Fungal populations in the bedroom dust of children in Havana, Cuba, and its relationship with environmental conditions

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Abstract

The study of the fungal community composition in house dust is useful to assess the cumulative exposure to fungi in indoor environments. The objective of this research was to characterize the fungal diversity of house dust and its association with the environmental conditions of bedrooms. For this, the dust was collected from 41 bedrooms of children between the ages of 8 and 9 with a family history of asthma, residents of Havana, Cuba. The fungal content of each sample was determined by two methods: plate culture with Malt Extract Agar and by direct microscopy. An ecological analysis was carried out from the fungal diversity detected. To describe the factors associated with the fungi detected, bivariate logistic regression was used. Through direct microscopy, between 10-2311 fragments of hyphae and spores corresponding mainly to *Cladosporium*, *Coprinus*, *Curvularia*, *Aspergillus/ Penicillium*, *Xylariaceae*, and *Periconia* were identified. Through the culture, 0–208 CFU were quantified, where *Aspergillus*, *Cladosporium*, and *Penicillium* predominated. The culturability evidenced the differences between the quantification determined by both methods. A positive relationship was found between the type of cleaning of the furniture, the presence of trees in front of the bedroom, indoor relative humidity, indoor temperature, the presence of air conditioning by air conditioning and natural ventilation with specific spore types and genders. The use of two different identification methods allowed to detect a greater fungal diversity in the residences evaluated. Monitoring the exposure to these fungal allergens in childhood can help to prevent sensitization in the allergic child, the development of asthma and other respiratory diseases.

Introduction

Household dust contains organic and inorganic matter, and is the largest reservoir of microorganisms in indoor environments (Dassonville et al. 2008; Macher 2001). Piecková and Wilkins (2004) reported that dust inside houses can be made up of 82% organic matter, in contrast to 18% that contains dust from outside. In addition, it can be the source of nutrients for various micromycetes. The fungal genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* have predominated in studies that analyze dust from residences located in different climatic regions (Shinohara et al. 2018; Shan et al. 2019; Andersen et al. 2021). However, these investigations have also raised the relationship of high fungal diversity with extrinsic factors such as season of the year, winds and rainfall, and with intrinsic factors like human activity, temperature, relative humidity (RH), and indoor nutrient source (Ja Cho et al. 2008; Dallongeville et al. 2015). Additionally, the longevity of fungal propagules is also variable. The hyphal fragments are the first to lose their viability, while the spores persist for a longer time, even some survival structures can remain viable for decades (Rintala et al. 2012).

Davies (1958) and Van der Werff (1958) were among the first to investigate the presence of fungi in house dust through their isolation in culture media. Subsequently, several authors pointed out the need to expressly refer to the methodology used in the isolation and characterization of fungi from dust, since the method of sample collection and processing can impact the qualitative and/or quantitative data (Duce et al. 1986; Gravesen 1987; Angulo et al. 1993). Some studies analyzed the fungal diversity of house dust

by isolating and culturing viable propagules in agarized culture media (Miller 2001; AIHA 2005). Other investigations have been based on identification of spores by direct microscopy (Niemeier et al. 2006). A single method cannot describe and quantify fungi's species richness in full, because each method has advantages and disadvantages (Nevalainen et al. 2015). Culture-based methods allow the identification of the isolates down to species (Cabral, 2010). However, the medium composition, the incubation temperature and the light conditions regulate the formation of colonies in the medium. The availability of space in the culture plate can also limit growth, especially of slower growth organisms (Mensah-Attipoe and Toyinbo 2019). In that sense, An et al. (2018) reported that only 10 to 40% of the propagules collected are cultivable. For their part, direct microscopy-based methods allow the detection of both cultivable and non-cultivable spores, and their identification can generally only be done at the genus level (Niemeier et al. 2006). Currently there are no standardized or definitive methods for this analysis, which allow to directly correlate human exposure to fungi and the development allergies (Park et al. 2018).

