ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Airborne fungi isolated from different environments of a primary school in the city of Manaus, Amazonas, Brazil

Fungos anemófilos isolados de diferentes ambientes de uma escola primária na cidade de Manaus, Amazonas, Brasil

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ABSTRACT

Introduction: Airborne fungi can cause respiratory diseases, including pulmonary mycoses. The objective of this study was to isolate and identify airborne fungi from external and internal environments at a full-day primary school in Manaus, Brazil, and ascertain the influence of seasonality on the incidence of these microorganisms. **Methods:** Airborne fungi were collected by exposing Sabouraud agar plates at various external and internal locations in the school. **Results:** A total of 2,386 fungal colonies were isolated, 1,041 in the rainy season and 1,345 during the dry season. Of these, 1,858 were identified and distributed into 34 genera. The most prevalent were *Cladosporium* sp. (22.6%); *Aspergillus* sp. (17.14%); *Penicillium* sp. (8.55%); *Curvularia* sp. (6.83%); and *Drechslera* sp. (5.7%). During the dry season, the most prevalent genre was *Aspergillus* (19.3%), while in the rainy season, *Cladosporium* predominated (34.6%). **Conclusion:** Seasonality influenced fungal incidence, especially of the genus *Cladosporium*, which increased significantly during the rainy season. *Cladosporium* can be considered a bioindicator of the rainy season in the Brazilian Amazon.

Keywords: Fungi, air, diversity, environmental.

RESUMO

Introdução: Os fungos presentes no ar, denominados anemófilos, possuem uma ampla diversidade em locais de clima tropical e são causadores de micoses pulmonares e outras doenças do aparelho respiratório. O objetivo do estudo foi isolar e identificar os fungos do ar de uma escola de ensino fundamental de tempo integral, a partir de ambientes externos e internos, e verificar se a sazonalidade influencia a incidência desses microrganismos. **Métodos:** Para coleta dos fungos do ar, placas de Petri contendo Sabouraud foram expostas nos ambientes externos e internos da escola. **Resultados:** Foram isoladas 2.386 colônias de fungos, sendo 1.041 na estação chuvosa e 1.345 na estação seca. Foram identificados 1.858 fungos, que puderam ser distribuídos em 34 gêneros. Os gêneros mais frequentes foram *Cladosporium* sp. (22,6%), *Aspergillus* sp. (17,14%), *Penicillium* sp. (8,55%), *Curvularia* sp. (6,83%) e *Drechslera* sp. (5,7%). Durante o período seco, o gênero mais frequente foi o *Aspergillus* (19,21%), e no período chuvoso, o gênero *Cladosporium* (34,8%). **Conclusão:** A sazonalidade influenciou principalmente o gênero *Cladosporium*, que obteve aumento significativo na estação chuvosa, constituindo um biomarcador dessa estação.

Descritores: Fungos, meio ambiente e saúde pública, biodiversidade.

Introduction

Atmosphere constitutes a way of spreading a peculiar type of microbiota, where the dispersion of the reproductive cells of most fungi and many other organisms depend on air transportation. The fungi present in the air are called “contaminants” or “airborne”.[1-3]

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High levels of air pollution pose a risk to the respiratory health of human populations. In constant contact with the atmosphere, humans can be sensitized by fungal spores present in the air and develop allergic diseases in the respiratory tract and elsewhere in the body. Airborne fungi thus act as bioallergens; allergic conditions such as rhinitis, allergic asthma, allergic sinusitis, allergic bronchopulmonary mycoses, and others are manifestations of airborne fungal contamination. Populations of these fungi are susceptible to large variation with temperature and relative humidity, wind speed, and the seasons.

Airborne fungi may be classified as yeasts or filamentous fungi, according to the morphology and texture of the colonies. Yeast colonies are usually pasty and mucoid, while filamentous fungi are cottony, velvety, and variously pigmented.[5] Recent research in Brazil has sought to characterize populations of these fungi in various locations. In a study conducted in the state of Paraíba, *Cladosporium* sp. was identified as the most prevalent airborne fungus in a pulp and fruit industry.[6] In a hospital in the city of Ariquemes, Rondônia, the most commonly isolated airborne fungi were of the genus *Fusarium*,[7] and in a private hospital in the municipality of Sinop, Mato Grosso, 18 genera of filamentous fungi were identified, with *Cladosporium* being most frequent.[8]

In the Amazon region, special conditions of temperature and humidity facilitate the existence of an abundant microbiota. Since the 1970s, with the emergence of HIV/AIDS, interest in infectious diseases has been increasing, and airborne fungi have been identified as bioallergens. These species are important for public health in all age group, are known to cause allergic disease, and can easily cause opportunistic infection in immunologically impaired individuals. Hence, identifying the airborne fungal microbiota of specific regions has become a constant concern. In the Amazon, with the exception of the work of Fonseca and Conceição[9] and Furtado and Ferraroni[10], there has been little research in this field, especially in the city of Manaus.

