

Review Article

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Airborne Fungi in Indoor Hospital Environments

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ABSTRACT

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The relationship between air quality and human health has been indisputably evidenced in the literature. In indoor environments this quality can be influenced by the presence of microorganisms. In these places, fungi find every favorable condition for their growth and development. Factors such as temperature, relative humidity, infiltrations in the building structure and availability of nutrients may be associated with the success of the presence of fungi in indoor air. Fungi can put at risk the health of occupants of a given environment, favoring the emergence of allergies and serious infections. In the hospital context, patients, professionals and visitors can have their health compromised by the presence of airborne fungi that can cause serious damage. Allergens produced by various species of fungi are known to affect susceptible people and are related to allergic diseases like asthma and rhinitis. In this review we highlight the participation of some fungi as one of the main indoor air contaminants and the risk of aerial exposure to these microorganisms in hospital environments.

Introduction

The activities we develop in our daily routine contribute to our long stay indoors. In these places the air that circulates is the result of a cooling process, which provides better well-being to those who are present. However, the quality of the air circulating in these

environments can have a significant effect on the health of occupants and, instead of providing comfort, influence a situation of discomfort and even the emergence of diseases related to the environment (Sterling *et al.*, 1991; Wolkoff, 2018). Buildings where the air quality is inadequate and the reflection of this inadequacy may cause direct effects on

the health of workers or residents are thus referred to as "sick buildings", being involved in a condition called the Sick Building Syndrome (SBS).

Sick Building Syndrome (SBS) is a generic term that describes the situation in which the occupants of a given building experience non-specific symptoms which relate directly to the time in which they develop their activities inside the building (Joshi, 2008; Ghaffarianhoseini *et al.*, 2018). The precondition to classify a building as "sick" relates to the presence of pollutants in the air that circulates, resulting in the occupants being exposed to an environment of poor air quality. The symptoms experienced by the occupants of such sites are usually headaches, disturbances in the eye (irritation, pain, itching, constant tearing or dryness), nasal problems (nasal irritation, nasal cold or runny nose), drowsiness, fatigue, lack of concentration, colds, sore throats, backaches, cold extremities, tension, dry skin, dizziness, muscle aches, weakness, difficulty breathing and wheezing (Joshi, 2008; Schirmer *et al.*, 2011). Indoor air quality in several different types of environments can be influenced by many factors, of which chemical and biological contaminants can significantly harm the health of exposed individuals.

Different chemical pollutants with various properties and different concentrations in the environment have been reported as compromising factors to the indoor air quality (WHO, 2010). Some of the major chemical pollutants detected in indoor air are carbon dioxide, inorganic gases, ozone and organic compounds (Luengas *et al.*, 2015). Bessonneau and collaborators evaluated the chemical contamination of indoor air in a school hospital in France, the results showing compounds such as alcohols, esters and ketones as the most prevalent in the air samples analyzed. Additionally, aromatic

hydrocarbons, aliphatic hydrocarbons, aldehydes and terpenes were also found in the samples (Bessonneau *et al.*, 2013).

Biological pollutants that are often related to contamination of indoor air include: animal allergens (dust mites and certain proteins), pollen grains, bacteria, endotoxins, fungi and their spores, as well as mycotoxins, which are products of the secondary metabolism of many species of fungi (Luengas *et al.*, 2015; Kim *et al.*, 2017). The presence of biological agents such as bacteria, fungi and viruses in a particular environment and their interference in the quality of the air that circulates in these locations will significantly depend on the factors favoring growth and establishment of these species in the indoor environment. Temperature, moisture and nutrient availability are the major factors commonly related to their establishment (Haleem-Khan and Karuppayil, 2012; Nazaroff, 2013).

With respect to air quality in hospital settings, there are many concerns about the influence and effects that poor-quality air can cause in individuals that are exposed to it, especially patients whose hospital stay should be related to their evolution and recovery. For example, maintaining a well-ventilated space can be of great impact and significance in terms of indoor hospital air quality, since it results in the protection of visitors, health professionals and patients from the most diverse types of pollutants that circulate in the environment via aerial dispersion (Li *et al.*, 2007).

When it comes to patients with immune system impairment, the risk of developing infections caused by spores or fungal fragments present in the air increases substantially. Measures that contribute to the reduction of microbiological contamination of the air in hospital environments represent an extreme value in the prevention of nosocomial infections of the most susceptible individuals

(Gangneux *et al.*, 2006), as inhalation of ambient air is one of the main routes of exposure to fungal pathogens (Peláez *et al.*, 2012). Holý and collaborators (2015) carried out a study where they evaluated the microbial contamination of the indoor air collected in a Transplant Unit in a university hospital, as well as verifying the performance of an air filtration system against the observed contamination. The authors verified that the air filtration equipment was efficient to improve the air quality in the analyzed environment, particularly when they found very little evident microbial growth from the samples collected following the use of the filtration device. Since fungi can be considered one of the main contaminating microorganisms in indoor air (Quadros *et al.*, 2009), the application of actions to monitor these microbial contaminants in the air and the role of these measures in the containment of certain diseases caused by these pathogens are noteworthy (Holý *et al.*, 2015).