The analysis of the fungal community of house dust is important for allergology since the fragments of hyphae and spores can cause hypersensitivity reactions. This is due to the presence of enzymes, cell wall components, and toxins with allergenic properties (Levetin et al. 2016). The main immunological mechanisms of fungal allergies are type I, II, III and IV hypersensitivity reactions (Simon-Nobbe et al. 2008). Among the allergic diseases caused by these hypersensitivity reactions are rhinitis, asthma, and atopic dermatitis (Denning et al. 2014; Rudert and Portnoy 2017).

Frankel et al. (2012) state that the study of house dust is useful to measure respiratory exposure to fungi in indoor environments and its relationship with asthma. This practice assumes that fungi in dust are a proxy for past or ongoing airborne exposure (Ja Cho et al. 2008). The accumulation of the daily average exposure to fungi is more hazardous to human health than the exposure of a single day (Khan and Karuppayil, 2011). Several investigations suggest that exposure to fungal allergens during early childhood may increase the risk of sensitization in genetically predisposed children (Jacob et al. 2002; Ege et al. 2011). This implies that for children with a family history of asthma, once sensitized, lower concentrations of allergens may be sufficient to trigger a hypersensitivity reaction, which can lead to symptoms of this disease (Dannemiller et al. 2014). Additionally, early exposure to damp environments, visible fungal growth, and mold odor is associated with the development of asthma in children (Nastasi et al. 2020). There is also evidence that this relationship is stronger when a high fungal concentration is detected in the asthmatic child's room (Karvonen et al. 2015).

In Cuba there is a high prevalence of allergic diseases. In this sense, bronchial asthma is the seventh cause of death among lower respiratory diseases, and Havana is the province with the highest prevalence of this illness. In pediatric age, more than 60% present a moderate quality of life and out of every 1000 children between the ages of 5–9 years, 107 are asthmatics (Statistical Yearbook of Health, 2020). However, studies on the fungi present in the indoor dust of homes of infants with a history of allergic diseases are scarce. Therefore, the objective of this work was to characterize the fungal diversity of house dust and its association with the environmental conditions of bedrooms of children with a family history of asthma.

Materials And Methods

Study area and sample collection

Dust from 41 bedrooms of children between 8 and 9 years old with a family history of asthma, belonging to the project cohort of the HINASIC (National History of Wheezing in Cuba) was analyzed (Venero et al. 2013). These houses are located in urban and suburban areas of Havana, Cuba. Dust collection was carried out with a sterile brush in places in the room with evidence of accumulation. The sample was stored at 4 °C overnight until it was processed.

Environmental Assessment Of Bedrooms

In each house, the construction materials and structure of the house was recorded according to the criteria proposed by Palacio and Prieto (2004) regarding the type of materials present in the floor, walls and ceiling. The presence of humidity problems in the bedrooms was recorded. Humidity problems were defined as any visible or perceived outcome of excess moisture that can cause problems in buildings, such as mold, leaks or material degradation, mold odor or excess moisture, directly measured in terms of relative humidity or moisture content, and visible fungal growth on walls and ceilings (Heseltine and Rosen 2009). The quality of ventilation, the use of air conditioning systems, proximity to groves and the presence of dusty surfaces, the frequency and type of cleaning of the room and its furniture were also taken into account. As environmental parameters, the values of relative air humidity and interior temperature (T^a) were recorded.

Analysis Of Dust Samples

For the analysis of the dust through the culture method, from each sample, serial dilutions were made, by suspending 1g of the collected dust in a solution of Tween 80 (0.02% in sterile distilled water). Then 0.5 mL of the last dilution, were inoculated by spreading, in Petri dishes with Malt Extract Agar. These plates were then incubated at 28 ± 0.5 °C for five days in the dark (Miller, 1988; Ja Cho et al. 2008).

Subsequently, the fungal colonies (CFU) that grew on both plates were quantified and isolated. The criteria of Carmichael et al. (1980), Barnett and Hunter (1998) and Seifert et al. (2011) were used for the taxonomic identification of genera.

Following the culture-based method, each dust suspension was vortex homogenized for 30 seconds, then centrifuged at 1008 xg for 20 minutes and the supernatant was discarded. The pellets were suspended in 200 µl of glycerogelatin (20 g Gelatin, 70 ml distilled water, 60 ml glycerin, 2.4 g phenol) at 40°C. Of this new homogenized suspension, 50 µl were gently pipetted onto a 75 x 25 mm and 1 mm thick slide. The sample was evenly distributed and gently covered with a 22 x 22 mm coverslip, so that no air bubbles were formed, it was kept at 15°C until solidified and the coverslip was firmly attached to the slide.