Within this context, the objective of this study was to identify the airborne fungal microbiota of a primary school in Manaus, isolating them from external and internal environments, and assess potential seasonal influences on the incidence of these microorganisms.

Methods

Study setting

The study was conducted at an all-day primary school with an enrollment of approximately 600 students divided across 16 classrooms, ranging from 40 to 45 students per class. All classrooms were air-conditioned. Waste disposal and cleaning of the classrooms, restrooms, cafeteria, and gym were carried out daily. Eight external environments and 29 internal environments were selected for airborne fungus collection, for a total of 37 collection sites.

Isolation of fungi

To collect airborne fungi, the method described by Flores and Onofre,[2] which is based on the sedimentation of aerial fungal spores on Petri dishes, was employed. At each collection site, two plates containing 20% Sabouraud agar culture medium (Dicf®) were placed in a horizontal position at a height of 1 m from the ground and exposed for 5 minutes. Samples were collected once monthly in October, November, and December 2008 (dry season) and February, March, and April 2009 (rainy season). After exposure, the plates were incubated at room temperature for 5 to 7 days.

Identification of fungi

The slide-culture method was used for fungal identification.[11] Colonies were preserved in mineral oil, as described elsewhere.[12]

Diversity analysis

The frequency, genus richness (number of genera), biological diversity, and similarity of the community were estimated for the rainy and dry seasons.

Diversity was calculated from the Shannon-Weaver index (H').[13] The higher the index, the greater the heterogeneity and diversity. The formula used was:

$$H' = -\Sigma\ (p_i)\ (\ln p_i)$$

where $p_{in} = n_i / N$

n_i = is the individual number of i^{th} genus; and

N = number of all genera.

The evenness (or equitability) index represents the uniformity of the number of individuals per genus.[14] As it tends to 0, it means a single genus dominates the community; as it approaches 1, it means other genera

have the same abundance. The index is expressed by the formula:

$$E = H'/In\ S$$

where

H' = Shannon-Weaver Index based on number of individuals; and

S = number of genera present in the sample.

To characterize the differences between fungal communities in the rainy and dry seasons, Sorensen's index (S') was calculated[15], where 0 means no similarity and 1 means absolute similarity. This index was calculated by the following formula:

$$S' = 2c/(a+b)$$

where

a = the total number of genera collected in condition A;

b = the total number of genera collected in condition B; and

c = the number of genera common to both conditions.

Results

A total of 2,386 fungal colonies were isolated from the air: 1,041 in the dry season and 1,345 during the rainy season (Table 1). We were able to identify 1,858 colonies, distributed across 32 different genera (mainly *Cladosporium*, *Aspergillus*, *Penicillium*, *Fusarium* and *Curvularia*). The remaining 393 cultures could not be identified (Table 1). Overall, fungal colonies from the genera *Cladosporium*, *Aspergillus*, *Penicillium*, *Curvularia*, and *Drechslera* accounted for 54-67% of all cultures isolated from the internal and external collection sites during the dry and rainy seasons.

During the rainy season, *Cladosporium* was the most prevalent genus in both the external environment and the internal environment. During the dry season, *Curvularia* was most prevalent in the external environment, while *Aspergillus* dominated at internal collection sites.

Seven genera were present only in the dry season: *Pithomyces*, *Colletotrichum*, *Pestalotiopsis*, *Phoma*, *Dactylaria*, *Diplodia*, *and Zygosporium*. Genera that occurred only during the rainy season were *Trichosporon*, *Mucor*, *Beltrania*, *Cordana*, and *Cryptococcus*. *Aspergillus*, *Penicillium*, *Curvularia*, and *Drechslera* all declined in frequency from the dry season to the wet season.

Richness, diversity, and evenness findings are described in Table 1. In the dry season, the diversity index (H') was 2.5100 and the evenness index (E) was 0.410164, respectively. In the rainy season, the diversity index (H') was 1.8963 and the evenness index (E) was 0.2379. For the two analyzed conditions, the Sorensen index (S') was 0.7931. The dry season exhibited greater richness than the rainy season, even though the amount of fungi isolated during the rainy season exceeded that isolated during the dry season.

