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Fungal air quality in medical protected environments

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1. Introduction

Fungi are ubiquitous in indoor environments and are responsible for a wide range of diseases, from localized non-invasive pathologies to invasive and disseminated infections. These infections occur predominantly among highly immunosuppressed patients (patients with acute leukaemia, haematopoietic stem cell or solid organ transplantation) and can have devastating consequences. *Aspergillus* remains the most common mould to cause invasive infections, but other fungi are emerging as serious pathogens and threats in immunosuppressed patients. Most invasive fungal infections are acquired from air. It is therefore imperative to adopt, in clinical environments, preventive measures in order to reduce airborne fungal concentrations and, concomitantly, the risk for development of a fungal infection. At present, there are no methods and equipments that can completely eliminate fungi from indoor medical environments. Exposure to moulds in medical units is inevitable but the presence of air filtration systems, isolation, and adoption of environmental protective measures do mitigate patient exposure. Airborne mycological investigations should inform about indoor air quality and therefore should be routinely carried out in hospitals or other institutions where immunosuppressed individuals are treated. It is important to improve the methods already available to study indoor fungi in clean environments, and it is critical to define indicators of indoor air quality in medical environments. The present chapter deals with the biology of indoor fungi in medical environments, and the strategies and technical progresses that are at present available to prevent and control fungal diseases and to improve air quality in medical facilities.

2. Indoor fungi and medical environments

2.1 Main fungi in indoor environments

Fungi are ubiquitous in all atmospheres. In general, both outdoor and indoor atmospheres are dominated by species of *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, and by yeasts and *Mycelia Sterilia*. *Cladosporium* is always the dominant fungus in outdoor atmospheres and in indoor atmospheres of normal and healthy buildings (except hospitals

where *Aspergillus* and *Penicillium* are usually dominant). The abundance of the other fungi varies with the season and place. In relation to outdoor environments, indoor atmosphere typically display lower diversity and abundance of fungi (Dacarro et al., 2003). The following genera can be represented indoors, but are always in clear minority: *Absidia*, *Acremonium*, *Arthrinium, Aureobasidium*, *Beauveria*, *Botrytis*, *Candida*, *Chaetomium*, *Chrysosporium*, *Epicoccum*, *Fusarium*, *Gliocladium*, *Mucor*, *Nigrospora*, *Paecilomyces*, *Phoma*, *Rhizopus*, *Scopulariopsis*, *Sporobolomyces*, *Stemphylium*, *Syncephalastrum*, *Trichoderma, Ulocladium* and *Verticillium* (Dacarro et al., 2003; Flannigan, 1997; Horner et al., 2004; Jo & Seo, 2005; Martinez et al., 2004; Sautour et al., 2009; Shelton et al., 2002).

Climate and human activities are the main factors that influence the composition of outdoor atmosphere. In the temperate climates, these display a typical pattern around the year. On the contrary, climate is not determinative in the mycoflora of indoor atmosphere, but human activities and the quality and maintenance of the building do play a major role in these environments. For these reasons, dominant fungi indoors vary between buildings and can be used as monitors of indoor air quality (Araujo et al., 2008a).

In the atmosphere, fungi are present in bioaerosols. Bioaerosols contain bacterial and fungal cells and cellular fragments, and products of microbial metabolism. Fungal spores constitute a significant fraction of bioaerosol microbial particles, and are often 100-1000 times more numerous than other bioparticles, like pollen grains. The particulate fraction in a bioaerosol is generally 0.3-100 µm in diameter. Fungal spores larger than 10 µm are deposited in the nasopharynx and can unchain nasal and ocular disorders. The respirable size fraction of 1-10 µm is of primary concern. Spores and fragments smaller than 10 µm (especially those smaller than 6 µm) can be transported to the lower airways and lungs, and trigger allergic reactions or infect tissues (Martinez et al., 2004; Stetzenbach et al., 2004). Bioaerosols that range in size from 1 to 5 µm generally remain in the air, whereas larger particles are deposited in the surfaces. Physical and environmental factors affect the settling of bioaerosols. Air currents, relative humidity and temperature are the most important environmental parameters affecting bioaerosol settling. The most significant physical parameters are particle size, density and shape (Martinez et al., 2004; Stetzenbach et al., 2004).

A human inhales on average 10 m^3 of air per day, and spends 80-95 % of their time indoors. Indoor air pollution is therefore frequently reported to cause health problems (Dacarro et al., 2003).