Properly labeled preparations were observed with a bright field optical microscope at 400X magnification, observing equidistant transects until the coverslip area was completed. The spores were microscopically identified to either genus or group level, taking into account the distinctive morphological characteristics

of the spores (color, shape, wall, septation, size and other characters). The quantitative results were expressed as total spores + hyphal fragments count (Sivasubramani et al. 2004; Niemeier et al. 2006).

Ecological Analysis

The hierarchical location of the fungi detected in the dust samples, for each sampling methodology, was determined using the Olmstead-Tukey correlation method. It was represented by quadrant graph (Sokal and Rolf, 2012), based on the calculation of the relative abundance of each detected fungus, expressed as Log (RD + 1) and the relative frequency (RF). Each detected fungus was classified as: Dominant: relative frequency and relative abundance higher than the average values; Constant: relative frequency above average and relative abundance below average; Occasional: relative frequency below average and relative abundance above average; Rare: relative frequency and relative abundance lower than the average values.

The relationship between culturable fungal propagules (CFU) and total spores and hyphal fragments determined in each room indicates culturability (Niemeier et al. 2006) and was calculated using the following equation:

$$\text{Culturability dustborne fungi (\%)} = \frac{\text{Culturable fungi count} \times 100\%}{\text{spores} + \text{hyphal fragments count}}$$

Statistical analysis

Continuous or categorical variables were established based on the characteristics of the bedrooms, the behavior of the inhabitants and the environmental parameters obtained from the responses to the questionnaire and the environmental evaluation. The continuous variables were transformed to dichotomous using the median as the cut-off point of the distribution. Spore types and indoor genera with a frequency greater than 40% were dichotomized using the median value for statistical analysis; due to the existence of dispersion in the quantification values and to the fact that standard limit values are not reported in the consulted literature. The bivariate associations between the fungal levels and the variables evaluated were examined in a 2x2 contingency table, using the Chi-square test (χ^2). Bivariate logistic regression model was used to analyze the associations. The studied associations were indicated by odds ratio (OR), and their 95% confidence intervals (CI). In all cases, the alpha value equal to 0.05 was used to indicate statistical significance.

Results

Through direct microscopy 10–2311 spores and hyphal fragments were counted in the dust from the studied bedrooms. The average was 227 total spores + hyphal fragments. By means of the culture method, 0–208 CFU were quantified, with a mean of 24 CFU. The culturability showed the differences between the quantification determined by both methods. In 39 bedrooms (95.12% of the total) this index

ranged between 0–100% (50–81% in 2; 27.8–49.2% in 11; 1.43–16.67% in 18; less than 1% in 8). Only in 2 bedrooms (C9 and A22) the culturability was higher than 100% (Table 1).

Table 1

Quantification of the total spores + hyphal fragments and culturable fungal propagules (CFU) obtained by the methods of direct microscopy and culture in the sampled houses. Culturability of fungal propagules.

Methods				
Date	Code	Direct microscopy	Culture	Culturability
		(spores + hyphal fragments)	(CFU)	
8/2/2018	C1	29	2	6.90
8/2/2018	C2	483	2	0.41
15/02/2018	C3	24	10	41.67
1/2/2018	C4	619	5	0.81
1/2/2018	C5	33	10	30.30
8/2/2018	C6	542	9	1.66
15/02/2018	C7	169	9	5.33
8/2/2018	C8	1048	100	9.54
1/2/2018	C9	14	66	471.43
1/2/2018	C10	118	1	0.85
22/02/2018	C11	667	22	3.30
22/02/2018	C12	2311	33	1.43
15/02/2018	C13	110	0	0.00
3/3/2018	C14	63	19	30.16
22/02/2018	C15	236	100	42.37
22/03/2018	A1	204	100	49.02
22/03/2018	A2	161	15	9.32
29/03/2018	A3	316	1	0.32
29/03/2018	A4	24	12	50.00
29/03/2018	A5	251	2	0.80
22/03/2018	A6	36	10	27.78
17/05/2018	A7	62	22	35.48
17/05/2018	A8	66	6	9.09