Fungi in indoor air environments

Indoor air environments represent one of the many places of occurrence and importance with regard to the presence of fungi. Their adaptive characteristics favor their global dispersion, allowing the survival of these microorganisms in several habitat types (Nevalainen *et al.*, 2015; Coombs *et al.*, 2018). Monitoring of airborne fungi has already been recorded in the 19th century (Maddox, 1870), as well as at the beginning of the second half of the 20th century. This initial research on the topic aimed to investigate concentrations of fungi in the outside air (Morrow *et al.*, 1942; Hirst, 1952; Hamilton, 1959). Since the early days, researchers were aware of the relationship between the concentrations of fungal spores in outdoor air and their presence in indoor air, as well as the health risks of exposure to these spores in the air from both outdoor and indoor

environments (Richards, 1954). The presence of fungi in hospital air was first demonstrated by Noble and Clayton (1963) and later in a study by Lidwell and Nobe (1975) that demonstrated the performance of air-conditioning devices as a source of fungi for ambient air. The presence of fungi as contaminants or biocontaminants that affect indoor air quality has been widely discussed in the literature (Miller, 1992; Gorny *et al.*, 2002; Dubey, 2011; Caillaud *et al.*, 2018).

It is very clear today that most of the fungi present in indoor air come to these sites mainly through external air, which is an important source of biological contamination for indoor air (Lee *et al.*, 2006; Crawford *et al.*, 2015; Abassi and Samaei, 2018). Although external air contributes significantly to the composition and increase in the concentration of fungal spores in indoor air, this is not the only way indoor air contamination can occur. Domestic and everyday activities may have a considerable impact on the concentrations of spores in the air (Lehtonen and Reponen, 1993; Awad *et al.*, 2018). Manipulation of organic material (firewood and potted plants), handling of bedding, dog and cat hair, human skin, hair and nails, as well as clothing and human occupation can positively influence the spores in the indoor air (Reponen *et al.*, 1992; Pitkaranta *et al.*, 2008).

The factors considered preponderant in influencing the presence and development of fungi in environments with indoor air are: humidity, temperature and nutrient availability (TANG *et al.*, 2015). Baughman and Arens (1996) argue that building materials such as wood, cellulose, wallpaper, organic insulation materials, textiles, glues and paints may contain nutrients such as carbohydrates and proteins which serve the fungal growth very well. Still, according to these authors, materials such as concrete, metals, glass fibers, plastics and other synthetic products,

although not readily used by fungi, can contain organic remains that serve as a source of nutrients. In general, the temperature and humidity values for indoor fungi growth may vary depending on the species considered. Indoor air environments usually have optimum temperature values for fungi growth, ranging from 10-35 °C (WHO, 2009). For relative humidity, values below 75% are reported as limiting for fungal growth in buildings (Rowan *et al.*, 1999). The critical relative humidity conditions for microbiological growth in building materials were defined by Johansson and collaborators (2005), who established that depending on the group of materials (wood, concrete and others) the fungal growth could occur in values of minimum relative humidity of 75% and maximum of 95%. Polizzi and collaborators (2011) analyzed the metabolic response of some species of fungi found in indoor air under different environmental conditions and found that temperatures of 25 °C and 30 °C, as well as values of relative humidity in the range of 97-100% were considered ideal conditions for the growth of *Penicillium* spp., *Aspergillus* spp. and *Periconia* spp. Fungal growth in building materials was observed at temperatures and relative humidity values of 5 °C to 91% RH, 10 °C in 90-95% RH, 20 °C in 86-90% RH, and 25 °C in 78 -86% RH (Nielsen *et al.*, 2004).

