



Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com



ORIGINAL ARTICLE/ARTICLE ORIGINAL

Environmental and molecular study of fungal flora in asthmatic patients

H. Trablesi^a, I. Hadrich^a, S. Neji^a, N. Fendri^a, D. Ghorbel^a,
F. Makni^a, H. Ayadi^{b,*}, S. Kammoun^b, A. Ayadi^a

^a Laboratory of Fungal and Parasitic Molecular Biology, School of Medicine, University of Sfax, Sfax, Tunisia

^b Department of Pneumology, UH Hedi Chaker, University of Sfax, Sfax, Tunisia

Received 25 July 2017; received in revised form 17 September 2017; accepted 18 September 2017

KEYWORDS

Asthma;
Environment;
Fungal contamination;
ITS sequencing

Summary The aim of the present study was to investigate the epidemiological and fungal environmental profile in asthmatic patients. We conducted a prospective study involving 49 patients with allergic asthma. One hundred and forty-five clinical samples and 289 environmental samples were performed. Only 30 patients accepted to participate to the environmental study at their home. For specific IgE antibodies, ELISA assay was conducted for 21 patients. Molecular ITS sequencing was performed for 37 isolates. The frequency of attacks was significantly associated with the seasonality, which was closely related to climate ($P = 0.024$), exposure to animals (cats, $P = 0.025$), plants (olive, $P = 0.018$), physical effort ($P = 0.04$) and the number of permanent occupants in house (> 6) ($P = 0.026$). Fungal contaminants were detected from 78.6% of biological samples and 97.8% of environmental samples. Antibodies corresponding to the studied allergens were detected in 10 patients (10/21). PCR sequencing allowed as rectified morphological identification for 27.02% (10/37) strains of *Aspergillus*. The allergy in molds is an indisputable reality that is necessary to look for in front of any severe asthma. So, it is important to establish clearly a relationship between exposure to fungi and health disorders in order to set up specific and effective preventive measures.

© 2017 Elsevier Masson SAS. All rights reserved.

* Corresponding author.

E-mail address: ali.ayadi@rns.tn (H. Ayadi).

<http://dx.doi.org/10.1016/j.mycmed.2017.09.001>

1156-5233/© 2017 Elsevier Masson SAS. All rights reserved.

Introduction

Asthma is a clinical syndrome characterized by episodic reversible airway obstruction, increased bronchial reactivity, and airway inflammation. Asthma results from complex interactions among inflammatory cells, their mediators, airway epithelium and smooth muscle, and the nervous system.

It is a major public health problem, affecting more than 20% of the pediatric population and 5–10% of adults [1,2]. It is also the most common chronic childhood disease in developed nations [2,3]. The increasing prevalence of respiratory allergic diseases noted in recent years in industrialized countries raised questions about environmental changes that could be responsible [1,3].

Causes or triggers of asthma can be divided into allergic and non-allergic etiologies. Aeroallergens can include seasonal pollen, mold spores, dust mites, animal allergens, and food (especially in children) [4,5]. Indoor asthmatics environment play a major role in modulating asthma severity. Therefore, many surveys opted to study risk factors for asthma including a questionnaire about housing characteristics [1,6].

There is consistent evidence that environmental factors acting during early childhood play a key role in the pathogenesis of atopic disease [7]. Because of the clinical, social and economic importance of asthma and other atopic disease, environmental factors must be sought in order to take the necessary preventives measures.

Mold is one of the many triggers of asthma exacerbations in some individuals with atopic asthma, although no asthma exacerbation threshold for mold exposure is known to exist. However, it seems likely that all molds are not equally relevant to the disease.

Studies in different countries showed that occupants of damp or moldy buildings are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma [8,9]. However, associations between symptoms and concentrations of fungi seem to be dependent on distinctive genera or species [10,11].

Identifying specific molds that are potentially associated with asthma could narrow down our search for the most important molds.

In present study, we conducted an epidemiological and environmental survey for fungal flora in asthmatic patients.

Materials and methods

Patients study

We released a prospective study (February 2014–October 2014) involving 49 patients with allergic asthma hospitalized in Pneumology department (University Hospital Hedi Chaker Sfax, Tunisia). The stages of asthma of patients were classified according to the Global Initiative for Asthma (GINA) 2008 [12].

