicillium chrysogenum</i>	40/60 (67)	39.6 $\pm$ 1.8	8 $\pm$ 9	37/60 (62)	39.9 $\pm$ 1.9	10 $\pm$ 17
<i>Alternaria alternata</i>	17/60 (28)	39.4 $\pm$ 1.2	2.8 $\pm$ 3.9	12/60 (20)	39.8 $\pm$ 1.8	3 $\pm$ 4
<i>Stachybotrys chartarum</i>	1/60 (2)	40.2	12.6	1/60 (2)	40.5	5
	APH			CH-APH		
<i>C. sphaerospermum</i>	16/50 (32)	36.5 $\pm$ 0.9	5.3 $\times$ 10 ⁴ $\pm$ 8.8 $\times$ 10 ⁴	12/50 (24)	37 $\pm$ 0.8	1 $\times$ 10 ⁴ $\pm$ 8 $\times$ 10 ³
<i>A. versicolor</i>	10/50 (20)	38.3 $\pm$ 2.3	2 $\times$ 10 ² $\pm$ 2.9 $\times$ 10 ²	10/50 (20)	39.6 $\pm$ 1.5	56 $\pm$ 50
<i>P. chrysogenum</i>	34/50 (68)	40.6 $\pm$ 1.6	5.1 $\pm$ 5.4	22/50 (44)	40.4 $\pm$ 1.6	4 $\pm$ 5
<i>A. alternata</i>	16/50 (32)	39.6 $\pm$ 1.1	2.9 $\pm$ 3.2	14/50 (28)	39.6 $\pm$ 1.4	3 $\pm$ 3
<i>S. chartarum</i>	0/50 (0)	/	/	0/50 (0)	/	/
	MDH			CH-MDH		
Surface sample	Number of positive	Mean Ct $\pm$ SEM	Mean sp-eq per 25 cm ² $\pm$ SEM	Number of positive	Mean Ct $\pm$ SEM	Mean sp-eq per 25 cm ² $\pm$ SEM
<i>C. sphaerospermum</i>	48/60 (80)	28.1 $\pm$ 5.1	8 $\times$ 10 ⁷ $\pm$ 2 $\times$ 10 ⁷	17/60 (28)	33 $\pm$ 5.6	3 $\times$ 10 ⁶ $\pm$ 4 $\times$ 10 ⁶
<i>A. versicolor</i>	43/60 (72)	30.8 $\pm$ 6.9	6 $\times$ 10 ⁴ $\pm$ 2 $\times$ 10 ⁵	25/60 (42)	37.9 $\pm$ 3.3	53 $\pm$ 228
<i>P. chrysogenum</i>	38//60 (63)	34.5 $\pm$ 5.5	4 $\times$ 10 ³ $\pm$ 10 ⁴	24/60 (40)	40.1 $\pm$ 2.7	2 $\pm$ 10
<i>A. alternata</i>	35/60 (58)	34 $\pm$ 4.7	55 $\pm$ 192	22/60 (37)	36.1 $\pm$ 3.7	8 $\pm$ 30
<i>S. chartarum</i>	9/60 (15)	36.2 $\pm$ 6.9	4 $\times$ 10 ³ $\pm$ 10 ⁵	4/60 (7)	38.1 $\pm$ 2.1	1.4 $\pm$ 1.5
	APH			CH-APH		
<i>C. sphaerospermum</i>	34/50 (68)	31.7 $\pm$ 5.6	2 $\times$ 10 ⁷ $\pm$ 10 ⁸	27/50 (54)	34.5 $\pm$ 3.6	10 ⁶ $\pm$ 5 $\times$ 10 ⁶
<i>A. versicolor</i>	34/50 (68)	36.2 $\pm$ 3.9	118 $\pm$ 334	20/50 (40)	38.1 $\pm$ 2.2	5 $\pm$ 7
<i>P. chrysogenum</i>	22/50 (44)	37.8 $\pm$ 3	5 $\pm$ 16	20/50 (40)	39.3 $\pm$ 2.4	1 $\pm$ 2
<i>A. alternata</i>	28/50 (56)	35.6 $\pm$ 3.9	4 $\pm$ 8	23/50 (46)	35.8 $\pm$ 5.3	5 $\pm$ 8
<i>S. chartarum</i>	7/50 (14)	39 $\pm$ 2	1 $\pm$ 0.8	1/50 (2)	37.8	1.2

Mean Ct, Mean Ct of PCR-positive samples only.

Quantification of fungal DNA in MDH vs CH

Air samples

Significantly higher amounts of DNA from *C. sphaerospermum* were found in air samples from MDH than in those from CH (Table 2, *P* < 0.001). This result was confirmed by culture-based methods (mean = 592 vs 72 CFU per m³, *P* < 0.001) (Table 2). There was no statistical difference for DNA amounts from other species (*A. versicolor*, *A. alternata*, *S. chartarum* and *P. chrysogenum*).