Once present in the environment and compromising air quality, the determination of a limit value of fungal concentration that is not enough to cause health risks to exposed individuals would be extremely relevant. Nowadays, the existence of an international standard guide concerning the maximum acceptable levels and values of fungal bioaerosols related to good air quality in indoor environments has not been established. Rao *et al.*, (1996) reviewed and compared the regulations and quantitative standards for fungi in existing indoor air environments and

concluded that a better characterization of fungi sources for these sites as well as more data related to the effects of acute and chronic exposure to these pathogens could be of great assistance in the elaboration of a reliable standard document. According to the American Conference of Governmental Industrial Hygienists (ACGIH), the absence of data related to the exposure and response to concentrations of bioaerosols, as well as the non-availability of a standard collection method in analyses of fungal bioaerosols make it difficult to draw up a common standard (ACGIH 2009). A review containing some recommendations from governmental and private organizations about concentrations of fungi in the air can be found in the review published by Rao *et al.*, (1996). At present, there is an establishment of values of acceptable concentrations for various fungal bioaerosols whose variation depends on the country in question. Table 1 describes some limiting concentrations for indoor fungi in some countries. For hospital environments, the World Health Organization has defined a maximum acceptable value of 50 CFU / m³ for fungi (WHO, 1988).

Fungal bioaerosols in hospital environments

Nosocomial infections represent a major challenge and concern for hospitals, especially due to the high costs that they generate and the consequences for public health (Scott 2009; Singh *et al.*, 2018). It is estimated that 10 to 20% of hospital infections are due to airborne pathogens (Eickhoff, 1994; Dürmaz *et al.*, 2005). In hospitals, the main source of microorganisms for ambient air is infected patients themselves (Hambraeus, 1988; Lemaire *et al.*, 2018). Although this is true for many bacterial and viral pathogens, the main source of fungi in the air in hospital environments, however, is outdoor air (Beggs, 2003; Abassi and Samaei, 2018). The opening

of doors, windows and even the inflow and outflow of the hospital may favor increased concentration of fungal spores in the indoor air. Human occupation in the hospital (professionals and visitors), materials brought in by the occupants (personal objects, food and fruit) are also sources of fungi for indoor air (Nuruka *et al.*, 2014). Ventilation systems have also been considered as likely sources of fungal contamination of indoor air (Batterman and Burge, 1995; Ahearn *et al.*, 2004; Sowiak *et al.*, 2018).

Since the conditions of the hospital environment are related to comfort and well-being for human coexistence and in these environments there is considerable presence of pathogenic fungi, these same conditions can also act favoring substantially the colonization and establishment of many of these microorganisms. Conditions like heat, moisture, shade, substrate material (e.g. carpets, furniture and concrete), as well as the presence of food can allow the development and permanence of viable fungi in these locations for extended periods of time (Kowalski, 2012). Neely and Orloff (2001) investigated the growth of some fungi of medical importance in samples of tissues and plastic materials, both for hospital use, verifying that *Aspergillus* and *Fusarium* grew and remained viable in the analyzed samples, indicating the high capacity of hospital environments to provide suitable conditions for the development of these pathogens. *Aspergillus fumigatus*, a species commonly found in indoor air environments, has also been found to develop for long periods of time (more than 30 days) in hospital cloth samples (Koca *et al.*, 2012).

Ventilation, heating and air-conditioning systems in hospital environments can provide a microenvironment that is highly conducive to fungus growth and favors the spread of many infectious agents such as fungi in

buildings (Ahearn *et al.*, 2004). High relative humidity or condensation of water inside surfaces or ducts, filters and collecting trays in ventilation systems can promote fungal growth, just as the accumulation of dust and dirt may contain compounds that provide important nutrients to the metabolism of these microorganisms (Nevalainen *et al.*, 2015). The material composition of air filters combined with the presence of moisture in them can also act in favor of the development of many fungi (Kemp *et al.*, 2001; Viegas *et al.*, 2018). Fungal growth in air-conditioners have been reported in the literature (Li *et al.*, 2010, 2012; Viegas *et al.*, 2018) as well as the capacity of the air filters to retain bacteria and fungi in ventilation systems (Moritz *et al.*, 2001; Aquino *et al.*, 2018). The first research related to fungal contamination of filters used in hospital ventilation systems was made by Arnow *et al.*, (1978). In the results, they could verify the growth of *A. fumigatus* in the analyzed filters as well as relate the observed growth to some cases of aspergillosis. Later, outbreaks of aspergillosis were associated with contamination of hospital air filters, as well as dust and carpet samples in the same environment (Arnow *et al.*, 1991). Simmons *et al.*, (1997) analyzed several types of air filters in seven hospitals in order to investigate their colonization by fungi. Species of the genera *Aspergillus*, *Acremonium*, *Alternaria*, *Cladosporium* and *Penicillium*, which are fungi frequently present in indoor environments, were also found in the samples analyzed.