Methods				
17/05/2018	A9	107	1	0.93
14/06/2018	A10	131	7	5.34
14/06/2018	A11	136	16	11.76
28/02/2019	A12	10	3	30.00
7/3/2019	A13	114	2	1.75
7/3/2019	A14	92	6	6.52
7/3/2019	A15	27	2	7.41
7/3/2019	A16	78	13	16.67
28/03/2019	A17	204	2	0.98
28/03/2019	A18	55	3	5.45
28/03/2019	A19	83	2	2.41
30/05/2019	A20	158	5	3.16
30/05/2019	A21	79	64	81.01
30/05/2019	A22	58	208	358.62
30/05/2019	A23	106	30	28.30
20/06/2019	A24	65	24	36.92
20/06/2019	A25	188	15	7.98
20/06/2019	A26	36	16	44.44

Thirty-three fungal genera were detected (5 by means of culture-based method, 22 through direct microscopy, and 6 by both methods). In addition, 5 spore types were defined by direct microscopy, which grouped several spores with similar morphology (*Aspergillus/ Penicillium*, *Xylariaceae*, *Periconia*, *Torula*, and *Sporidesmium*). Other propagules were also identified through this independent culture methodology, corresponding to hyaline hyphal fragments, pigmented hyphal fragments, ascospores, basidiospores, and unidentified spores. *Aspergillus*, *Cladosporium* and *Penicillium* were classified as Dominant among the fungi detected through the culture-based method (Fig. 1). However, *Cladosporium*, *Coprinus*, *Curvularia*, and *Aspergillus/ Penicillium*, *Xylariaceae*, and *Periconia* prevailed through direct microscopy method (Fig. 2). *Trichoderma* was classified as Constant and the rest of the genera and spore types counted were classified as Rare. The hyaline hyphal fragments presented a RF 53.66%, the pigmented ones RF 73.17% and the conglomerates RF 34.15%.

The results of the environmental evaluation showed that the highest percentage values corresponded to bedrooms with humidity problems, poor ventilation, and lacking air conditioning (Table 2). In the bedrooms with air conditioning, its use was not continuous. In addition, in most cases, the frequency of cleaning the furniture and the bedroom was less than 3 times a week and in a wet way. The average T^a and RH were 29.2 °C and 59.6%, respectively.

Table 2
Characteristics of the houses, behavior of the inhabitants and
environmental parameters determined in the study

Characteristic	N	%
		n = 41
House quality		
Good	19	46
Bad	22	54
Humidity problems		
Yes	21	51
No	20	49
Visible fungal growth		
Yes	9	22
No	32	78
Presence of air conditioning systems		
Yes	9	22
No	32	78
Stuffiness		
Yes	22	54
No	19	46
Bedroom in front of grove		
Yes	8	20
No	33	80
Number of objects in the bedroom with dust		
≤ 6	24	59
> 6	17	41
Furniture cleaning frequency		
≤ 3/for week	25	61
> 3/for week	16	39
Floor cleaning frequency		

Characteristic	N	%
≤ 3/for week	24	59
> 3/for week	17	41
Furniture cleaning type		
Dry	8	20
Damp	33	80
Bedroom cleaning type		
Dry	20	49
Damp	21	51
Average indoor RH, %	59.61	
Average indoor temperature, °C	29.34	

Bivariate logistic regression was used to evaluate the association between total fungal propagules and those with a relative frequency greater than 40% (from the culture-based method: *Aspergillus*; from the direct microscopy method: *Aspergillus/ Penicillium*, *Cladosporium*, *Coprinus*, *Curvularia*, *Nigrospora*, hyaline hyphal fragments, and pigmented hyphal fragments). *Cladosporium*, *Aspergillus/ Penicillium*, hyaline hyphal fragments, and pigmented hyphal fragments were positively associated with total spores + hyphal fragments. Furthermore, *Aspergillus* was positively related to total viable fungal propagules (Table 3).

