

Prevalence of *Aspergillus* sp in Portuguese Infant and Elementary Schools

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Abstract

Background: Long-term exposure to a contaminated indoor environment may result in different health complaints. The filamentous fungi indoor air quality assessment of infant and elementary Portuguese schools and *Aspergillus* sp. identification was performed.

Methods: Environmental exposure was performed in seven infant and elementary schools, situated in Vila Nova de Gaia, during January 2011, following filamentous culture characterization and identification for *Aspergillus* sp.

Results: Filamentous cultures were more prevalent than yeasts, being Ascomycetes, more widespread than Zygomycetes. Elementary classrooms were more environmental contaminated than infant level classrooms.

Conclusion: *A. terreus* and *A. fumigatus* cultures isolation occurred from samples exposed in two elementary classrooms and were related with school habitability and hygienisation conditions.

Keywords: Filamentous fungi; *Aspergillus* sp; Infant and elementary classrooms

Introduction

Incidence of respiratory infections in immunocompromised individuals, caused by filamentous fungi, has increased significantly over the past few years [1,2]. Staying long periods of time in an indoor closed environment can contribute to infection acquisition since the lack of aeration, the poor conservation ventilation, temperature regulating systems and relative air humidity may contribute to the prevalence and multiplication of filamentous fungi in both public and private buildings [3,4]. Resulting spores spread in the environment, reaching to man and causing disease, being gravity dependent of individual immunological state, airborne spores concentration and exposition time [5]. Acute respiratory diseases [e.g. invasive aspergillosis] may result from long-term exposure by individuals [6,7] and chronic respiratory diseases [e.g. allergic bronchopulmonary aspergillosis] result as an opportunistic infection, mainly in immunocompromised patients; when no treatment is available, it can be also fatal [8,9]. Children are a very sensitive group since they stay long periods of time inside classrooms, being dependent of indoor air quality. *Aspergillus* sp. are the most pathogenic moulds and their spores inhalation can result in respiratory diseases; subsequent colonization of pre-formed pulmonary cavities by hyphae can result in aspergilloma or aspergillosis diseases [2,10,11]. Even though, their identification is still difficult and laborious since filamentous fungi diagnosis is based on both macroscopic and microscopic evaluation of cultures, being dependent on the experience of the evaluator. Despite the importance for public health the study and characterization of filamentous fungi presence in public buildings, few studies have been developed in Portugal. The aim of this study was to evaluate the filamentous fungi indoor air quality from infant and elementary Portuguese schools, situated in Vila Nova de Gaia. Also, *Aspergillus* sp. identification was performed.

Materials and Methods

Study was performed at December 2010 in seven schools (I-VII), situated in Vila Nova de Gaia (a satellite city of Oporto, the second largest Portuguese city); except for school I (3 elementary classrooms), all the others had 1 infant (I-VII) and 4 elementary classrooms for each, resulting in a total of 34 samples (sample per school, classroom

and school year). Environmental samples were collected by passive exposure of Sabouraud Dextrose Agar (SDA - OXOID, Hampshire, UK) plates during 30 min at environmental temperature. Data concerning habitability of each classroom were collected (area; type of deck; number of windows and doors; exposure to sunlight; presence of air conditioning and fans; patches of water and moisture). Following exposition, SDA plates were incubated (25°C/ 5 days) and, for each developed filamentous cultures, macroscopic fungal colony morphology was performed (colour -top and base plate-, elevation, texture and form). Cultures with gray-white (or gray-brown) and pepper like stippled surface colonies were suspected to be Zygomycetes; cultures with powdery or granular surface, as also green, gray, yellow or brown pigmentation were supposed to be Ascomycetes. Subsequently, humidity chambers were executed and incubated (37°C/ 2 days), following culture microscopic (40x) characterization of spores, hyphae and fruiting bodies, namely sporangium (typical Zygomycete) and conidial heads (typical Ascomycete), was performed.

Results

Yeasts and filamentous colonies were obtained from all the samples. For filamentous cultures we found similar results in school III and V (Table 1). Macroscopic morphology studies found diverse filamentous cultures; although, cultures from different schools and classrooms presented similar colour, elevation and textures; by microscopy, all the isolated filamentous cultures presented fruiting bodies (Sporangium and conidia). Consequently, we prepared cultures according to their macroscopic and microscopic characteristics. Zygomycetes were more prevalent in schools IV and VI (infant and elementary levels); also, in

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Schools	Yeasts (n(%))		Filamentous (n(%))		Total (N)
	Infant Level	Elementary Level	Infant Level	Elementary Level	
I	2 (4)	5 (10)	11 (21)	34 (65)	52
II	5 (8)	24 (35)	2 (3)	37 (54)	68
III	15 (15)	32 (31)	14 (14)	41 (40)	102
IV	8 (8)	17 (16)	12 (11)	69 (65)	106
V	13 (19)	23 (33)	11 (15)	23 (33)	70
VI	2 (2)	16 (20)	7 (9)	57 (69)	82
VII	0 (0)	26 (13)	0 (0)	182 (87)	208

Table 1: Number of yeast and filamentous colonies obtained from all the studied schools (I - VII), after environmental exposition of SDA plates in all the infant and elementary education levels classrooms.

