Original Article

Seasonal Distribution of *Alternaria, Aspergillus, Cladosporium* and Penicillium Species Isolated in Homes of Fungal Allergic Patients

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Abstract. *Background:* Allergy to airborne fungi can cause rhinitis and severe asthma, hence the exposure to spores inside home is an important factor of sensitization. The aim of this study was to determine the distribution and prevalence of species of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* inside and outside of homes of patients allergic to fungi and to evaluate seasonal variations.
Methods: Air samples were collected in 22 selected homes of patients with allergy to fungi using a volumetric method of impacting plates with culture media. The isolated species were identified and statistical analysis of the presence of the four fungi was carried out.
Results: A total of 431 indoor and 150 outdoor exposed plates were cultured, leading to isolation of 11 843 colonies of fungi (range 0 – 1 666 colony-forming units per cubic meter (CFUs/m^3). 85.5% of total colonies belonged to the four genera considered. The highest presence of *Aspergillus, Cladosporium* and *Penicillium* in indoor environment was registered in autumn. Alternaria was more frequent in summer. In the outdoor environment, *Penicillium* was more abundant in winter and *Aspergillus* in summer ($P = .002$).
The largest numbers of isolations were of *Cladosporium* and *Penicillium* during all four seasons, indoors as well as outdoors. Alternaria was present in all the homes studied both in summer and in autumn.
The most prevalent species were: *Alternaria alternata, Cladosporium herbarum, Cladosporium cladosporioides, Aspergillus* niger and *Penicillium chrysogenum.*
Conclusions: The quantitative analysis of the four taxa related with respiratory allergies demonstrated considerable seasonal variability. Statistical differences between the indoor and outdoor prevalence were detected only in *Alternaria.* In summer and autumn, the greater level of exposure to the four studied taxas occurred inside homes.

Key words: Fungal allergy. Indoor seasonal distribution. *Alternaria. Aspergillus. Cladosporium. Penicillium.*

Resumen. *Antecedentes:* La sensibilización a hongos atmosféricos puede ocasionar rinitis y asma severa, por lo que la exposición a esporas fúngicas en el interior del domicilio es un factor importante de sensibilización. El objetivo de este estudio ha sido determinar la distribución y prevalencia de *Alternaria, Aspergillus, Cladosporium* y *Penicillium* en el interior y exterior del domicilio de pacientes alérgicos a hongos, y conocer sus variaciones estacionales.
Métodos: Se han tomado muestras del aire atmosférico en 22 domicilios de enfermos alérgicos a uno o más de las cuatro especies consideradas, utilizando un método volumétrico de impactación en dos medios de cultivo. Se analiza estadísticamente la presencia de los hongos aislados.
Resultados: Se cultivaron 431 placas interiores y 150 exteriores, aislándose 11.843 colonias (rango 0 – 1.666 UFC/m^3). En el interior de los domicilios, en otoño se registró la mayor presencia de *Aspergillus, Cladosporium*

y *Penicillium*. El género *Alternaria* fue más frecuente en verano. En el exterior *Penicillium* resultó más abundante en invierno y *Aspergillus* en verano (p=.002). El 85.5% de las colonias correspondió a alguno de estos géneros.
El mayor número de aislamientos fue de *Cladosporium* y *Penicillium* en las 4 estaciones tanto en el interior como en el exterior. *Alternaria* estuvo presente en todos los domicilios estudiados tanto en verano como otoño.
Las especies fúngicas más prevalentes fueron: *A. alternata, C. herbarum, C. cladosporioides, A. niger* y *P. chrysogenum.*
Conclusiones: El análisis cuantitativo de los cuatro taxones más relacionados con alergias respiratorias demuestra una importante variabilidad estacional.
Solamente se hallaron diferencias estadísticas entre la prevalencia interior y exterior en *Alternaria*. En verano y otoño se produjo el mayor nivel de exposición intradomiciliaria a los cuatro taxones estudiados.