2.2 The variability of indoor concentrations - outdoor air and other routes

Shelton et al. (2002) presented an exhaustive study of the mycoflora of outdoor and indoor atmospheres in all USA regions. More than 12,000 samplings were carried out, both in outdoor and indoor atmospheres in more than 1,700 buildings. The median of total indoor concentrations was 82 colony forming units (CFU) x m^{-3}, and of *Cladosporium*, *Penicillium*, *Aspergillus* and *Mycelia Sterilia*, was 40, 30, 20 e 30 CFU x m^{-3}, respectively. The median of the ratio indoor/outdoor was 0.16.

There are two main sources for indoor fungi. Outdoor sources are usually dominant. Most fungi present indoors come from outdoors (Flannigan, 1997; Horner et al., 2004). Another source is indoor environment itself. Fungi can grow in building materials, foodstuffs, flower pots, pet bedding materials, and house dust (Chao et al., 2001; Pasanen et al., 1992a, 1992b).

If suitable conditions exist, growth and sporulation in these substrates can be significant, and constitute a major source of fungi indoors (Pasanen et al., 1992a).
The mycoflora composition of outdoor and indoor atmosphere displays high variability. Fungi in the atmospheres vary along the year and during the day. For this reason, a reliable estimate of fungal levels in the atmosphere demands multiple determinations carried out in different seasons (Jantunen et al., 1997). Temporal variability is a major problem in assessing human exposure to indoor fungi. This variability is mainly due to the release of fungi from carpets and walls or other surfaces. This release depends on the type and degree of activity of occupants in the dwelling or building. All activities in buildings disturb settled fungal particles, but cleaning, constructional work and any other major dust-raising activities have a particular impact (Flannigan, 1997). To circumvent this temporal variability of indoor mycoflora, it has been suggested that floor dust should be sampled instead of the air, since it provides a long-term accumulation of previously airborne fungi. However, although house dust fungi reflect atmospheric populations, there are qualitative differences between these two mycofloras, probably resulting from the differences in the environments. Sampling of dust should not be used as a substitute for air sampling. In addition, viable counts for settled dust are much higher than corresponding air sampling counts for aerosolized dust, suggesting that many microbes in dust either form aggregates or are carried on dust particles which settle very rapidly (Flannigan, 1997).

2.3 Fungal growth, sporulation and adaptation to xerophylic conditions

Fungi from the atmosphere and indoor environments are influenced by temperature and humidity (atmospheric relative humidity and substrate moisture content). The optimum temperature for growth and sporulation is usually around 25-30 °C. Lower or higher temperatures result in lower growth and sporulation rates. A remarkable exception comprises fungi that can infect humans, such as those involved in aspergillosis and candidiasis, which display an optimum temperature around 37 °C (Araujo & Rodrigues, 2004). Temperature is usually not limiting in indoor environments, since most indoor fungi can grow in a wide range of temperatures (Douwes, 2009; Verhoeff, 1993).
Humidity is the most important factor determining fungal growth in indoor environments (Nielsen, 2003). Atmospheric relative humidity influences directly the release of conidia from conidiophores, and concomitantly, the concentration of spores in the atmosphere. Different patterns are displayed by *Cladosporium* and *Penicillium*. Whereas in *Cladosporium*, spore release is favoured by low humidity, the opposite behaviour is displayed by *Penicillium* (Pasanen et al., 1991). These differences influence the seasonal patterns of outdoor fungi. *Cladosporium* have maxima in the summer, but *Penicillium* display higher concentrations in the wetter months (Flannigan, 1997; Sautour et al., 2009; Verhoeff, 1993).
Fungal growth in building materials is more dependent on the moisture content of the substrate than on atmospheric relative humidity. The minimum moisture content of building materials allowing fungal growth is near 76 % (for atmospheric relative humidity, this value is near 82 %). Wood, wood composites (plywood, chipboard), and materials with a high starch content are capable of supporting fungal growth, at the lowest substrate moisture content. Plasterboard reinforced with cardboard and paper fibres, or inorganic materials coated with paint or treated with additives that offer an easily-degradable carbon source, are excellent substrates for fungal growth when substrate moisture content reaches 85-90 % (Nielsen, 2003; Pasanen et al., 1992b). All fungi need nutrients for growth and

sporulation. When growing in indoor substrates such as food, nutrients are not limiting, but on the surface of certain building materials, nutrients may limit fungal growth (Pasanen et al., 1992b). Local differences in ventilation and surface temperature can generate micro-climates with very high substrate moisture content, although the room can have a low atmospheric relative humidity. For this reason, a measurement of indoor atmospheric relative humidity is a poor predictor of indoor fungal growth (Nielsen, 2003).