Only 30 patients accepted to participate in the environmental study at their home. The demographic data included: sex, date of birth, gender, underlying diseases. The additional data collected regarding housing were: type of home, building material, floor construction, age of home, number

of bedrooms, number of people usually living in the home, and type of floor covering in each room of the house. Antibiotic and antifungal therapy history was also noted for each patient.

In total, 145 biological samples: nasal samples (75) by swab and sputum (70) in sterile jars were taken for all patients. For environmental study, we performed 289 samples: by swabbing ($n = 135$) (bed, table, rug, carpet, floor, wall, covering, headset), and by using contact Petri ($n = 154$) on doors, sheets and walls. For air sampling ($n = 75$), we used a bioimpactor (180 liters of air/minute) (SAS super 180). A total of 509 samples were then collected from patients and their environment. Culture plates in Sabouraud dextrose agar were incubated at 25 °C for 4 to 15 days.

The number of colonies per plate of each fungal genus/species was counted. The results were expressed as number of colony forming units per cubic meter (CFU/m³) in air samples.

For the morphological identification of different fungi, we used the Biforma manual (*cahier de formation Bioforma: Molds of medical interest*).

For measurement of fungal specific IgE antibodies, we used ELISA assay combining enzyme-labelled conjugates and chromogenic substances (Kit EUROIMMUN «Antibodies of class IgE against inhalation allergens»: EUROLINE Inhalation 2).

Mold sensitization study was released for 21 patients by dosage of specific IgE of the following allergens: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*.

Molecular study

Identification of *Aspergillus* isolates and DNA isolation

Fungi strains were identified on the basis of macroscopic and microscopic morphological characteristics.

The morphological identification of 37 *Aspergillus* isolates was confirmed by the rRNA ITS1–5.8S–ITS2 region sequence analysis, as previously described by De Hoog et al. [13]. DNA was extracted by using a QIAmp kit (QIAGEN), following the manufacturer's instructions, and eluted with 50 µL sterile water.

Statistical analyses

Statistical analyses were carried out using IBM SPSS software version 20. Bivariate analysis was performed using Chi² tests in the case of pairs of categorical variables or two tailed *t*-tests in the case of a categorical and a continuous variable. We studied the frequency of attacks according to the different risk factors and triggering factors (physical effort, dust, smoking, pollen, strong odors, perfumes, detergents, felines, olive, cold, heat, and climate change, exposure mold), sex, age, symptoms, seasonality characteristic of the home, improvement in case of change of habitat and humidity.

Results

Patients study

There were 13 men and 36 women (sex-ratio = 0.36). The average age was 50 years (extremes: 28–91 years).

All our patients were classified as uncontrolled asthma. Triggering factors were: dust (86.6%), physical effort (16.6%), humidity (23.3%), cold (23.3%) and irritant exposure (20%).

A significant association was found between the frequency of attacks and the seasonality, which was closely related to climate ($P = 0.024$), exposure to animals (cats, $P = 0.025$) and plants (olive, $P = 0.018$), physical effort ($P = 0.04$) and the number of permanent occupants in house (> 6) ($P = 0.026$). For associated symptoms, we observed a correlation between rhinitis and type of house (urban or rural house) ($P = 0.045$).

We noted a significant correlation between the frequency of attacks and *Alternaria* concentration in the air ($r = 0.456$, P -value = 0.011).

Antibodies corresponding to the studied allergens were detected in 10 (10/21) patients. The specific IgE anti-*Aspergillus* were present in 28.6% of patients, those anti-*Alternaria* in 23.8%, those anti-*Cladosporium* in 19%, and those anti-*Penicillium* in 9.5%.

The IgE concentration ranged between 0.35 and 0.7 kU/L. These values were small quantity of antibody and corresponded to sensitization often without clinical symptoms.

Fungal study

Different fungal contaminants were detected from 78.6% and 97.8% of the biological samples and environmental samples respectively. *Aspergillus* was the most dominant genus. *A. flavus* was the most dominant species of *Aspergillus* in clinical (58%) and environmental samples (32.60%) (Table 1). The frequency of fungal flora is detailed in Table 1.

The average concentration of total fungi was 26.33 CFU/m³ (6–163) in environmental samples. The highest average concentration was noted for *A. flavus* (48.3 CFU/m³) followed by *Penicillium* (42.8 CFU/m³) and *A. fumigatus* (35.6 CFU/m³).