Table 3

Association between the characteristics of the houses, the behavior of the inhabitants and the environmental parameters with the quantification of the spores + hyphal fragments and viable fungal propagules.

Total fungi and fungal taxa (FR > 40%)	Variable	OR	(95% IC)	p
Total viable fungal propagules	Relative Humidity (> 60 vs. ≤60)	4.04	(1.07–15.27)	0.0349
<i>Aspergillus</i>	Relative Humidity (> 60 vs. ≤60)	4.40	(1.10–17.68)	0.0314
	Temperature (> 29°C vs. ≤29°C)	3.78	(0.99–14.48)	0.0474
	Total viable fungal propagules (> 10 CFU vs. ≤10 CFU)	13.33	(2.80–63.44)	0.0004
Total spores + hyphal fragments	Floor cleaning method (dry vs. wet)	3.71	(1.02–13.51)	0.0426
	Bedroom in front of grove (yes vs. no)	10.77	(1.18–98.03)	0.0146
<i>Aspergillus/ Penicillium</i>	Total spores + hyphal fragments (> 107 vs. ≤107)	9.60	(2.31–39.95)	0.0010
<i>Cladosporium</i>	Total spores + hyphal fragments (> 107 vs. ≤107)	5.83	(1.52–22.41)	0.0080
	Floor cleaning method (dry vs. wet)	5.83	(1.52 – 22.41)	0.0080
	Furniture cleaning method (dry vs. wet) ^a			
<i>Coprinus</i>	Stiffness (yes vs. no)	0.12	(0.03–0.54)	0.0025
	Bedroom in front of grove (yes vs. no)	12.25	(1.34–111.9)	0.0093
	Presence of Air Conditioning systems (yes vs. no) ^b			
<i>Nigrospora</i>	Bedroom in front a grove (yes vs. no)	6.00	(1.04–34.75)	0.0319
Hyaline hyphal fragments	Total spores + hyphal fragments (> 107 vs. ≤107)	5.83	(1.52–22.41)	0.0080
	Presence of Air Conditioning systems (yes vs. no)	13.33	(1.48–120.2)	0.0064

Total fungi and fungal taxa (FR > 40%)	Variable	OR	(95% IC)	p
Pigmented hyphal fragments	Total spores + hyphal fragments (> 107 vs. ≤107)	4.64	(1.24–17.37)	0.0194
	Furniture cleaning method (dry vs. wet)	12.25	(1.34–111.9)	0.0093

Statistical analysis was also performed to determine the relationship between the fungi detected by both methodologies and the characteristics of the bedroom, RH, and T^a. *Aspergillus* and total viable fungal propagules were positively associated with RH, and *Aspergillus* was positively associated with T^a. All the fungi detected by the direct microscopy method and included in this statistical analysis, were associated with at least one of the characteristics of the bedroom, with the exception of *Curvularia* and *Aspergillus/Penicillium*. Pigmented hyphal fragments were positively associated with dry cleaning of furniture. On the other hand, total spores + hyphal fragments counts were positively related to dry cleaning of the bedroom floor. *Coprinus*, *Nigrospora*, and total spores + hyphal fragments were positively associated with the report of a grove near the bedroom. Hyaline hyphal fragments were positively associated with air conditioning. In all the houses that had air conditioning, *Coprinus* was not detected. Furthermore, the detection of *Coprinus* spores was negatively associated with poor ventilation.

Discussion

The study of the fungal composition of house dust is a useful measure of long-term cumulative exposure to fungi in indoor environments and is less influenced by indoor activities and turbulence than the airborne mycobiota (Jacob et al. 2002). There is no standardized method for analyzing these samples. In some investigations they use culture-based methods and other culture-independent methods such as direct microscopy as well as biochemical and molecular methods (Choi et al. 2014; Dallongeville et al. 2015; An et al. 2018; Andersen et al. 2021). However, due to the limitations each one, the best option is a combination of methods. For this reason, this research used the combination of culture and direct microscopy methods and provide detailed information on the culturable and non-culturable allergenic mycobiota present in a residence (Nevalainen et al. 2015).