Several species of filamentous fungi can be found in aerial contamination investigations in hospital settings. Hong *et al.*, (1999) analyzed air samples from 83 hospital sites and verified that species of the genera *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* were the most frequent isolated fungi. Research on fungi concentrations in the indoor air of a Pediatric Hospital Unit revealed that

Cladosporium, *Alternaria*, *Penicillium*, *Aspergillus* and *Acremonium* were the most prevalent genera (Okten and Asan 2012). Quadros and collaborators (2009), when investigating aerial microbiological contamination of a neonatal ICU, an adult ICU and two operating rooms, found that *Aspergillus* and *Penicillium* were the most common fungal genera, although *Cladosporium* and *Acremonium* were also present at a lower frequency. Qudiesat and collaborators (2009) evaluated the quality of air as well as the amount of aerial microorganisms in two hospitals. They found that in both hospitals *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.* and *Alternaria spp.* were the air contaminant fungal species. The authors also verified that the concentration of fungi and bacteria in the air of both environments were influenced by human occupation (Qudiesat *et al.*, 2009).

Major fungi in hospital interior environments and their chemical allergens

Although many species of fungi are found in studies on air quality analysis in hospital settings, only *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria* and *Fusarium* will be highlighted. In addition, the allergens produced by these fungi, whose presence in the environment can immensely affect the health of the exposed individuals, will also be briefly addressed as they are responsible for worsening the health status of immune-impaired patients.

Genus *Aspergillus*

Species of the genus *Aspergillus* can be found in several environments, including indoor hospital environments (Kousha *et al.*, 2011; Asif *et al.*, 2018). Only few species are considered human pathogens (Paulussen *et al.*, 2016), among which we can highlight *Aspergillus fumigatus*, *Aspergillus flavus*,

Aspergillus nidulans, *Aspergillus terreus*, and *Aspergillus niger* (Hedayati *et al.*, 2007; Kwon-chung and Sugui 2013; Hachem *et al.*, 2014; Vermeulen *et al.*, 2014; Veraldi *et al.*, 2016). Barrs and collaborators (2013) also reported *Aspergillus felis* as a causative agent of invasive aspergillosis in humans. *A. fumigatus* is undoubtedly the species with the greatest impact in terms of infection of individuals with impaired immune system, as well as in the etiology of diseases caused by fungi that are transported by air (Moreno-gonzález *et al.*, 2016).

Air exposure to fungal material can significantly affect the health of individuals in a particular environment, especially in hospitals, where conditions favor nosocomial infections. Clinical manifestations caused by *Aspergillus* can be categorized into allergic reactions, chronic pulmonary aspergillosis and invasive aspergillosis (Paulussen *et al.*, 2016).

The presence of *Aspergillus* in hospital environments has been well described in the literature. Holý *et al.*, (2015) reported microbiological contamination of air by *Aspergillus sydowii*, *Aspergillus versicolor* and *A. terreus* in a Transplant Unit of a university hospital. Several sites of a Hospital in India were used for air collection in order to carry out a monitoring study of contamination by filamentous fungi. Out of all isolated fungi, the genus *Aspergillus* was the most frequent, and the species *A. niger* and *A. flavus* stood out as the main air contaminants in the analyzed sites (Kushawaha *et al.*, 2015). Interestingly, both species are related to aspergillosis and superficial infections in humans. In their study, Martins-Diniz and collaborators (2005) showed the presence of *Aspergillus* in the samples analyzed, which may compromise the recovery of patients, especially those immunologically impaired. The occurrence of *A. fumigatus* and *A. versicolor* isolated from poorly maintained

air-conditioners located in operating rooms was reported by Gniadek and Macura (2011).

The presence of *Aspergillus* in hospital environments raises great concern, especially for its ability to produce allergens that are released into the air, which can affect the health of sensitized individuals. The increase in the concentration of *Aspergillus* spp. indoors will cause the exacerbation of asthma, for example (Zubairi *et al.*, 2014). *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae* and *A. versicolor* are the species that produce allergens, according to the WHO/IUIS Allergen Nomenclature Sub-committee (www.allergen.org), the allergen website approved by the World Health Organization (Table 2). Out of all, *A. fumigatus* is the one presenting the highest amount of allergens described, while for *A. flavus* and *A. versicolor* there is only one allergen description for each species. Allergens from *A. flavus* and *A. niger* were identified, and the allergic response to them was confirmed by allergy skin test and serum IgE test (Verman *et al.*, 2015). In tests using serum from asthmatic patients, a 34-kD alkaline serine protease was identified as *A. oryzae* allergen (Shen *et al.*, 1998). Allergens of *A. fumigatus* Asp f 18 and Asp f 34 have been identified in serum of asthmatic patients and with allergic bronchopulmonary aspergillosis (ABPA), respectively (Shen *et al.*, 2001; Glaser *et al.*, 2009). A review covering various aspects of allergens produced by *A. fumigatus* can be seen in the study published by Kurup (2005).