Molecular study

The alignment of the 37 *Aspergillus* sequences with sequences deposited in the database (GenBank) was realized by the Blastn program. PCR sequencing rectified mycological identification of species for 27.02% (10/37) strains of *Aspergillus*. In 27 cases, the molecular study gave the exact identification of *Aspergillus* species (Table 2).

Three patients (patient 3, patient 4 and patient 36) were hosted by the same species collected from the environment and clinical samples.

Discussion

Exacerbation of asthma can be triggered by a number of weather and environmental factors. Viewing the important role of environmental factors in the worsening of asthma, several studies have focused on the study of asthmatic environment in different regions by looking for the impact of climate, humidity, life style, exposure to allergens, mold and animals on the status of the disease [1,14,15]. Air borne fungal spores have been implicated as causative factor in respiratory allergies particularly asthma.

In our study, women were more affected by asthma. These results were confirmed by Johnston et al. [16].

Table 1 Frequency of fungal flora in environmental and clinical samples collected from the 49 asthmatic patients.

	Clinical samples (%)	Environmental samples (%)
<i>Aspergillus</i>		
<i>A. flavus</i>	58	32.60
<i>A. fumigatus</i>	26	24.90
<i>A. niger</i>	5	21.30
<i>A. parasiticus</i>	4	4.50
<i>A. nidulans</i>	1	4.10
<i>A. candidus</i>		2.30
<i>A. versicolor</i>		0.49
<i>A. terreus</i>		0.33
<i>A. oryzae</i>		0.33
<i>A. section flavi</i>	1	3.74
<i>A. section fumigati</i>	1	1.15
<i>A. section nigri</i>		
<i>Aspergillus</i> sp	4	3.11
Total	89	67
<i>Penicillium</i>	5	9
<i>Alternaria</i>	4	8
<i>Chrysosporium</i>	1	
<i>Aureobasidium</i>	1	
<i>Cladosporium</i>		5
<i>Fusarium</i>		3
<i>Rhizopus</i>		3
<i>Bipolaris</i>		2
Others		3

However, Palmer et al. noted that the male was still associated with an increased risk of asthma [17].

In this study, we found that the severity of asthma was related to physical effort. This can be explained by the effect of physical activity on airway obstruction. Driessen et al. showed that physical exercise in 72 asthmatic children caused a limitation of inspired airflow, which leads to an aggravation of asthma [18].

Our study found a relationship between asthma frequency and exposure to animals (cats) and plants (olive). Others authors have shown that exposure to ornamental plants amplifies the worsening of asthma [19]. Several epidemiological studies suggest a link between exposure to allergens of cats and dogs and exacerbation of asthma [20,21]. Indeed, Matsui et al. have demonstrated an increased risk of asthma in patients who are in direct contact with cats [14].

In the present study, a significant correlation between the frequency of attacks and the number of permanent occupants (> 6) ($P = 0.026$) was noted. However, Kuehni et al. found that the aggravation of all forms of asthma in children does not depend on the size of the family [22].

For associated symptoms of asthma, a correlation between rhinitis and type of habitat ($P = 0.045$) was found. Rhinitis was also observed among patient's residents in urban areas. This can be explained by the high pollution rate in these regions.

There was a correlation between the sensitivity against *Aspergillus* and the severity of asthma [23,24]. However, no significant differences were observed with *Alternaria* [24]. In fact, the effect fungi colonization of sputum in asthma

Table 2 Comparison of mycological and molecular study and specific Ig E isolated in asthmatic patients.