In most of the bedrooms (39), a greater number of propagules identified by direct microscopy (total spores + hyphal fragments) was detected than by culture. As stated by Niemeier et al. (2006) this is due to the fact that in the count by direct microscopy all the fungal propagules are observed, regardless of their viability; while the culture method only detects those that are capable of growing in the culture medium used and under the established incubation conditions. The inclusion of the counting of the hyphal fragments in the direct microscopy method allowed us to reduce the discrepancies in the culturability values, since new colonies can also be obtained from them. Although many investigations only quantify spores, there is evidence to suggest that hyphal fragments are more common than spores

in indoor environments and that they have a high content of allergenic proteins (Green et al. 2006; Lin et al. 2016). Also, Afanou et al. (2018) indicated that hyphal fragments contain immunostimulatory macromolecules, which include antigens, allergens, mycotoxins, and β -1,3-glucans. In the Caribbean area, sensitization to hyphal fragments is comparable to basidiospores incidence, even more prevalent than conidial sensitization (Rivera-Mariani and Bolaños-Rosero, 2012).

The composition of house dust is variable in different geographical areas and influences the diversity of microbial communities. *Aspergillus*, *Cladoporium*, *Penicillium*, *Coprinus*, *Curvularia*, as well as *Aspergillus/ Penicillium*, *Xylariaceae*, and *Periconia* were detected in most of the houses analyzed in the present investigation. This result contrasts with other studies that refer only to the prevalence of *Aspergillus*, *Cladoporium*, *Penicillium* or *Aspergillus/ Penicillium* (Niemeier et al. 2006; Shinohara et al. 2018; Stamatelopoulou et al. 2020). In this study, the presence of eighteen genera and three spore types in house dust is reported for the first time in Cuba (*Cercospora*, *Coprinus*, *Corynespora*, *Ganoderma*, *Helminthosporium*, *Lasiodiplodia*, *Leptosphaeria*, *Leptosphaerulina*, *Dydimosphaeria*, *Massarina*, *Monodictys*, *Paraphaeosphaeria*, *Pestalotiopsis*, *Pleospora*, *Spegazzinia*, *Stachybotris*, *Tetraploa*, *Venturia*, spore types *Bipolaris/Dreschlera*, *Sporidesmium*, and *Xylariaceae*). This result is a contribution to the knowledge about fungal diversity within homes in this neotropical country. Other propagules such as *Alternaria*, *Arthrinium*, *Fusarium*, *Nigrospora*, *Chaetomium*, *Trichotecium*, *Trichoderma*, and spore type *Torula* have been found in indoor dust in schools, libraries, and archives in Spain and Poland (Angulo et al. 1993; Karbowska-Berent et al. 2011).

The genera *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus* are considered potent allergenic agents associated with exacerbation of asthma in children as well as with the worsening of its symptoms (Pongracic et al. 2010; Zubairi et al. 2014; Tham et al. 2017a). In addition, these genera are related with the hospitalization of children for asthma. This relationship has also been reported for *Chaetomium*, *Coprinus*, *Ganoderma*, *Leptosphaeria*, *Pleospora*, *Periconia*, *Bipolaris/ Dreschlera*, ascospores, and basidiospores (Tham et al. 2017a, b). Likewise, there are reports of an association of asthma aggravation with *Cercospora*, *Chrysonilia*, *Epicoccum*, *Fusarium*, *Helminthosporium* and *Rhizopus* and of type I allergies with *Arthrinium*, *Corynespora*, *Leptosphaerulina*, *Nigrospora*, *Pithomyces*, *Spegazzinia*, *Stachybotris*, *Tetraploa*, *Torula*, *Trichoderma*, *Trichothecium*, and *Xylariaceae* (Jenkins 1981; Kurup 2003; Green 2005; Simon-Nobbe et al. 2008; Monzon et al. 2009; Karne et al. 2013; Njokuocha et al. 2016; Rudert and Portnoy 2017; Almaguer et al. 2018). There is no evidence that the genera *Dydimosphaeria*, *Lasiodiplodia*, *Massarina*, *Monodictys*, *Paraphaeosphaeria*, *Pestalotiopsis*, and *Venturia* are allergenic (Levetin et al. 2016; Baxi et al. 2016). Although in Cuba there is no association between exposure to fungi and asthma, there is evidence of sensitization to *Penicillium*, *Cladosporium*, and *Alternaria* in schoolchildren aged 6–7 years (Díaz et al. 2010). Barrios et al. (2019) reported the presence of *Acremonium*, *Candida*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Curvularia*, *Chrysonilia*, *Fusarium*, *Torula*, and non-sporulated mycelium in the nasal mycobiota of patients with respiratory allergies between the ages of 1–70 years living in Havana. In the aforementioned investigation, monosensitization to strains of environmental fungi of the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, and *Fusarium* was also demonstrated.