Surface samples

Significantly higher amounts of DNA from *C. sphaerospermum*, *P. chrysogenum*, *A. versicolor* and *A. alternata* were found in surface samples from MDH than in those from CH (Table 2, *P* = 0.006 for *A. alternata* and *P* < 0.001 for the three others species). Culture after swabbing also showed significantly higher amounts of the same four

species in MDH (Table 2, *P* < 0.001 for the four species). *Stachybotrys chartarum* was detected only using Rt-PCR in nine of 60 surface samples from MDH and in four of 60 surface samples from CH (Table 1).

Quantification of fungal DNA in APH vs CH

Air samples

Significantly higher amounts of DNA from *A. versicolor* were found in air samples from APH than in those from CH (Table 2, *P* = 0.03). This result was not confirmed by culture-based method. Amounts of DNA from the other species did not differ statistically.

Surface samples

Significantly higher amounts of DNA from *A. versicolor* and *C. sphaerospermum* were found in surface samples from APH than in those from CH (Table 2, *P* < 0.001 for

Table 2 Quantification of the five fungal species in air and surface samples from moisture-damaged homes (MDH, $n = 30$) and allergic patients homes (APH, $n = 25$) vs respective paired control homes (CH, $n = 30 + 25$)

Air sample	MDH vs CH					
	Real time PCR (mean Ct $\pm$ SD)			Culture (filtration) (mean CFU m ⁻³ $\pm$ SD)		
	MDH	CH	<i>P</i> value	MDH	CH	<i>P</i> value
<i>Cladosporium sphaerospermum</i>	41.8 $\pm$ 4.1	44.1 $\pm$ 2.5	<0.001	592 $\pm$ 1738	72 $\pm$ 191	<0.001
<i>Aspergillus versicolor</i>	43.1 $\pm$ 3.2	43.8 $\pm$ 2.4	NS	618 $\pm$ 2461	341 $\pm$ 1637	NS
<i>Penicillium chrysogenum</i>	41.4 $\pm$ 2.9	41.8 $\pm$ 2.9	NS	1309 $\pm$ 2631	2350 $\pm$ 3774	NS
<i>Alternaria alternata</i>	43.4 $\pm$ 2.6	43.9 $\pm$ 2.2	NS	0	0	Not carried out
Surface sample	Culture (swabbing) (mean CFU per 25 cm ² $\pm$ SD)					
<i>C. sphaerospermum</i>	31.6 $\pm$ 8.3	41.5 $\pm$ 6.3	<0.0001	91 $\pm$ 97	20 $\pm$ 59	<0.001
<i>A. versicolor</i>	35.1 $\pm$ 8.7	41.9 $\pm$ 4.2	<0.0001	23 $\pm$ 62	3 $\pm$ 22	<0.001
<i>P. chrysogenum</i>	38.8 $\pm$ 6.4	42.9 $\pm$ 3	<0.0001	59 $\pm$ 88	5 $\pm$ 25	<0.001
<i>A. alternata</i>	38.4 $\pm$ 6.5	41.6 $\pm$ 4.9	0.006	8 $\pm$ 38	0.09 $\pm$ 0.9	<0.001
Air sample	APH vs CH					
	Real time PCR (mean Ct $\pm$ SD)			Culture (filtration) (mean CFU m ⁻³ $\pm$ SD)		
	APH	CH	<i>P</i> value	APH	CH	<i>P</i> value
<i>C. sphaerospermum</i>	42.3 $\pm$ 4	43.1 $\pm$ 3.4	NS	195 $\pm$ 804	111 $\pm$ 341	NS
<i>A. versicolor</i>	42.7 $\pm$ 3.5	43.9 $\pm$ 2.2	0.03	176 $\pm$ 360	192 $\pm$ 302	NS
<i>P. chrysogenum</i>	42.1 $\pm$ 2.4	42.9 $\pm$ 2.5	NS	854 $\pm$ 2329	785 $\pm$ 1806	NS
<i>A. alternata</i>	43.3 $\pm$ 2.6	43.5 $\pm$ 2.6	NS	3 $\pm$ 36	4 $\pm$ 39	NS
Surface sample	Culture (swabbing) (mean CFU per 25 cm ² $\pm$ SD)					
<i>C. sphaerospermum</i>	35.9 $\pm$ 7.8	39.3 $\pm$ 6.0	0.008	44 $\pm$ 77	16 $\pm$ 51	0.004
<i>A. versicolor</i>	39.0 $\pm$ 5.3	42.2 $\pm$ 3.7	0.0006	7 $\pm$ 36	0.08 $\pm$ 0.4	0.034
<i>P. chrysogenum</i>	41.7 $\pm$ 4.2	42.7 $\pm$ 3.2	NS	15 $\pm$ 49	6 $\pm$ 29	NS
<i>A. alternata</i>	39.7 $\pm$ 5.6	40.8 $\pm$ 5.8	NS	6 $\pm$ 34	4 $\pm$ 27	NS

Mean Ct was calculated assigning a Ct value of 45 cycles to the PCR-negative samples.