ID patient	Sequence_ID	Isolation source	Culture	ITS sequencing	Accession number	<i>Penicillium notatum</i>	<i>Cladosporium herbarum</i>	<i>Aspergillus fumigatus</i>	<i>Alternaria alternata</i>
Patient 1	TN-5	Air	<i>A. versicolor</i>	<i>A. unguis</i>	KX610751				
	TN-42	Air	<i>Aspergillus</i> sp	<i>A. sepultus</i>	KX610733				
	TN-44	Environmental	<i>A. section flavi</i>	<i>A. ochraceus</i>	KX610749	Neg	Neg	Pos	Pos
	TN-10	Air	<i>A. section flavi</i>	<i>A. pallidofulvus</i>	KX610754				
	TN-107	Nasal	<i>A. flavus</i>	<i>A. flavus</i>	KX610723				
Patient 2	TN-7	Environmental	<i>Aspergillus</i> sp	<i>A. tamarii</i>	KX610719				
	TN-33	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610724	Pos	Pos	Pos	Neg
	TN-49	Air	<i>Aspergillus</i> sp	<i>A. chevalieri</i>	KX610737				
	TN-1	Environmental	<i>A. candidus</i>	<i>A. candidus</i>	KX610753				
Patient 3	TN-3	Nasal	<i>A. fumigatus</i>	<i>A. fumigatus</i>	KX610739	Neg	Neg	Neg	Neg
	TN-121	Environmental	<i>A. fumigatus</i>	<i>A. fumigatus</i>	KX610740				
Patient 4	TN-68	Nasal	<i>A. flavus</i>	<i>A. flavus</i>	KX610725				
	TN-27	Air	<i>A. flavus</i>	<i>A. flavus</i>	KX610722	Neg	Pos	Pos	Pos
	TN-30	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610732				
Patient 5	TN-57	Air	<i>A. flavus</i>	<i>A. flavus</i>	KX610728	Neg	Neg	Pos	Pos
Patient 6	TN-69	Air	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610744				
	TN-80	Environmental	<i>Aspergillus</i> sp	<i>A. quadrilineatus</i>	KX610735	Neg	Neg	Neg	Pos
	TN-85	Environmental	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610745				
Patient 7	TN-203	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610729	Neg	Pos	Neg	Neg
	TN-205	Environmental	<i>Aspergillus</i> sp	<i>A. quadrilineatus</i>	KX610736				
Patient 8	TN-24	Air	<i>A. versicolor</i>	<i>A. unguis</i>	KX610752	Neg	Neg	Neg	Neg
	TN-25	Air	<i>A. oryzae</i>	<i>A. sepultus</i>	KX610734				
	TN-26	Air	<i>A. section flavi</i>	<i>A. ochraceus</i>	KX610750				
	TN-43	Suptum	<i>Aspergillus</i> sp	<i>A. quadrilineatus</i>	KX610755	Neg	Neg	Neg	Neg
Patient 10	TN-100	Nasal	<i>A. fumigatus</i>	<i>A. fumigatus</i>	KX610741	Neg	Neg	Neg	Neg
Patient 11	TN-136	Environmental	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610748	Neg	Neg	Neg	Neg
	TN-139	Air	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610747				
	TN-144	Environmental	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610746				
Patient 12	TN-281	Nasal	<i>A. fumigatus</i>	<i>A. fumigatus</i>	KX610742	Neg	Neg	Neg	Neg
	TN-263	Air	<i>A. section flavi</i>	<i>A. tamarii</i>	KX610720				
	TN-275	Environmental	<i>A. section flavi</i>	<i>A. tamarii</i>	KX610721				
Patient 13	TN-225	Suptum	<i>A. flavus</i>	<i>A. flavus</i>	KX610726				
	TN-230	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610730				
	TN-236	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610731	Neg	Neg	Pos	Pos
	TN-235	Environmental	<i>A. flavus</i>	<i>A. flavus</i>	KX610727				
Patient 14	TN-301	Nasal	<i>Aspergillus</i> sp	<i>A. chevalieri</i>	KX610738	Neg	Neg	Pos	Neg
	TN-306	Environmental	<i>A. nidulans</i>	<i>A. nidulans</i>	KX610743				

Neg: negative; Pos: positive.

remains not clear [25]. Recently, Zubairi et al. found an association of higher concentration of indoor *Aspergillus* spp. with asthma exacerbation in adults [26].

Moreover, the internal fungal profile is governed by the outside dispersion of the sources of fungi [27]. This profile varies according to the media and sampling method used, the geographical location, the season, the temperature, the humidity, the ventilation and the behavior of the occupant [28].

In our study, molds were detected in 97.8% of the environmental samples with the predominance of *Aspergillus* genus. The average concentration of total fungi was 26.33 CFU/m³ (6–163). Studies performed in different countries provide variable results. In Eastern France, higher indoor molds concentrations of *Aspergillus*, *Penicillium* and *Cladosporium* were found and exceeding the values of 1000 CFU/mm³ [29]. Shelton et al. recorded the concentration of 500 CFU/m³ in the United States [30]. Takahashi observed a concentration range of 13–2750 CFU/m³ in Japan [31] whereas, De Ana et al. isolated 11 843 fungal colonies in different homes of allergic patients in Spain [32].