The genera *Cladosporium*, *Aspergillus*, and *Penicillium* showed a preponderance for internal environments, while *Curvularia* sp. and *Drechslera* sp. preferred external environments; these two genera, in addition to sharing great micromorphological similarity, were both more frequent in open areas.

The number of fungal colonies isolated in the indoor environments was two- to threefold that in the outdoor environments. Overall, the genus *Cladosporium* was predominant in internal and external environments. In the dry season, the genus *Aspergillus* was the most abundant in both environments, while in the rainy season, *Cladosporium* was the most abundant in both environments.

Discussion

The study of airborne fungi has great relevance for the treatment of allergic diseases. Knowledge of which fungi are implicated in these conditions should help develop more efficient therapies. A greater understanding of yet-unexplored airborne fungi in the Amazonian environment is particularly relevant and important.

The genus *Cladosporium* predominated over all others in this sample, with a sharp rise in incidence in the rainy season. This was the airborne fungus most often observed by Fonseca and Conceição[9] in the city of Manaus. The highest incidence also was observed in the rainy season, in the months of September, October, and December 1975 and in January 1976 (Table 1). A previous study evaluated dispersion of the genus *Cladosporium* in western Cuba and noted that some *Cladosporium* species do not need high humidity for spore dispersion; however, increasing relative humidity caused an increase in the frequency of some species of the genus.[16] It can be assumed that the *Cladosporium* species of the Amazon region depend on favorable weather conditions to spread their spores.[1]

Table 1
Frequency and number of fungi isolated during the dry and rainy seasons at a primary school in the city of Manaus, Amazonas, Brazil (2008-2009)

	Frequency of fungi isolated and identified									
Seasons	**Dry**				**Rainy**					
Environment	**External**		**Internal**		**External**		**Internal**		**Total**	
Genus	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**
Cladosporium sp.	7.5	18	6.87	55	30.13	113	36.4	353	22.6	539
Aspergillus sp.	13.75	33	20.97	168	10.13	38	17.52	170	17.14	409
Penicillium sp.	6.25	15	10.61	85	2.4	9	9.8	95	8.55	204
Curvularia sp.	18.75	45	9.0	72	6.13	23	2.4	23	6.83	163
Drechslera sp.	12.92	31	6.5	52	11.2	42	1.13	11	5.7	136
Oidiodendron sp.	1.25	3	6.62	53	6.9	26	4.5	44	5.3	126
Candida parapsilosis	1.25	3	2.25	18	1.1	4	3.2	31	2.35	56
Aspergillus niger	4.58	11	4.24	34	0.0	0	0.52	5	2.1	50
Fusarium sp.	7.5	18	2.0	16	0.27	1	0.52	5	1.68	40
Nigrospora sp.	1.7	4	1.9	15	0.0	0	0.31	3	0.92	22
Spegazzinia sp.	0.41	1	1.74	14	0.0	0	0.21	2	0.71	17
Trichoderma sp.	0.41	1	0.87	7	0.27	1	0.82	8	0.71	17
Paecilomyces sp.	0.41	1	0.87	7	0.27	1	0.1	1	0.42	10
Trichocladium sp.	0.83	2	0.62	5	0.0	0	0.1	1	0.33	8
Arthrinium sp.	0.0	0	0.75	6	0.0	0	0.1	1	0.29	7
Pithomyces sp.	0.41	1	0.75	6	0.0	0	0.0	0	0.29	7
Nodulisporium sp.	0.41	1	0.25	2	0.0	0	0.21	2	0.21	5
Trichosporon sp.	0.0	0	0.0	0	0.0	0	0.52	5	0.21	5
Acremoniela sp.	0.0	0	0.25	2	0.0	0	0.21	2	0.17	4
Candida tropicalis	1.25	3	0.0	0	0.0	0	0.1	1	0.17	4
Alternaria sp.	0.0	0	0.25	2	0.0	0	0.1	1	0.13	3
Monodictys sp.	0.41	1	0.12	1	0.0	0	0.1	1	0.13	3
Mucor sp.	0.0	0	0.0	0	0.53	2	0.1	1	0.13	3
Rhizopus sp.	0.0	0	0.25	2	0.27	1	0.0	0	0.13	3
Beltrania sp.	0.0	0	0.0	0	0.53	2	0.0	0	0.08	2
Colletotrichum sp.	0.0	0	0.25	2	0.0	0	0.0	0	0.08	2
Cordana sp.	0.0	0	0.0	0	0.0	0	0.21	2	0.08	2
Cryptococcus sp.	0.0	0	0.0	0	0.0	0	0.21	2	0.08	2
Pestalotiopsis sp.	0.41	1	0.12	1	0.0	0	0.0	0	0.08	2
Phoma sp.	0.83	2	0.0	0	0.0	0	0.0	0	0.08	2
Scopulariopsis sp.	0.0	0	0.12	1	0.0	0	0.1	1	0.08	2
Dactylaria sp.	0.0	0	0.12	1	0.0	0	0.0	0	0.04	1
Diplodia sp.	0.41	1	0.0	0	0.0	0	0.0	0	0.04	1
Zygosporium sp.	0.0	0	0.12	1	0.0	0	0.0	0	0.04	1
Yeast	5.83	14	7.62	61	4.27	16	3.81	37	5.36	128
Not sporulated	11.7	28	13.48	108	25.6	96	16.6	161	16.47	393
Discarded	0.83	2	0.5	4	0.0	0	0.1	1	0.29	7
Total	100	240	100	801	100	375	100	970	100	2,386
Richness (R)	30				28					
Shannon-Weaver (H')	2.5100				1.8963					
Evenness (E)	0.410164				0.2379					
Sorensen (S')	0.7931									