Xerophilic fungi are well adapted to indoor environments, since these fungi grow and sporulate with low atmospheric relative humidity and substrates with low moisture content. Indeed, the majority of *Aspergillus* and *Penicillium* species are xerophilic and able to growth in substrates with water activity lower than 0.80 (Pasanen et al., 1992a; Verhoeff, 1993). Most of the other indoor fungi (namely *Cladosporium*, *Stachybotrys*, *Chaetomium*, *Trichoderma* and *Ulocladium*) are much less tolerant to xerophilic conditions (Pasanen et al., 1992b). Because of their low water activity requirements (compared with bacteria), fungi are the principal contaminant in various types of indoor substrates. They tend to colonize a wide variety of humid building materials wetted by floods or by plumbing leaks (Dacarro et al., 2003).

2.4 Fungal fragments and allergenicity

Until 1990-2000, it was thought that indoors fungi exist only as spores and hyphae. Work published by several teams showed that fungi from the atmosphere, growing in culture media or building materials, subjected to air currents, release cellular fragments (presumably hyphal and spore fragments). The presence of these fragments in indoor air was confirmed experimentally.

For three common species from the atmosphere, growing in culture medium or building material, Górny et al. (2002) showed that when the colonies were subjected to air currents, the number of released fragments was higher that the number of spores. Fragments released from fungi growing in culture medium were not influenced by air velocity. Kildesø et al. (2003) reported the release of spores and fragments from colonies of three different species. When *Penicillium chrysogenum* was subjected to air currents, only spores were released from the colonies, but with *Aspergillus versicolor*, 1 µm fragments were also released, in addition to individual spores. With *Trichoderma harzianum*, three types of particles were released from the colonies: groups of spores; individual spores; and fragments. The release of fragments and spores from indoor fungi (*A. versicolor* and *Stachybotrys chartarum*) growing on the surface of white ceiling tiles, wall-papered gypsum board and culture medium, and subjected to air currents, was recently reported by Seo et al. (2009). One month-old cultures released more spores than fragments, but after six month incubation, the number of released fragments exceeded the number of spores. The mass of released fragments and spores (assessed by the amount of (1,3)-β-D-glucan) generally increased with age of the cultures.

The presence of fungal fragments in indoor atmosphere, predicted by these studies carried out *in vitro* (in laboratory conditions), was confirmed by field determinations. Reponen et al. (2007) reported a study carried out in five mould-contaminated single houses in Louisiana and Southern Ohio. Indoor total spore concentrations were very high and higher than outdoor concentrations (both in winter and summer). Assessed by the 1,3-β-D-glucan concentration, the ratio between fragments and spores ranged from 0.011 to 2.163, the highest average (1.017) being for indoor samples collected in the winter. Considering that fragments are much smaller than spores, the corresponding number of fragments in indoor air in these houses was certainly much higher than the number of spores. It was concluded

that, in mouldy houses, fungal fragment mass can be as high as spore mass, and fragment number can exceed total spore number.

Long-term mould damage in buildings may increase the contribution of submicrometer-sized fungal fragments to the overall mould exposure. The health impact of these particles may be even greater than that of spores, considering the strong association between numbers of fine particles and adverse health effects reported in other studies (Reponen et al., 2007; Seo et al., 2009).

However, there are at present no detailed morphological and cultural studies of these fragments released by fungal colonies subjected to air currents, and therefore important questions remain open. Are these particles, fragments of spores or of hyphae? Are they viable and able to grow in culture media and in the respiratory tract?

It has been demonstrated that *in vitro*, depending of the fungal species and tested antibody, immunological reactivity of fungal fragments is 2 to 5 times higher than conidia, (Górny et al., 2002). In several moulds responsible for releasing airborne allergens, Green et al. (2005b) found that many of the allergens were in hyphal fragments. Germinated conidia and hyphae may be more allergenic than fungal conidia, but personal exposure to fungal allergens may be difficult to evaluate (Górny et al., 2002; Green et al., 2005a). Common fungal allergens described in the literature include *Aspergillus* Asp f 1, Asp f 3, Asp f 6, and *Alternaria* Alt a 1 (Chapman et al., 2001; Crameri & Blaser, 2002). Few enzyme-linked immunosorbent assays (ELISA) are commercially available for quantification of these allergens in environmental and house-dust samples. Very often, the allergens are not detected by available immunological methods and protocols. Chapman et al. (2001) reported that in order to detect allergens in spore suspensions, it was necessary to use heavily concentrated suspensions (>100,000 conidia x ml^{-1}). This may hampered the direct detection of allergens in atmospheric sampling.