Allergen sensitization is associated with asthma morbidity. A better understanding of allergen sensitization patterns among asthmatic children could help clinicians for more effective health care. The IgE assay realized for 21 patients showed that approximately the half of these patients was sensitized to one or several allergens. The frequencies published in different studies varied between 16% and 38% [33,34]. Recently, Beck et al. showed that more than 50% of included patients were sensitized to *Alternaria* and *Aspergillus* [35].

Arroyave et al. demonstrated that sensitization to particular indoor environmental allergens (*Aspergillus* and *Alternaria*) was found to be a risk factor for asthma emergency department visits. These outcomes increased as the concentration of IgE to these allergens increased [36].

Moreover, Vojdai et al. have demonstrated that most patients with high levels of antibodies against various mold antigens also exhibited elevated antibodies against purified mycotoxins, indicating that the patients had been exposed to mold spores and mycotoxins [37].

Our molecular study allowed us to identify a rare *Aspergillus* species, such as *A. unguis*, *A. sepultus* and *A. pallidofulvus*, which are new *Aspergillus* species. We isolated *A. tamari* frequently confused with *A. flavus*. Recently, this species was isolated in a patient with *Aspergillus* infection and in patient with cutaneous aspergillosis [38,39].

We also isolated *Bipolaris* from the bedroom of five patients. In fact, this species was isolated from lung biopsy of a patient with asthma [40].

These results confirmed the usefulness of molecular techniques for accurate identification of strains. The sequencing of ITS regions has proved effective in the precise identification of *Aspergillus* species [41,42].

In others studies, specific qPCR were used to analyze the microorganisms composition of indoor dust dwelling and to evaluate fungi exposure and risks of developing asthma [43,44].

Our study allowed us to evaluate the risk of fungal contamination in asthmatic population and this can be useful to prevent the exacerbation of asthma in our population. We

then advice to use the personal protection with masks, to assure the humidity control and if necessary changing the habitat.

In conclusion, the allergy in molds is an indisputable reality that is necessary to look for in front of any severe asthma. However, the exposure level to fungal allergens is difficult to evaluate and the knowledge on the risks associated with indoor molds are still limited. So, it is important to establish clearly a relationship between exposure to fungi and health disorders in order to set up specific and effective preventive measures.

Contribution of authors

The authors alone are responsible for the content and writing of the paper.

Disclosure of interest

The authors declare that they have no competing interest.

References

- [1] Agarwal R, et al. Cut-off values of serum IgE (total and *A. fumigatus*-specific) and eosinophil count in differentiating allergic bronchopulmonary aspergillosis from asthma. *Mycoses* 2014;57:659–63.
- [2] Cai X, et al. Transient receptor potential vanilloid 2 (TRPV2), a potential novel biomarker in childhood asthma. *J Asthma* 2013;50:209–14.
- [3] Delmas MC, et al. [Prevalence and control of asthma in young children in France]. *Rev Mal Respir* 2012;29:688–96.
- [4] Bunyavanich S, et al. Peanut, milk, and wheat intake during pregnancy is associated with reduced allergy and asthma in children. *J Allergy Clin Immunol* 2014;133:1373–82.
- [5] Calderon MA, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol* 2015;136:38–48.
- [6] Jacquemin B, et al. The role of air pollution in adult-onset asthma: a review of the current evidence. *Semin Respir Crit Care Med* 2012;33:606–19.
- [7] Dick S, et al. A systematic review of associations between environmental exposures and development of asthma in children aged up to 9 years. *BMJ Open* 2014;4:e006554.
- [8] Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air* 2007;17(4):284–96.
- [9] The European health report. Health and health systems; 2009.
- [10] Stark PC, et al. Fungal levels in the home and allergic rhinitis by 5 years of age. *Environ Health Perspect* 2005;113(10):1405–9.
- [11] Rosenbaum PF, et al. Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma. *J Expo Sci Environ Epidemiol* 2010;20(6):503–15.
- [12] Bateman ED, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143–78.
- [13] de Hoog GS, et al. Molecular analysis and pathogenicity of the *Cladophialophora carrionii* complex, with the description of a novel species. *Stud Mycol* 2007;58:219–34.
- [14] Matsui EC, et al. Asthma in the inner city and the indoor environment. *Immunol Allergy Clin North Am* 2008;28:665x–86x.
- [15] O'Connor GT, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: the Inner-City Asthma Study. *J Allergy Clin Immunol* 2004;114:599–606.