The environmental evaluation of houses and the detection of fungi present in house dust can contribute to applying measures that reduce fungal concentrations and therefore reduce exposure to their propagules (Baxi et al. 2016; Kader et al. 2018). This research showed that several factors can favor fungal contamination inside of the bedrooms. Some of these factors are the way of cleaning the furniture and the room, the presence of air conditioning, the presence of trees in front of the bedrooms, T^a, and RH.

Pigmented hyphal fragments, *Cladosporium*, and total spores + hyphal fragments were associated with dry cleaning of bedroom furniture and floor. Shinohara et al. (2018) found that the type of cleaning (dry or wet) can influence the fungal concentrations in the dust. If water is used to clean, a large part of the dust is eliminated. On the other hand, if the cleaning is dry, the increase of the fungal concentrations in the air is favored, which can then be settle to form dust accumulations over time. According Chew et al. (2016) some occupant activities such as wet cleaning can release large amounts of water into the air. If the bedroom does not vent outside, conditions of localized high humidity can develop and produce greater risk for condensation and fungal damage.

Aspergillus, *Penicillium*, *Trichoderma*, and *Fusarium* have been isolated from the air and dust of the filters of air conditioning systems (Hamada and Fujita 2002; Khan et al. 2009; Kelkar et al. 2005; Viegas et al. 2018). Therefore, it is probable that the hyaline hyphal fragments that were associated with the total spores + hyphal fragments and the presence of air conditioning could correspond to these genera, which are allergenic fungi related to asthma. On the other hand, discontinuous use of air conditioning during the day could lead to water condensation, increased RH and T^a, ideal conditions for fungal growth on various substrates (Sowiak et al. 2018). For homes in warmer climates, condensation can also be a problem and it is difficult to determine if air conditioning is helping to decrease humidity or adding fungi and dampness problems (Chew et al. 2016).

Although saprophytic and phytopathogenic fungi are found in plants. Their propagules can penetrate into houses through ventilation due to the interchange indoor/outdoor (Ponce-Caballero et al. 2013; Tang et al. 2015). *Nigrospora* species have been isolated as endophytes from leaves and stems of various plants, or as saprobes from detritus, dead larvae or leaf litter. *Nigrospora* species have also been commonly recorded as plant pathogens on many important economic crops, fruits and ornamentals plants (Sharma et al. 2013; Wang et al. 2017). *Coprinus* has not been registered as a plant pathogen or saprophyte, however it is usually found in grassy places, particularly under trees and shrubbery (Ulje and Bas 1988). This genus predominates in the Cuban atmosphere, which may explain its negative relationship with poor ventilation in the bedroom (Almaguer et al. 2014). *Coprinus* spore can directly aggravate respiratory diseases (Rivera-Mariani and Bolaños-Rosero, 2012) since their small size allows them to penetrate deeper into the airways (Levetin and Horner, 2002).

T^a and RH are the most important abiotic factors for the study of the indoor fungi (Abbasi and Samaei, 2019). In our study, the temperature was between 23–34 °C and of RH ranged from 40–76%, which favors growth and sporulation of some *Aspergillus* species and other fungi (Visagie et al. 2014; Rudramurthy et al. 2019; Abbasi and Samaei 2019). Korpi et al. (1997) showed that the fungal

concentrations increase one hundred times if the powder is incubated for 25 days with 96–98% RH without the addition of any nutrient. In the settled dust of very humid indoor environments, the so-called “first colonizers” such as *Aspergillus* can grow and develop (Karbowska-Bernet et al. 2011). In other latitudes, Delanoë et al. (2020) found a significant positive correlation between the RH recorded in houses with moisture damage in France and species of this genus. So, it could be expected that in tropical climates this relationship could be even stronger.