Genus *Penicillium*

The genus *Penicillium* has species whose occurrence has been verified in a wide variety of habitats, especially with great prevalence in indoor air environments (Visagie *et al.*, 2014). In hospital settings, its presence as a contaminant of indoor air has been constantly verified. Sepahvand *et al.*, (2013) found

Penicillium as the most prominent genus of isolated fungi present in the microflora of indoor air in five hospitals. Similarly, a study conducted in order to evaluate the presence of fungi in indoor air of a hospital Oncology Unit also pointed this genus as the most frequent in air samples (Okten *et al.*, 2015). In critical public and private hospitals areas, analysis of samples collected in filters and air-conditioning palettes showed contamination with *Penicillium* sp. (Santana and Fortune 2012), which in this type of environment, due to the precarious health condition of many patients, may represent a risk to their recovery.

Penicillium mainly affects immunosuppressed individuals, being this impaired immune system a result from a primary infection with human immunodeficiency virus (HIV) or caused by some sort of treatment (Barcus *et al.*, 2005). The first report of human infection by this fungus was observed in 1973, when *Penicillium marneffeii* isolation from the spleen of a patient with Hodgkin's disease was described (Disalvo *et al.*, 1973). In fact, *P. marneffeii* has been the most frequent species reported in human infections (Vanittanakom *et al.*, 2006; Yu *et al.*, 2018), although rare infections by other species have also been reported (Geltner *et al.*, 2013; Oshikata *et al.*, 2013; Radulesco *et al.*, 2018).

The importance of *Penicillium* as a fungus that produces allergens and its presence in indoor air environments has currently received great attention. A review published by Sharpe and collaborators (2015) highlights the relationship between the exposure to fungal allergen from some species in indoor air environment and asthma cases. For example, the severity of asthma was associated with *Penicillium* exposure (Pongracic *et al.*, 2010). The official website of Allergens highlights *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Penicillium citrinum*,

Penicillium crustosum and *Penicillium oxalicum* as allergenic species. A 68 kDa allergen, produced by *P. chrysogenum* (previously named *Penicillium notatum*), was characterized by molecular biology and identified as N-acetyl-glucosaminidase (Shen *et al.*, 1995). Allergens produced by *P. oxalicum* and *P. chrysogenum* were immunologically reactive with serum from asthmatic individuals, as well as showing homology with allergen produced by *P. citrinum* and a vacuolar serine protease from *A. fumigatus* (Shen *et al.*, 1999). Molecular cloning of genes encoding allergens in *P. brevicompactum* revealed the presence of an allergenic clone named Pen b 26 which was reactive with serum from sensitive individuals, the allergen being identified as a ribosomal protein (Sevinc *et al.*, 2005). Isolation and characterization of an allergen (Pen cr 26) produced by *P. crustosum* was reported by Sevin *et al.*, (2014). Pen cr 26 presented strong sequence homology with Pen b 26; however, the authors described the existence of antigenic differences among IgE epitopes, which led them to consider Pen cr 26 as a hypoallergenic variant of Pen b 26. Table 3 shows the allergens recognized and approved by WHO/IUIS Allergen Nomenclature.

Genus *Cladosporium*

Cladosporium involves fungi with a wide worldwide distribution and which are also isolated from materials commonly found in environments such as paints, wood, textiles and other organic compounds (Andersen *et al.*, 2000; 2011). Conidia of species of this genus are commonly isolated from the air (Pavan and Manjunath 2014; Weryszko-Chmielewska *et al.*, 2018). Although the highest prevalence of *Cladosporium* is verified in atmospheric air (Zoppas *et al.*, 2011), its isolation in indoor air environments has also been verified (Nambu *et al.*, 2009). The occurrence of this fungus as one of the most prevalent in the air of hospital

environments has been verified in the literature. By analyzing the presence of contaminating fungi in the air-conditioning devices in intensive care units and operating rooms, the authors found *Cladosporium* as one of the most prevalent genera (ABOUL-NASR *et al.*, 2014). These results emphasize the contribution of air-conditioning equipment to the contamination of air in hospital units, which significantly compromises patient recovery. Although *Cladosporium* species are rare as human pathogens, they are involved in cutaneous infections, phaeohyphomycosis and pulmonary infections (Viheira and Pacheco 2001; Tasic and Tasic 2007; Castro *et al.*, 2013), some of them presenting significant aggravation, which can represent an enormous risk to hospitalized patients. Additional studies have also observed the presence of *Cladosporium* as one of the main air pollutants in hospital environments (Lobato *et al.*, 2009; Maldonado-Vega *et al.*, 2014; Chaivisit *et al.*, 2018).