- [16] Johnston NW, Sears MR. Asthma exacerbations. 1. Epidemiology. Thorax 2006;61:722–8.
- [17] Palmer LJ, et al. Environmental factors and asthma and allergy in schoolchildren from Western Australia. Eur Respir J 1999;14:1351–7.
- [18] Driessen JM, et al. Inspiratory airflow limitation after exercise challenge in cold air in asthmatic children. Respir Med 2012;106:1362–8.
- [19] Aydin O, et al. Allergic sensitization to ornamental plants in patients with allergic rhinitis and asthma. Allergy Asthma Proc 2014;35:e9–14.
- [20] Chew FT, et al. Presence of domestic pets and respiratory symptoms in asthmatic children. Ann Acad Med Singapore 1997;26:294–8.
- [21] Melen E, et al. Influence of early and current environmental exposure factors on sensitization and outcome of asthma in pre-school children. Allergy 2001;56:646–52.
- [22] Kuehni CE, et al. Are all wheezing disorders in very young (preschool) children increasing in prevalence? Lancet 2001;357:1821–5.
- [23] Agbetile J, et al. Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV1. Clin Exp Allergy 2012;42:782–91.
- [24] Zou H, et al. Correlation between fungal sIgE and bronchial asthma severity. Exp Ther Med 2013;6:537–41.
- [25] Fairs A, et al. IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010;82:1362–8.
- [26] Zubairi AB, et al. Association of airborne *Aspergillus* with asthma exacerbation in Southern Pakistan. Asia Pac Allergy 2014;2:91–8.
- [27] Adams RJ, Weiss ST, Fuhlbrigge A. How and by whom care is delivered influences anti-inflammatory use in asthma: results of a national population survey. J Allergy Clin Immunol 2003;112:445–50.
- [28] Das S, et al. Proteomics blood testing to distinguish chronic rhinosinusitis subtypes. Laryngoscope 2008;118:2231–4.
- [29] Reboux G, et al. Indoor mold concentration in Eastern France. Indoor Air 2009;19:446–53.
- [30] Shelton BG, et al. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol 2002;68:1743–53.
- [31] Takahashi T. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. Mycopathologia 1997;139:23–33.
- [32] de Ana SG, et al. Seasonal distribution of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* species isolated in homes of fungal allergic patients. J Investig Allergol Clin Immunol 2006;16:357–63.
- [33] Maurya V, et al. Sensitization to *Aspergillus* antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. Chest 2005;127:1252–9.
- [34] Shah A, Panjabi C. Asthma, hypersensitivity, and coitus. Am J Respir Crit Care Med 2004;170:1135.
- [35] Beck AF, et al. Allergen sensitization profiles in a population-based cohort of children hospitalized for asthma. Ann Am Thorac Soc 2015;12:376–84.
- [36] Arroyave WD, Rabito FA, Carlson JC. The relationship between a specific IgE level and asthma outcomes: results from the 2005–2006 National Health and Nutrition Examination Survey. J Allergy Clin Immunol Pract 2013;1:501–8.
- [37] Vojdani A, et al. Antibodies to molds and satratoxin in individuals exposed in water-damaged buildings. Arch Environ Health 2003;58:421–32.
- [38] Tam EW, et al. Misidentification of *Aspergillus nomius* and *Aspergillus tamarii* as *Aspergillus flavus*: characterization by internal transcribed spacer, beta-Tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. J Clin Microbiol 2014;52:1153–60.
- [39] Sharma S, et al. Primary cutaneous aspergillosis due to *Aspergillus tamarii* in an immunocompetent host. BMJ Case Rep 2013;010128.
- [40] Karim M, et al. Disseminated bipolaris infection in an asthmatic patient: case report. Clin Infect Dis 1993;17:248–53.
- [41] Henry T, Iwen PC, Hinrichs SH. Identification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. J Clin Microbiol 2000;38:1510–5.
- [42] Balajee SA, et al. *Aspergillus* species identification in the clinical setting. Stud Mycol 2007;59:39–46.
- [43] Rocchi S, et al. Microbiological characterization of 3193 French dwellings of Elfe cohort children. Sci Total Environ 2015;505:1026–35.
- [44] Scherer E, et al. Assessment of pets (cats and dogs) in homes using electrostatic dust collectors and QPCR: new tools to evaluate exposure and risk of allergies. Int J Environ Health Res 2016;26:589–99.