Palabras clave: Alergia a hongos, distribución estacional, interiores, *Alternaria, Aspergillus, Cladosporium, Penicillium.*

Introduction

Sensitization to certain airborne fungi has been shown to be a risk factor for severe respiratory disease in children and adults [1-3]. In recent years, there has been an increased interest in better characterizing the properties of the fungi present indoors, in analyzing the influence that they have as a risk factor for asthma [4-6], and in determining how variations in exposure levels to fungi at home influence the risk of developing or exacerbating asthma [7].

At present, little is known about the distribution of fungi inside homes. Years ago some studies were carried out in some areas of Spain but the methods used were rudimentary [8]. Furthermore, since that time many key factors have changed, including life-style habits, the addition of air conditioning and air heating units and the construction of buildings with new materials and ventilation systems. In addition, other external factors such as increased pollution may also have contributed to changes in the prevalence and distribution of indoor fungi.

Barcelona is located on the Mediterranean coast at 41.4° Latitude N and 2.2° Longitude E. It occupies a surface area of 100.4 km^2 with 1 593 000 inhabitants according to the 2005 census. Until now no information has been available on the prevalence of indoor moulds in this city.

This study was therefore undertaken to determine the presence and seasonal distribution of the four fungi considered to be most allergenic (*Alternaria, Aspergillus, Cladosporium and Penicillium*) in the air, inside and outside the homes of fungal allergic subjects living in Barcelona. Quantification of spores using a standardized volumetric method and techniques based on mycological cultures as well as the identification of the isolated fungi at the species level were relevant associated objectives.

Material and methods

Home selection

From a list of 40 patients diagnosed with respiratory allergy to moulds, 22 were randomly selected for this study. All the patients were informed as to the nature of the study and all accepted voluntarily to participate. The location of the homes is presented in Figure 1. The type of homes were: house N=3, lower floors in a block of flats N=5; middle-level floors N=4; high floors, including attics N=8.

All the 22 homes of these patients were located in Barcelona city or its metropolitan area. Maximum distance between the homes was approximately 15 km.

The patients presented a clinical history of asthma and/or rhinitis and were positive to one or more allergens of the 4 fungi analyzed in this study, as determined by the skin prick test. Participants also presented a specific IgE equal or superior to class 2 of the UniCAP test (Pharmacia Diagnostics AB. Uppsala, Sweden). The distribution of allergies were: *Alternaria* (N=15), *Cladosporium* and *Alternaria* (N=5), and *Alternaria* + *Cladosporium* and *Penicillium* (N=1), *Alternaria, Cladosporium, Penicillium* and *Aspergillus* (N=1).

Atmospheric conditions

According to the information of the Servei de Metereologia de Catalunya [9], the average temperatures and rainfall during the seasons of the period studied were:

Spring: 13 ±3°C, 60 ±40 mm; Summer: 23 ±1°C , 40 ±30 mm; Autumn: 13 ±5°C, 30 ± 20 mm; Winter: 8±2°C, 20 ± 20 mm.

Sample collection

The research team travelled to the homes of the patients to collect samples during four seasons of one year, from the spring of 2004 until the winter of 2005. Samples were taken on different days during the first two months of each season.

In each home, a sample from the patient's bedroom was collected and from two other rooms, where the patient spent most time or which showed a problem of dampness.