The presence of *Cladosporium* in environments with indoor air is considerably influenced by atmospheric air. Spores of this fungus have allergens that can affect the health of sensitive individuals and have been associated with exacerbation of asthma in children (Raphoz *et al.*, 2010). According to WHO/IUIS Allergen Nomenclature, *Cladosporium cladosporioides* and *Cladosporium herbarum* are the species whose allergens have been identified so far. Out of these species, *C. herbarum* receives greater attention due to the greater number of allergens it produces (Table 4). Many of the allergens produced by *Cladosporium* show cross-reactivity with allergens of other fungi, especially with species of the genus *Alternaria* (Achatz *et al.*, 1995). Chou *et al.*, (2008) reported the identification of a serine protease as the main allergen of *C. cladosporioides*. The authors also observed the reactivity of this allergen with serum of asthmatic patients, as

well as the cross-reactivity of this allergen with *Aspergillus* ssp. allergens and *Penicillium* ssp. NADP-dependent mannitol dehydrogenase was recognized by IgE antibodies in 57% of *C. herbarum*-sensitive patients, having been considered the main allergen of this species (Simon-Nobbe *et al.*, 2006). Monosensitization to *Cladosporium* allergens has rarely occurred, which can be attributed largely to cross-reactivity with allergens of other species.

Genus *Alternaria*

Alternaria is one among the most prevalent fungi in the atmospheric air, along with *Aspergillus* and *Cladosporium*. Although the highest concentrations of their spores are observed in the atmosphere, their presence in indoor air environments has also been reported (Sharma *et al.*, 2011; Fang *et al.*, 2013).

Alternaria alternata has been described as one of the most prevalent fungal species in indoor air environments in the United States (Woudenberg *et al.*, 2015). In the hospital environment, the presence of *Alternaria* in air samples collected in a neonatal unit has been

mentioned (Sakartepe *et al.*, 2016). The occurrence of *Alternaria* ssp. was also described in an Intensive Care Unit and apartments of a hospital unit in the city of Francisco Beltrão (Flores and Onofre 2010). A study by Godini *et al.*, (2015) found a high level of fungal bioaerosols in hospital air samples, with *Alternaria* ssp. being among the most prevalent fungi.

Despite being considered essentially a phytopathogen, species of this genus have been related to cases of infections in humans. Most of these infections are opportunistic, especially affecting individuals with impaired immune function (Moreno *et al.*, 2012). The clinical manifestations involve mainly cutaneous and subcutaneous infections, although other types of infections may also occur (Pastor and Guarro 2008). Patients who underwent transplantation may be susceptible to *Alternaria* infections, including those caused by more than one species (Brás *et al.*, 2015). Recently, *A. alternata* has been related to cases of cutaneous and visceral phaeohyphomycosis (Gomes *et al.*, 2011; Raza *et al.*, 2015).

Table.1 Limit concentrations for fungal bioaerosols in indoor air environments established in some countries. Adapted from Mandal and Brandl (2011)

COUNTRY	FUNGI CONCENTRATION IN THE ENVIRONMENT - CFU / m ³	REFERENCES
Brazil	750	(Nunes <i>et al.</i> 2005)
Canada	150	(Bartlett <i>et al.</i> 2002)
Germany	10000	(Ifa 2004)
Portugal	500	(Handles <i>et al.</i> 2010)
Russia	2000-1000 (Depending on the species of fungus)	(Eduard 2009)
Switzerland	1000	(Oppliger <i>et al.</i> 2005)
United States	1000	(Osha 2008)
European Union	10000 - In domestic dwellings	(Warner <i>et al.</i> 1994)
	2000 - Non-industrial environments	

Table.2 *Aspergillus* allergens recognized by WHO/IUIS Allergen Nomenclature Sub-committee