Indoor environments are influenced by the outside environment through the system of winds, among other abiotic factors.[17] This wind regime relationship was observed only in the dry season, when the *Curvularia* genus was the most frequent outdoors, but it did not influence indoor environments, where *Aspergillus* sp. was most common. In regions with a dry climate, the most frequent genera are *Aspergillus*, *Penicillium*, and *Curvularia*, which corroborates the results observed in this study during the dry season[18]. The predominance of *Aspergillus* during the dry season was also observed as far afield as Araraquara (state of São Paulo), Porto Alegre (state of Rio Grande do Sul), Fortaleza (state of Ceará), Paraíba, and Manaus.[9,18-20,21]

However, during the wet season, the genus *Cladosporium* occurs more frequently.[2,19] Previous research shows that the ideal climate conditions for the highest incidence of *Aspergillus* sp. are drier climes, while the rainy season is optimal for *Cladosporium.*[22] Occurrence of these genera was also observed in the Amazon state of Belém, with *Aspergillus* sp. being most frequent, while in the Northeast Brazilian state of Paraíba, *Cladosporium* was the main genus isolated.[3,6]

Climate factors that change from one season to the next, such as temperature and humidity, influence the incidence of some fungal genera. This influence was observed in Pelotas (state of Rio Grande do Sul),[23] where increased levels of fungi in the air were found to be influenced by seasonal change, confirming that abiotic factors influenced the frequency of isolated fungi.

The filamentous fungus Alternaria sp. is considered a powerful allergen. However, two studies done in Brazil, in the cities of Francisco Beltrão (Paraná) and Araraquara, found a low frequency of this fungus.

In our study, a higher diversity index (H') was observed in the dry season than in the rainy season, while the lowest evenness (E) was observed in the rainy season, due to the predominance of the genus *Cladosporium*. The Sorensen index endorsed high, but not absolute, similarity between fungal communities. Recently, higher indices of diversity and evenness were observed in the rainy season in a community study of freshwater fungi during rainy and non-rainy seasons in a small blackwater lake in the Amazon. Comparison of the two seasons using the Sorensen index yielded $S = 0.7931$, resulting from the overlap of 23 taxa.[26]

In the present study, seasonal variations mainly influenced the incidence of the gender *Cladosporium*. The absence of some genera and the emergence of others confirm the assumption that climate change influences the incidence of airborne fungi. For this reason, *Cladosporium* sp. can be considered a bioindicator of the rainy season.

Airborne fungi are involved in several allergic manifestations and are highly prevalent worldwide. It is estimated that 25% of the global population experiences symptoms of respiratory allergies related to airborne fungi.[27] In the Brazilian state of Maranhão, children have been found to exhibit allergic sensitivity to *Aspergillus* sp. and *Penicillium* sp.[28]

This is the first work to isolate airborne fungi from the environment in the Amazon and demonstrate the role of *Cladosporium*, a cosmopolitan fungal genus known to be involved in allergic processes,[16,27] as a bioindicator. Seasonality is known to influence some genera of airborne fungi, increasing or decreasing their incidence in the environment. We conclude that, in the atmosphere of the Brazilian Amazon city of Manaus, there is a high diversity of pathogenic airborne fungi that can cause respiratory allergies or mycoses.

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