In the human body, mucociliary clearance represents the first strategy for removal of airborne fungi from the respiratory tract. This can be followed by the activation of innate and adaptive immune responses. Occasionally, inflammation occurs and individuals may suffer mucous membrane irritation, chronic bronchitis and/or organic dust toxic syndrome. The most common inflammatory reactions to fungi are non-allergic, but an allergic response or a hypersensitivity pneumonitis can occur in individuals exposed to conidia, hyphae or fungal fragments (Eduard, 2009; Green et al., 2006).

More sensitized individuals may suffer from allergy following exposure to fungi. These patients usually present high IgE values and increased release of some inflammatory mediators. Houba et al. (1998) described baking workers with high IgE against common allergens. These professionals presented an increased risk for mould occupational allergy. Allergic bronchopulmonary aspergillosis (ABPA) is also an allergic response, but specific to *Aspergillus fumigatus* allergens present in the environment. The disease is more frequent among patients with asthma or with cystic fibrosis. The usual complains are breathless, pulmonary infiltrates, bronchiectasis and fibrosis (Stevens et al., 2003). Patients' serum display high levels of total IgE, specific *A. fumigatus* IgE and IgG antibodies, IL-2 receptor, and precipitins to *A. fumigatus*.

Besides an allergic response, hypersensitivity pneumonitis can occur upon exposure to fungi. This pathology, as described by the European Academy of Allergy and Clinical Immunology (www.eaaci.net), is generally associated with high IgG antibodies concentrations in response to alveolar or bronchiolar inflammation caused by fungi or other

allergens. On the contrary of allergy, this type of hypersensitivity to fungal allergens does not seem to be mediated by IgE. The patients may present neutrophilic inflammation with increased production of TNFα and IL-6, and symptoms such as fever, chilliness, dry cough, dyspnoea, changes in nodular bilateral x-ray, fatigue and headache (Eduard, 2009).
In some asthmatic patients, fungi seem to exacerbate symptoms, but in others this effect has not been found. Newson et al. (2000) described an association between airborne total fungal counts and incidence of severe asthma in England's Trent region. However, no specific fungal species were implicated. A twofold reduction of airborne exposure to allergens has been reported to reduce the risk of developing asthma and asthma severity (Peat & Li, 1999). Other studies reported no evidence of association between airborne fungi and asthma (Richardson et al., 2005). Thus, further studies are needed in order to clarify this problem.

2.5 Production of microbial volatile compounds and mycotoxins

Indoor atmosphere always contain a mixture of volatile organic compounds (VOCs), usually at low concentrations. It is not uncommon to detect 50 different compounds, each at a low concentration (usually below 5 μg x m^{-3}, but can exceed 100 μg x m^{-3}). Indoor atmosphere usually contain higher VOCs concentrations than outdoor atmospheres. VOCs belong to very different chemical groups, such as hydrocarbons, alcohols, acetones, S compounds, ethers, esters, N compounds, terpenes and acids (Jantunen et al., 1997; Portnoy et al., 2004).
Traditionally, sources for indoor VOCs were considered to be the outdoor air, the activities of people living and working inside the building, and the building materials and furniture. Modern buildings' atmosphere usually contain higher VOCs concentrations in relation to older constructions, due to VOCs' release from building materials (Jantunen et al., 1997). Studies carried out in the 1990s, showed that indoor fungi can also be a source for the production of VOCs. Some of the molecules produced by indoor fungi are not produced by other sources (Douwes, 2009; Verhoeff, 1993). Several authors have shown that fungi from the atmospheres growing in culture media, in building materials or in house dust, do produce an array of VOCs, and that these differed in the three growing conditions (Claeson et al., 2002; Fischer et al., 1999).
Far more difficult has been the detection and identification of fungal-produced VOCs directly in the atmosphere, due to their usually very low concentrations. The production of VOCs by fungi growing *in vitro* in the laboratory strongly suggests, but does not prove, that these compounds do exist in the atmospheres. Fischer et al. (2000) demonstrated that, in highly contaminated outdoor atmospheres, certain fungal-produced VOCs were detectable and identifiable. This was certainly related to the huge concentrations of fungi in the studied atmospheres.
When applied isolated, the negative effects on human body of several of these VOCs are known, and these could be used to establish safe limits, for indoor atmospheres. However, it is far more difficult to determine the effects of mixtures of compounds, the commonest situation in indoor atmospheres. For these there are no proposed safe limits (Jantunen et al., 1997).
A specific VOC fingerprint for each fungal species may be difficult to achieve, because emission patterns can vary between strains and the release of some compounds may be dependent on the growth phase. Using commercial materials (such as fibreglass, vinyl wallpaper, cork, ceiling tiles, and plasterboard) previously contaminated with conidial suspensions of *Aspergillus niger*, *Aspergillus versicolor* or *Penicillium brevicompactum*, Moularat