Fungus/Allergen	Size (kD)	Biological Activity	Sequence access number
<i>Aspergillus flavus</i>			
Asp fl 13	34	alkaline serine protease	*
<i>Aspergillus fumigatus</i>			
Asp f 1	18	Ribonuclease	M83781
Asp f 2	37	Unknown	U56938
Asp f 3	19	Peroxisomal protein	U58050
Asp f 4	30	Unknown	AJ001732
Asp f 5	40	Metalloprotease	Z30424
Asp f 6	26.5	Mn superoxide dismutase	U53561
Asp f 7	12	Unknown	AJ223315
Asp f 8	11	Ribosomal protein P2	AJ224333
Asp f 9	34	Unknown	AJ223327
Asp f 10	34	Aspartic protease	X85092
Asp f 11	24	Peptidyl prolyl isomerase	AJ006689
Asp f 12	90	Heat shock protein P90	U92465
Asp f 13	34	Serine alkaline protease	Z11580
Asp f 15	16	Unknown	AJ002026
Asp f 16	43	Unknown	AF062651
Asp f 17	-	Unknown	AJ224865
Asp f 18	34	Vacuolar serine protease	Y13338
Asp f 22	46	Enolase	AF284645
Asp f 23	44	Ribosomal protein L3	AF464911
Asp f 27	18	Cyclophilin / Isomerase	AJ937743
Asp f 28	13	Thioredoxin	AJ937744
Asp f 29	13	Thioredoxin	AJ937745
Asp f 34	20	Cell wall protein	AM496018
<i>Aspergillus niger</i>			
Asp n 14	105	B-xylosidase	AF108944
Asp n 18	34	Vacuolar serine protease	*
Asp n 25	66-100	3-phytase / Hidrolase	L20567
<i>Aspergillus oryzae</i>			
Asp o 13	34	Alkaline serine protease	X17561
Asp o 21	53	Taka Amilase A	M33218/ D00434
<i>Aspergillus versicolor</i>			
Asp v 13	43	Extracellular alkaline serine protease	GU827714

Table.3 *Penicillium* allergens recognized by WHO/IUIS Allergen Nomenclature Sub-committee

Fungus/Allergen	Size (kD)	Biological Activity	Sequence access number
<i>Penicillium brevicompactum</i>			
Pen b 13	33	Alkaline serine protease	*
Pen b 26	11	Ribosomal acid protein 60S	AY786077
<i>Penicillium chrysogenum</i>			
Pen ch 13	34	Alkaline serine protease	AF193420
Pen ch 18	32	Vacuolar serine protease	AF264027
Pen ch 20	68	N-acetyl-glucosaminidase	S77837
Pen ch 31		Calreticulin	AY850367
Pen ch 33	16	unknown	EF206657.1
Pen ch 35	36.5	Transaldolase	GQ925430
<i>Penicillium citrinum</i>			
Pen c 3	18	Peroxisomal membrane protein	AF144753
Pen c 13	33	Alkaline serine protease	*
Pen c 19	70	Heat shock protein P70	U64207
Pen c 22	46	Enolase	AF254643
Pen c 24		Elongation factor 1 β	AY363911
Pen c 30	97	Catalase	DQ288844
Pen c 32	40	Pectate lyase	EF159713
<i>Penicillium crustosum</i>			
Pen cr 26	11	Acid ribosomal phosphoprotein P1 60s	JN791438
<i>Penicillium oxalicum</i>			
Pen o 18	34	Vacuolar serine protease	AF243425

Table.4 *Cladosporium* allergens recognized by WHO/IUIS allergen nomenclature sub-committee

Fungus/Allergen	Size (kD)	Biological Activity	Sequence access number
<i>Cladosporium cladosporioides</i>			
Cla c 9	36	Vacuolar serine protease	EF407520
Cla c 14	36.5	Transaldolase	GQ906475
<i>Cladosporium herbarum</i>			
Cla h 2	45		*
Cla h 5	11	Acid ribosomal protein P2	X78223
Cla h 6	46	Enolase	X78226
Cla h 7	22	Protein YCP4	X78224
Cla h 8	28	Mannitol dehydrogenase	AY191816
Cla h 9		Vacuolar serine protease	AY787775
Cla h 10	53	Aldehyde dehydrogenase	X78228
Cla h 12	11	Acid ribosomal protein P1	X85180

Table.5 *Alternaria* allergens recognized by WHO/IUIS allergen nomenclature sub-committee

Fungus/Allergen	Size (kD)	Biological Activity	Sequence access number
<i>Alternaria alternata</i>			
Alt a 1	16.4 and 15.3 (30 non red)	Unknown	AY568627 U82633 U86752
Alt a 3		Heat shock protein P70	U87807 U87808
Alt a 4	57	Dissulfide isomerase	X84217
Alt a 5	11	Ribosomal protein P2	X78222 U87806
Alt a 6	45	Enolase	U82437
Alt a 7	22	Protein YCP4	X78225
Alt a 8	29	Mannitol dehydrogenase	AY191815
Alt a 10	53	Aldehyde dehydrogenase	X78227
Alt a 12	11	Acid ribosomal protein P1	X84216
Alt a 13	26	Glutathione-s-transferase	AY514673
Alt a 14	24 (reduced)	Mn-dependent superoxide dismutase	KC923297
Alt a 15	58	Serine protease	KJ558435 (awaiting release)

Table.6 *Fusarium* allergens recognized by WHO/IUIS allergen nomenclature sub-committee