SD, standard deviation; NS, not significant.

A. versicolor and $P = 0.008$ for *C. sphaerospermum*). Culture after swabbing also showed significantly higher amounts of these two species in APH (Table 2, $P = 0.034$ for *A. versicolor* and $P = 0.004$ for *C. sphaerospermum*). Amounts of DNA from the other species did not differ statistically. DNA from *S. chartarum* was detected in seven of 50 surface samples from APH and one of 50 surface samples from CH (Table 1).

Discussion

Our analysis of indoor fungal contamination, using Rt-PCR targeting five selected species associated with health injuries, clearly distinguished MDH from their paired controls. Amounts of DNA from *C. sphaerospermum* were higher in both air and surface samples in MDH. We also observed slightly elevated amounts of DNA from *A. versicolor* in both air and surface samples in APH compared to CH.

Species quantification is rapid and reliable with Rt-PCR. Detection and quantification of five targeted species in 20 samples were carried out in approx. 15 h (2 h for the DNA extraction and 2 h for each targeted amplification species). Analysis time could be further shortened by the use of a high throughput Rt-PCR system, which can analyse about 100 samples in a single run of 2 h. Rt-PCR is particularly helpful for detection of moulds that grow slowly or that require specific substrates for growth (Roe *et al.* 2001). *Stachybotrys chartarum* was detected in surface samples from 21 homes using Rt-PCR, whereas it was isolated only once by culture method, probably because no selective medium was used and competition with predominant species such as *C. sphaerospermum* may have occurred. Other advantages of Rt-PCR over culture methods are: (i) no significant expertise in mycology is required for species identification, (ii) dead spores that possess allergenic properties can be investigated and (iii) the detection and quantification can

be standardized for multicenter studies (Verhoeff *et al.* 1995; Vesper *et al.* 2007).

For those professionals concerned with environment mould-related pathologies, like biologists, physicians or members of social and health services, we needed to give an estimate of fungal contamination, not in Ct, but in sp-eqs per surface or volume unit. Although an extrapolation at low template concentrations was needed, we used the spore standard curve, in order to take in account the output of DNA per extraction in the same manner for standard sample and for environmental samples, whatever the initial concentration in the sample. Using this method of calculation, the detection limit was 1 spore per ml for all targeted species except *C. sphaerospermum*. The detection limit of *C. sphaerospermum* (100 spores per ml) persisted in spite of various trials to improve these results (prior enzymatic digestions, use of various purification kits). Designing a new set of primers/probe could be the way to improve the detection limit (Pietarinen *et al.* 2008).

Up until now, the evaluation of indoor mould growth in homes in France has been based on subjective data (mouldy odor and visible mould). The use of Rt-PCR to determine the fungal burden in homes is very informative (Vesper *et al.* 2007). Studying fungal contamination at the species level could also be of interest to assess the role of species-specific components such as cell wall antigenic proteins or mycotoxin. *Cladosporium* sp. growth is frequently a consequence of water damage, dampness and lack of heating (Mahooti-Brooks *et al.* 2004), and this was often mentioned in the initial complaints from the inhabitants of the MDH recruited for our study. The Rt-PCR quantification of *C. sphaerospermum* is an objective, reliable tool that could be included in a global indoor mould evaluation and could help to better target social service intervention.

Although mould contamination was not visible in APH, we showed slightly elevated but significant amounts of DNA from *A. versicolor* in both air and surface samples compared to CH. *Aspergillus versicolor* was the second most frequent species in homes of the allergic patients in Brussels and was previously suspected of having a role in allergy by Beguin and Nolard (1994). However, presence of *A. versicolor* in APH could also be explained by the cleaning habits of allergic patients. The indoor fungal ecological niche is a complex ecosystem where different fungal species interact with each other and with bacteria and mites (McGinnis 2007). Intensive cleaning, including frequent vacuuming and abundant use of antimite products, could modify species interaction and distribution and could alter the biodiversity. More severe pathologies have also been linked to the presence of *A. versicolor*, which can produce a carcinogenic toxin called sterigmatocystin (Kuhn and Ghannoum 2003).

Detection of toxic compounds like this in patients' indoor environment could also contribute to a better understanding of mould-related pathologies.

In conclusion, we showed that Rt-PCR quantification of targeted species was an easy and a reliable tool for characterizing MDH and APH. Further studies using targeted Rt-PCR, combined with an accurate recording of clinical data, which include a large number of homes from larger geographic areas, would increase our knowledge on the role of individual species in indoor mould-related diseases.

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