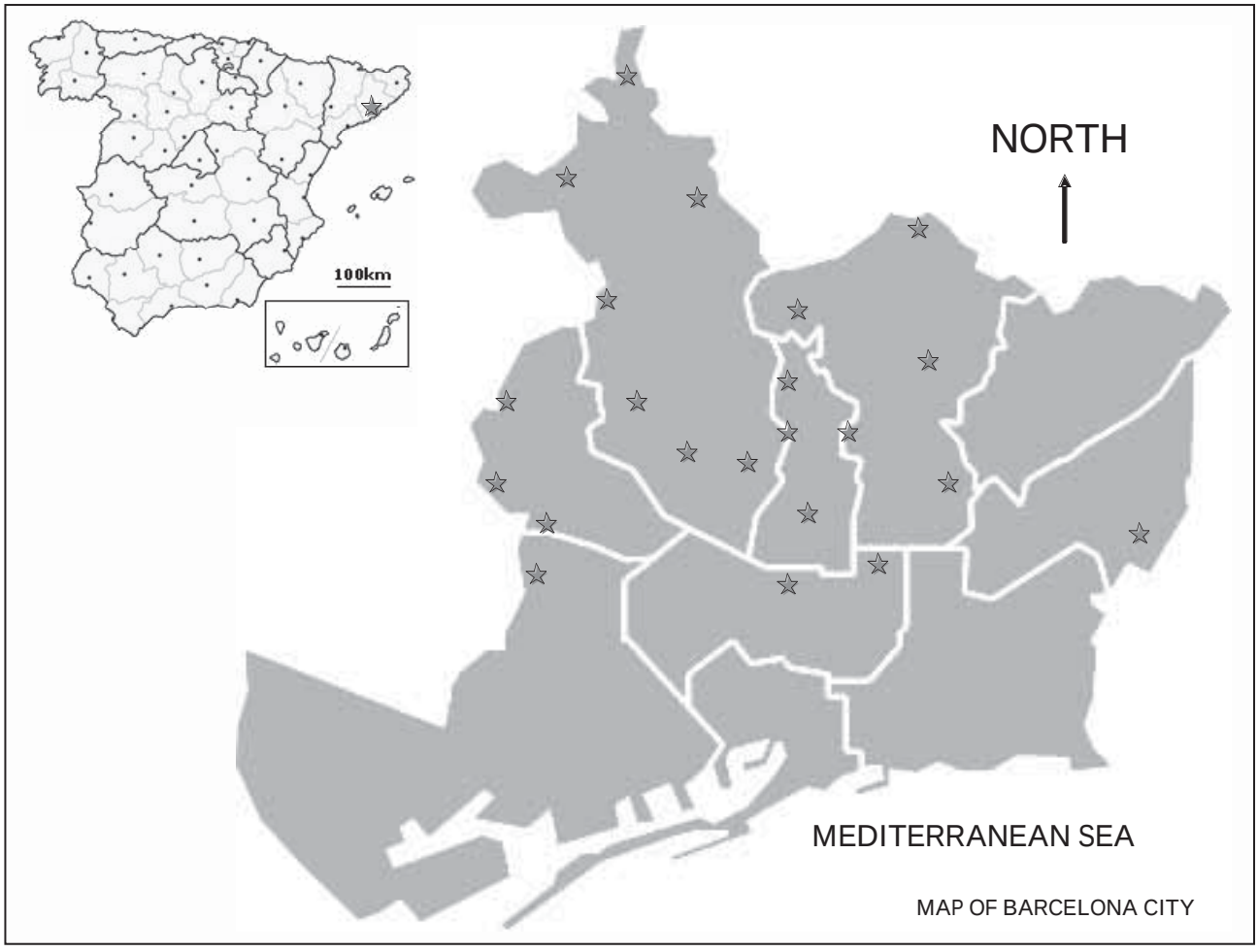

Figure 1. Localization of 22 selected homes for mycological study in Barcelona.

At the same time, samples from the immediate outdoor environment of the home (window, balcony or terrace) were collected using the same method as for indoors.

Samples were collected with a volumetric method that uses a portable air sampler able to aspirate spores from the environment (Microflow TM60 Microbiological Air Sampler, A.P. Buck, Inc. Orlando, Florida, USA). The air sampling period was 3 minutes and 20 seconds with a constant suction speed of 1.5 L/sec; in total, a volume of 300 L passed through an autoclavable head with multiple millimeter-size pores. The flow of air was projected onto the surface of two different "Rodac" 60 mm Petri dishes (NUNC TM Brand Prod, Denmark) each containing nutrient medium: Malt agar (Difco, Detroit, Michigan, USA) and Rose-Bengal agar with chloramphenicol, (Merck, Rahway, New Jersey, USA). The spore collector was placed in the centre of the room at an approximate height of 50 cm from the floor.

After sampling , Petri dishes were incubated at 25°C during 4 to 10 days.

For each sample, the number of exposed plates was 4 indoors and 2 outdoors in five homes, and 6 indoors plus 2 outdoors in the remaining 17 homes.