Filamentous Cultures (fruiting bodies characterization)					
Schools	Sporangium (n (%))		Conidia (n (%))		Total (N)
	Infant Level	Elementary Level	Infant Level	Elementary Level	
I	3 (7)	9 (20)	8 (18)	25 (55)	45
II	2 (5)	15 (39)	0 (0)	22 (56)	39
III	1 (2)	10 (18)	13 (24)	31 (56)	55
IV	6 (7)	57 (71)	6 (7)	12 (15)	81
V	1 (3)	14 (41)	10 (30)	9 (26)	34
VI	7 (9)	47 (59)	0 (0)	26 (32)	80
VII	0 (0)	10 (5)	0 (0)	172 (95)	182

Table 2: Filamentous cultures characterization obtained from all the infant and elementary education levels classrooms from all the studied schools (I - VII), according to their fruiting bodies (sporangium and conidia).

classrooms from school II (infant level) and school V (elementary level); additionally, Ascomycetes were prevalent at schools I and III (infant and elementary classrooms) and schools II and VII (elementary level). It is important to state that elementary classrooms of school VII which presented a greater number of filamentous cultures, being much higher than those obtained in other schools; also, in infant level classrooms no growth was observed (Table 2). Only two cultures were obtained from school VII, (elementary level classrooms) presented specific conidial columnar heads, been classified as *Aspergillus* sp. Even these two cultures presented different macroscopic morphology characteristics; one was *A. terreus* (granular surface, brown pigmentation and rugae radiation from the center) and *A. fumigates* (powdery surface and green pigmentation). All the other Ascomycetes cultures were considered to be *Penicillium* sp. since microscopic evaluation revealed chains of single-celled conidia, produced in a phialide. Schools were constructed with stone like building material except schools IV and VI (pre-manufactured wood). All the classrooms have at least one window and direct sunlight; although most do not have any ventilator and aeration systems, heater systems were observed at school I and II. Except school V, which presented the poorest state of preservation and inadequate cleaning products (no disinfectants), all the classrooms are clean twice for day with disinfectants products.

Discussion

Presence of filamentous fungi in indoor environment can result in acute respiratory infections, sinusitis and bronchitis in healthy individuals [12]. Also, exposure to *Aspergillus* sp. surface antigen may cause immediate reaction, usually defined as allergic bronchopulmonary aspergillosis [13]. At a very early age that children attend to public spaces and, during the school age, stay long periods of time inside classrooms, which is expected to carry the most

excellent environmental conditions [4,14]. On the other hand, the poor environmental indoor quality may result in health complaints from students [14,15]. With this study we would like to assess the indoor air quality (namely patches and moisture, humidity, ventilation, temperature and hygiene) of seven Portuguese elementary and infant schools, localized at Vila Nova de Gaia, a satellite city for Oporto. According to our results, a high prevalence of filamentous fungi was found in all indoor environments; even though, yeasts were more ubiquitous at school V. These facts might be a result from inadequate observed habitability conditions, such as: (a) lack of ventilators and aeration system of rooms; (b) poor state of preservation and inadequate cleaning products (no disinfectants); (c) degraded walls and with no ink; (d) presence of water infiltration patches. Also, schools IV and VI were constructed with pre-manufactured wood and presented spots of water infiltration and moisture, being those with more Zygomycetes. Despite the higher number of identified Ascomycetes, *Aspergillus* sp. culture isolation only occurred from samples obtained from school VII (elementary classrooms); no cultures were obtained from infant level. This might be due to the fact that this school presented the worse hygiene conditions, especially pots of water infiltration, walls degraded and without ink. Also, an inundation occurred on the previous day at the infant classroom, which has led to a greater hygiene and aeration of the room, being a possible explanation for the filamentous cultures nonexistence.

The indoor environment quality study is an important topic for public health, although results may be dependent of biological and environmental factors like building location, indoor environmental temperature and samples collection year's seasons, among others [2,5]. Studies performed at Portuguese schools founded higher fungi spores concentration inside classrooms, all exceeding the public wellbeing acceptable values [10,12,13]. In our study, we also found a large number of fungi and we isolated *Aspergillus* sp. cultures. As stated in previous studies, this could be a consequence the lack of ventilation and inside higher temperature and relative humidity, resulting in water patches which presence, according to their studies, is associated with an increase of indoor fungi [2,10,12,15]; also, when paint (a protection factor against the fungi appearance) is absent, there is a greater microorganism adherence [3]. The results were obtained, alerted the necessity to execute more studies at schools, in order to minimize children exposition to pathogenic fungi and avoid respiratory allergy.

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