Cladosporium, *Aspergillus*/ *Penicillium*, hyaline hyphal fragments, and pigmented hyphal fragments were associated with total spores + hyphal fragments, and *Aspergillus* with total fungal propagules. This indicates the predominance of these genera and spore types in the dust mycobiota in these bedrooms. It also shows the contribution of hyphal fragments in the fungal composition of this environment, which can constitute a source of growth if favorable conditions exist for their development.

The detection of these fungal propagules in the residences of children with a family history of asthma is important because several authors have shown that the reduction in exposure to fungal allergens in childhood can prevent sensitization in the allergic child, the development of asthma and other respiratory diseases (Abdo Rodriguez and Cué Brugueras 2006; Hu et al. 2017). Knowledge about the factors associated with fungal dust propagules in bedroom contributes to designing interventions in order to reduce exposure to indoor allergens controlling allergic diseases from childhood, particularly asthma (Stamatelopoulou et al. 2020).

Conclusions

Aspergillus, *Cladosporium*, *Penicillium*, *Coprinus*, *Curvularia*, and the spore types *Aspergillus*/ *Penicillium*, *Xylariaceae*, and *Periconia* predominated in the dust of the studied bedrooms. The type of cleaning of the furniture, the presence of bedroom in front of a grove, relative humidity, temperature, the presence of air conditioning, and ventilation was related to the presence of the fungal propagules detected in the bedroom dust of children with a family history of asthma.

Declarations

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Ethics approval and consent to participate

The study protocol was approved by National Institute of Hygiene, Epidemiology and Microbiology, the local Havana Scientific Committee in Cuba

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kenia C. Sánchez Espinosa, Teresa I. Rojas Flores, Sonia Rodríguez Davydenko, Silvia J. Venero Fernández and Michel Almaguer. The first draft of the manuscript was written by Kenia C. Sánchez Espinosa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Olmstead-Tukey diagram showed the relationship between frequency and abundance of the viable fungi identified in dust. The dashed lines correspond to the mean abundance (horizontal) and occurrence (vertical) and are used to define the occasional, dominant, rare and frequent. 1. *Aspergillus*, 2. *Chaetomium*, 3. *Chrysonilia*, 4. *Cladosporium*, 5. *Curvularia*, 6. *Fusarium*, 7. *Monodictys*, 8. *Nigrospora*, 9. *Penicillium*, 10. *Rhizopus*, 11. *Trichoderma*, 12. Non-sporulated hyaline mycelium, 13. Non-sporulated pigmented mycelium

Figure 2

Olmstead-Tukey diagram showed the relationship between frequency and abundance of the fungi spores identified in dust. The dashed lines correspond to the mean abundance (horizontal) and occurrence (vertical) and are used to define the occasional, dominant, rare and frequent. 1. *Alternaria*, 2. *Arthrinium*, 3. *Ascosporas*, 4. *Basidiosporas*, 5. *Cercospora*, 6. *Chaetomium*, 7. *Cladosporium*, 8. *Coprinus*, 9. *Corynespora*, 10. *Curvularia*, 11. *Dydimosphaeria*, 12. *Epicoccum*, 13. *Fusarium*, 14. *Ganoderma*, 15. *Helminthosporium*, 16. *Lasiodiplodia*, 17. *Leptosphaeria*, 18. *Leptosphaerulina*, 19. *Massarina*, 20. *Monodictys*, 21. *Nigrospora*, 22. *Paraphaerosphaeria*, 23. *Periconia*, 24. *Pestalotiopsis*, 25. *Pithomyces*, 26. *Pleospora*, 27. *Spegazzinia*, 28. *Sporidesmium*, 29. *Stachybotris*, 30. *Aspergillus/Penicillium*, 31. *Bipolaris/Dreschlera*, 32. *Torula*, 33. *Xylariaceae*, 34. *Tetraploa*, 35. *Trichotecium*, 36. *Venturia*, 37. Unidentified spores