Identification

Starting at 48 hours and during the following 8 days the plates were observed and the isolated colonies were counted and identified. Identification was based on the macroscopic and microscopic morphology of the colonies according to the criteria published in the specialized literature on the topic [10-12].

Statistical analysis

Presence of the fungi under study was defined as the identification of any species belonging to the four genera which were found to be growing in a plate, independently of the number of colonies.

Differences in the presence of genera according to the season and the indoor or outdoor location were determined, expressed as a percentage and analyzed using a χ^2 test or, alternatively, using the exact Fisher test. These analyses were performed with the SPSS v 14 (SPSS Inc., Chicago).

Results

A total of 581 exposed plates were collected, of which 431 were indoor air samples and 150 outdoor, and 11 843 colonies were isolated ranging from 0 to 1 666 CFUs/m^3 (colony-forming units per cubic meter). Overall, 36 different fungi genera were identified. However, for this study only the 4 genera considered to be most relevant for allergies (*Alternaria, Aspergillus, Cladosporium* and *Penicillium*) were evaluated.

10 132 colonies of the 4 genera were analyzed, which represented 85.5% of the total number of colonies 7328 of these were isolated from indoors and 2 804 from outdoors.

The greatest presence of the 4 genera was recorded in autumn (73%), followed by summer (64.3%), winter (55.7%) and spring (49.2%). Overall, *Cladosporium* constituted the majority (78%) followed by *Penicillium* (71%). *Aspergillus* and *Alternaria* displayed similar values of 46% and 44.5% respectively (Table 1).

Table 1. Number of colonies and presence of the four fungal genera in the exposed plates (N = 581)

Genera	Number of colonies	% Presence per plate
Alternaria	710	44
Aspergillus	770	46
Cladosporium	5761	78.1
Penicillium	2891	71.3

The presence of studied fungi was slightly higher in the outdoor air (66.2%) than indoor (57.8%) but the difference was not statistically significant.

Alternaria was present in 100% of homes in summer and autumn, but in spring and winter in only 54 % and 55% of homes, respectively. *Penicillium* and *Cladosporium* were found in 95% and 100% of the homes studied. The genus *Aspergillus* was isolated in approximately 70% (winter) and 89% (summer) of homes, as is shown in Figure 2, which also illustrates the distribution of these genera in the outdoor air.

Comparison of the indoor and outdoor environments (Figure 3), revealed *Alternaria* to be the only genus to exhibit statistically significant differences in spring (P=.002), autumn (P=.0039) and winter (P<.001).

When the plates exposed to indoor air were analyzed (Figure 3), *Alternaria* was isolated in 56% of all plates in summer, while in winter this fell to just 20.4%. The genus *Aspergillus* was more frequent in autumn (63.8%), while in winter it was isolated in only 33% of the plates. *Cladosporium* was also most common in autumn (91.5%), but least common in spring (59%). *Penicillium* was more frequent in autumn (81%) and to a lesser extent in summer (63.6%). The described seasonal differences were statistically significant for all four genera (Table 2).

In the outdoor air samples, no seasonal statistical variations of *Alternaria* and *Penicillium* were detected (P=.09 and P=.51, respectively) However, the lowest numbers of Alternaria were found in spring. *Penicillium* was more abundant in winter. In contrast, *Aspergillus* exhibited