Fungus/Allergen	Size (kD)	Biological Activity	Sequence access number
<i>Fusarium culmorum</i>			
Fus c 1	11	Ribosomal protein P2	AY077706
Fus c 2	13	Thioredoxin-like protein	AY077707
<i>Fusarium proliferatum</i>			
Fus p 4	37.5	Transaldolase	KF151224
Fus p 9	36.5	Vacuolar serine protease	KJ462778

The major importance of *Alternaria* is undoubtedly related to its position as one of the main genera of allergenic fungi, with a singular prominence for the *A. alternata* species. Association of *Alternaria* sp. with asthma, hypersensitive pneumonitis and allergic rhinitis (Pastor and Guarro, 2008), as well as the involvement in cases of respiratory arrest (O'hollaren *et al.*, 1991) has been verified in the literature. Pulimood *et al.*, (2007) found that the exposure of individuals susceptible to *Alternaria* may influence the increase in symptoms related to asthma. Exposure to spores of these fungi has also been associated with increased risks of hospitalizations for asthmatic children and adolescents (Tham *et al.*, 2016).

A. alternata is the only species of the genus that has allergens identified and approved by the WHO/IUIS Allergen Nomenclature Sub-Committee (Table 5). Out of all the identified allergens, Alt a 1 is the most important, having high reactivity with sera from sensitive individuals as well as a high allergenicity.

Twaroch *et al.*, (2012) have speculated that Alt a 1 is located in the cell wall of *Alternaria* spores, and in this way may contribute to the upper airway related symptoms of sensitive individuals. Recently, some published

reviews have specifically addressed the allergens produced by *A. alternata*, as well as the role of each one in the development of respiratory allergies caused by fungi (Kustrzeba-Wójcicka *et al.*, 2014; Gabriel *et al.*, 2016).

Genus *Fusarium*

Species of the genus *Fusarium* are widely distributed and recognized as an important plant pathogen. Many species are mycotoxin producers, which are toxic secondary metabolites whose toxicity can affect human health (Antonissen *et al.*, 2014). Among the many effects, exposure to these mycotoxins may affect the intestinal epithelium (Liew and Mohd-Redzwan, 2018), as well as lead to an effect on the immune system (Maresca 2013). *Fusarium* has been linked to a broad spectrum of infections in individuals with immune problems. In these individuals the clinical manifestations related to fusariosis involve endophthalmitis, sinusitis, pneumonia, skin problems, as well as fungemia (Nucci and Anaissie 2007). Immunosuppressed lung transplant patients have often been affected by fusariosis (Carneiro *et al.*, 2011).

The presence of *Fusarium* as an indoor air contaminant has been mentioned in the literature (Hsu *et al.*, 2012; Ziehe *et al.*,

2014). Likewise, contamination of the air in hospital environments by this fungus can also occur, significantly compromising patient recovery. Pantoja *et al.*, (2012), when analyzing the fungal biodiversity in the air of hospitals in the city of Fortaleza/Brazil, verified the presence of *Fusarium* spp. in all hospitals and in several of the sampled environments. Other studies on the biological contamination of air in hospitals have also shown the presence of this fungus (Awosika *et al.*, 2012; Emuren and Ordinioha 2016). The WHO/IUIS Allergen Nomenclature Sub-Committee identified and approved four *Fusarium* allergens (Table 6). The association of asthma with allergens of this fungus in sensitive individuals has been reported in the literature (Khosravi *et al.*, 2012). Hoff *et al.*, (2003) characterized and identified an allergen produced by *Fusarium culmorum*, which was reactive in 44% of sera from susceptible individuals. A transaldolase was identified as an allergen of *Fusarium proliferatum*, and this allergen was further verified to have cross-reactivity with the allergen (transaldolase) produced by *Cladosporium* as well as with human transaldolases (Chou *et al.*, 2014).

In conclusion, artificially air-conditioned environments represent one of many habitats where development and establishment of many fungal species can occur. Humidity, temperature, as well as nutrient availability and neglected maintenance of refrigeration systems are fundamental requirements for mold growth in indoor air-conditioned environments. In hospital settings, the presence of anemophilous fungi circulating throughout the hospital poses a great risk to all those present and this should be considered. Health professionals, visitors and patients, especially those patients who are immunologically compromised, can be significantly affected when exposed to fungal contaminants in the air. Many of the

anemophilous fungi commonly found in hospital air quality analyses are pathogenic and represent an even greater challenge because of the ability to produce allergens. Since many of the allergens produced are related to various respiratory illnesses in children and adults, maintaining a hospital environment free from contamination and with good air quality becomes a major challenge. In this context, a better understanding of all risks associated with exposure to fungi in indoor environments may lead to measures that contribute to minimizing the implications for human health, especially in maintaining a safer location.

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