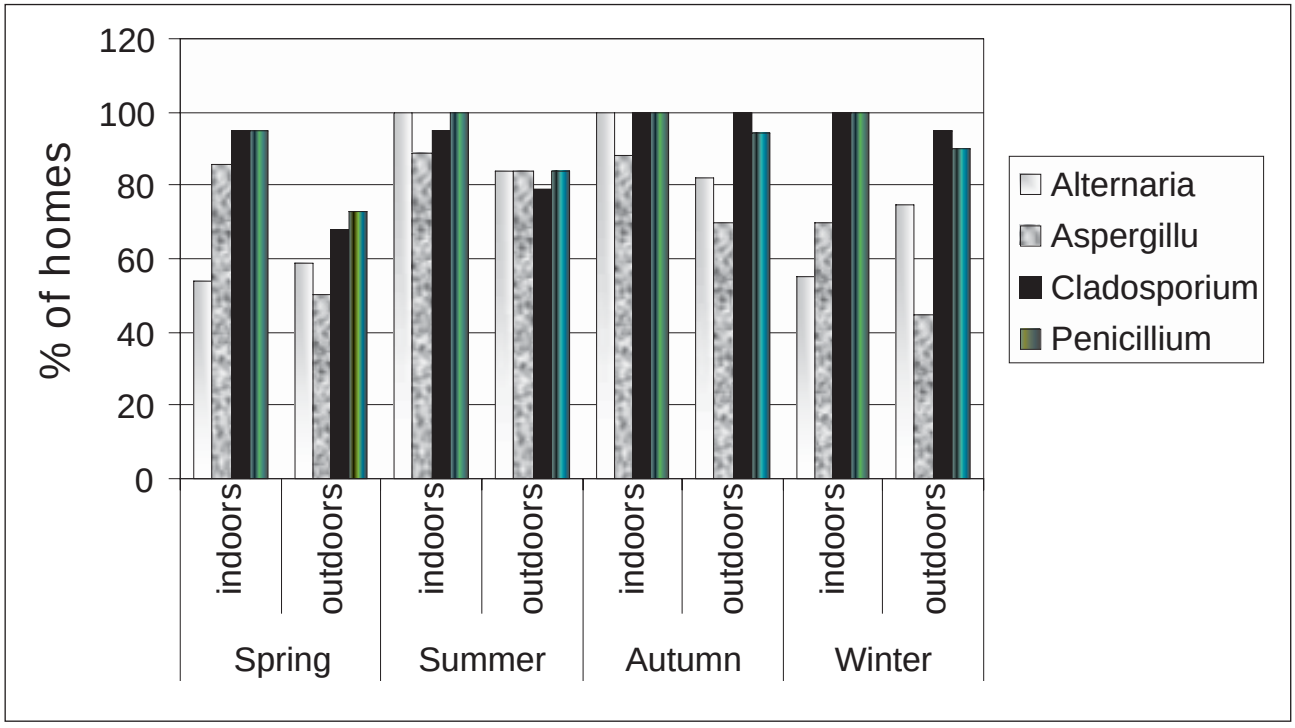

Figure 2. Percentage of homes where the 4 moulds genera were detected (N = 22), according to season and place.

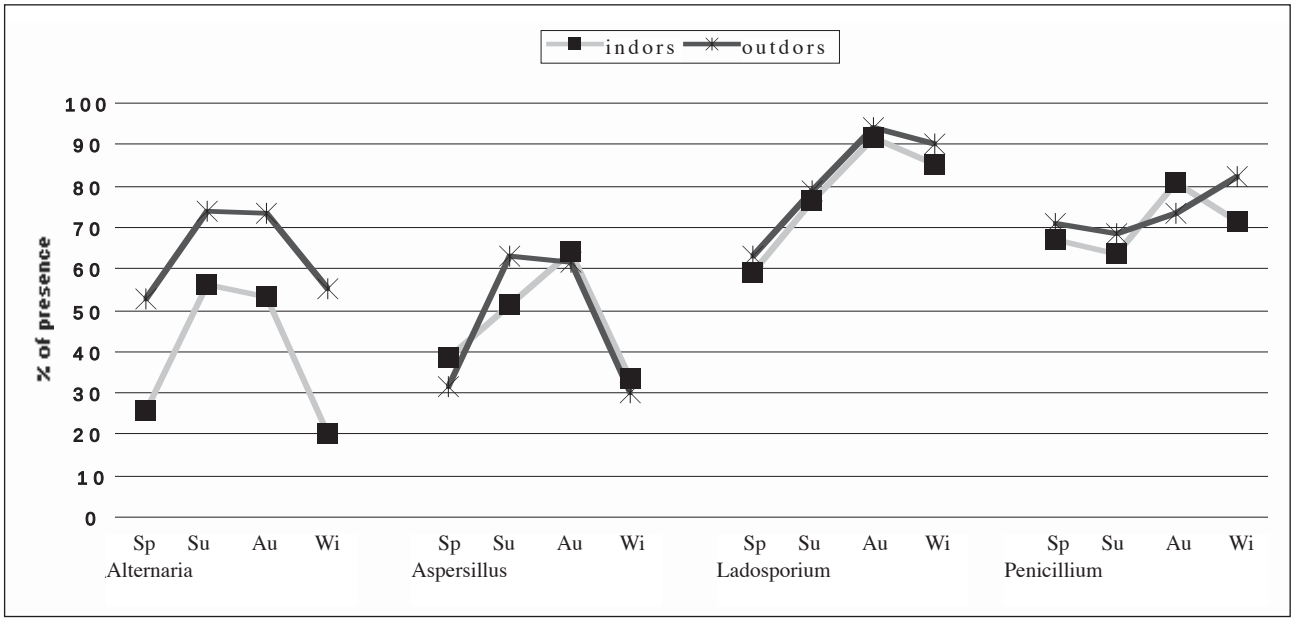

Figure 3. Percentage of *Alternaria, Aspergillus, Cladosporium* and *Pencillium* genera revealed, on plates exposed indoors and outdoors, in Spring (Sp), Summer (Su), Autumn (Au) and Winter (Wi).

Table 2. Statistical significance (p) of the presence of the 4 genera between the four seasons of the year

Genera	Indoor	Outdoor
Alternaria	<.001	.096
Aspergillus	<.001	.002
Cladosporium	<.001	.003
Penicillium	.047	.514

Table 3. Most frequent species of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* found indoors and outdoors. (Expressed as percentages)

Isolated species	Indoor N=431	Outdoor N=150
Alternaria alternata	37.1	62.66
Aspergillus flavus	9.5	9.3
Aspergillus niger	19.25	25.3
Aspergillus versicolor	16	9.3
Cladosporium cladosporioides	34.1	34.6
Cladosporium herbarum	38.5	39.3
Penicillium chrysogenum	26.2	22
Penicillium frequentans	10.2	12
Penicillium funiculosum	12.7	13.3

significant variations between the four seasons (*P*=.002), being more abundant in summer (63%) and less so in winter (30%). *Cladosporium* also displayed significant variations in the outdoor air (p=.003), being more frequent in autumn (94%) and less in spring (63%) This was the only genus that demonstrated the same pattern of distribution in both indoor and outdoor environments (Figure 3).

The species of the four genera that were most frequently isolated indoors are shown in Table 3. Particularly noteworthy is the presence of *C. herbarum, A. alternata, C. cladosporioides, P. chrysogenum, A. niger* and *A. versicolor*, with more than 15% in indoor air.

The range of CFUs/m^3 was markedly variable. The highest numbers of UFC/mL was found in *Penicillium* and *Cladosporium* with maximum values of 1 666 and 513 CFUs/m3, respectively (Table 4).

Table 4. Level of variability in the overall distribution of 4 fungal genera, expressed in colony-forming units per cubic meter (CFU s/m^3), N=581

Genera	Media	SD	SE	min	25%	50%	75%	90%	Max
Alternaria	4.07	7.25	0.30	0.0	0.0	0.0	6.6	13.3	63
Aspergillus	4.41	15.55	0.64	0.0	0.0	0.0	6.6	10.0	340
Cladosporium	33.05	51.08	2.11	0.0	3.3	13.3	43.3	93.3	513
Penicillium	16.5	81.00	3.36	0.0	0.0	3.3	13.3	23.3	1666.67

SD: standard deviation; SE: standard error; min: minimum concentration of CFUs/m^3; max: maximum concentration of CFUs/m^3; Percentiles 25%, 50%, 75% and 90%

Discussion

In atopic patients, a relationship has been shown to exist between the severity of asthma and rhinitis and exposure to fungi [4, 13]. Such exposure can occur as much outdoors as inside the home or place of work of the patient [4, 6, 14]. Higher concentrations of fungal spores increase the risk of developing asthma and of asthma persisting [7].

Although data on the presence of fungal spores in outdoor air exist and are partially available in some databases in Catalonia [15], the information on the fungi in indoor air is scarce. Studies carried out in different European countries and other areas of the world provide variable results regarding the distribution and concentration of spores indoors. Results have varied depending on the method used, season of the year, region and living conditions [16-23].

Solid knowledge on the presence of species in indoor air can only be achieved by using mycological methods, which are based on cultivation and subsequent identification of the fungi.

In Spain, very limited information exists on the distribution of fungi indoors. Moreover, the method of collection of airborne spores in these studies, in most of cases, was not volumetric and, therefore, was not quantitative [24]. The collection methods were usually based on gravimetric techniques or analysis of the domestic dust for the presence of fungi [8]. Except for some reports [24], the studies were limited to a single sample and, thus did not record changes that could have taken place during the year.

The data presented in our study were obtained using quantitative methods and were compared throughout the seasons of the year. Results highlight the elevated number of isolated colonies and their distribution in 36 genera of fungi, with the great majority of these colonies (85.5%) belonging to the four genera that are considered the causative agents of a large number of respiratory allergies [1, 25]. In the present study, the genera with highest presence were detected in the following order: *Cladosporium, Penicillium, Aspergillus* and *Alternaria.* The first two were also those recovered in more homes and those which produced the highest number of colonies.

Overall, no statistical differences were found among the presence of spores indoors and outdoors, with the exception of *Alternaria* which was more prevalent in outdoor air. Chew et al [17] analyzed the level of fungal spores inside homes, and found that characteristics of the habitat could predict higher or lower concentrations of spores in the dust, depending, for example, on the existence of carpets. When these authors compared the fungal levels in indoor and outdoor air, *Alternaria* was found to be more frequent in outdoor air and dust, while *Aspergillus versicolor* was more prevalent in house dust.

The study data show that inside homes three of the analyzed genera (*Cladosporium, Penicillium* and *Aspergillus*) were more prevalent in autumn, whereas *Alternaria* demonstrated a slight predominance in summer followed by autumn. These findings have also been reported in homes in the United States for *Cladosporium, Alternaria* and *Aspergillus*, but with no differences in the presence of *Penicillium* [16]. As was found in the present study, other authors have observed that in winter there is the smallest presence of fungi and the lowest number of colonies [16, 17].

The main goal of the present study was not to establish a relationship between the symptomatology and the presence or variation of indoor spores . However, we can state that during the sampling period, worsening of the symptoms was not observed.

The Commission of the European Community has established that fungal concentrations below 500 CFUs/m^3 are low, between 500 and 999 CFUs/m^3 are intermediate and, those above 1000 CFUs/m^3 are high [26]. In our study, only *Penicillium* exceeded maximum values and *Cladosporium* reached intermediate values.

The highest concentration of *Alternaria* found in outdoor air was 63 CFUs/m^3 and in indoor air was 36 CFUs/m^3. Both these values are lower than 100 CFUs/m^3 which have been related to hyperresponsiveness of airways in children sensitive to this fungus[1].

It has been proposed that fungal flora inside homes are representative of the outdoor atmosphere, since airborne spores penetrate through doors and windows [17]. However, considerable quantitative differences may exist as has been observed in the case of *Alternaria*.

Distribution according to species revealed that *A. alternata* was the most frequent fungi inside as well as outside homes; this fact can be correlated with the higher level of sensitizations to this fungi found in our geographical area [27].

Cladosporium herbarum was the most frequent species of this genus, followed very closely by *C. cladosporioides*. *Penicillium chrysogenum* was identified in 26.2% of all exposed plates and was most prevalent inside homes. Within the genera *Aspergillus*, *A. niger* was the most frequent species isolated, followed by *A. versicolor* that was also more prevalent indoors.

The analysis of results can vary considerably depending on whether the whole taxa (genera) is considered or the analysis is carried out according to species [28]. Specific antigens that are usually the most important allergens are not always shared by the species of the same genera. Hence, in environmental mycological studies, it is advisable to identify the species in order to obtain valid data that correlate with levels of sensitization and clinical manifestations.

The growing importance of respiratory allergies to fungi should be correlated with the presence of their spores, both outdoors and indoors, inside homes, study centres and work places [29]. The complexity of carrying out similar studies highlights the need for establishing valid approaches that would contribute and add to our aerobiological, epidemiological and clinical knowledge.

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