et al. (2008a) concluded that VOCs could be used for a preliminary characterization of the fungal diversity in air or dust samples. However, it was not possible to find VOCs specific for each fungal species (the profile changed with substrate). A second study with materials inoculated with conidial suspensions of the same three fungal species and placed in closed chambers showed that fungi, even before visible growth occurred, released 19 different VOCs, suggesting that identification of these molecules can be used for a rapid and reliable detection of the presence of fungal growth in materials (Moularat et al., 2008b). Schleibinger et al. (2005) studied the release of VOCs by *Penicillium brevicompactum*, *Aspergillus versicolor*, *Eurotium amstelodami* and *Chaetomium globosum* (two strains of each) growing in five different substrates. It was found that fungi released low amounts of VOCs, these encompassed a wide diversity of molecules, and there was a variation between the molecules released from the two strains tested for each species.

Mycotoxins are low molecular weight compounds, produced by fungi, toxic for animals and men, with no known function in fungal metabolism. Many mycotoxins are carcinogenic, teratogenic and mutagenic (Hintikka & Nikulin, 1998; Martinez et al., 2004; Portnoy et al., 2004). *Penicillium* and *Aspergillus* species are important fungi in indoor atmosphere, and many of these species were known mycotoxin-producers (Nielsen, 2003). It remained to be studied if *Penicillium* and *Aspergillus* present in the atmosphere also produce mycotoxins, and this was demonstrated from the beginning of the 1990s. When fungi common in the atmospheres and house dust were cultivated in building materials, several mycotoxins were produced *in vitro* (Nielsen, 2003; Nieminen et al., 2002). Mycotoxins have also been isolated directly from fungi-contaminated building materials and house dust (Hintikka & Nikulin, 1998).

However, the production of mycotoxins by indoor fungi growing in building materials is much lower (can be absent) than the production in culture medium, probably due to the much lower concentration of nutrients in the former conditions (Nielsen, 2003). Ren et al. (1999) even reported no mycotoxin production from several *Aspergillus* strains (isolated from indoor air) growing on building materials (although most of the strains did produce mycotoxins when grown in culture media).

As with the VOCs, it has been very difficult to detect, directly in the atmosphere, the presence of mycotoxins (Hintikka & Nikulin, 1998; Nielsen, 2003; Martinez et al., 2004). Papers by Fischer et al. (1999, 2000), among others (Hintikka & Nikulin, 1998), which are innovative in this subject, have since reported the detection directly in the filters of triptoquivaline and tripacidine, both produced by *A. fumigatus*, one of the most abundant fungi in the studied atmosphere. As for VOCs, the detection of these mycotoxins was most probably related to the very high concentration of spores in the analyzed atmosphere.

Trichothecenes are a family of mycotoxins produced by species of *Fusarium*, *Myrothecium, Trichoderma* and, specially important for indoor environments, *Stachybotrys*. Several tens of compounds have been described in this group. From these, stand out toxins T-2 and HT-2, nivalenol, desoxynivalenol and diacetoxyscirpenol. The effects of tricothecenes in humans and domestic and farm animals are well known for decades. The symptoms include internal burning, vomiting, diarrhoea with blood, cutaneous necrosis and internal haemorrhages, followed by death (Hintikka & Nikulin, 1998; Nielsen, 2003).

At high concentrations, mycotoxins induce acute intoxications, and the negative effects are relatively straightforward to examine and quantify. At low or very low concentrations, the problem is far more complicated. In very few cases (liver cancer induced by aflatoxins in

certain African regions, for instance), it has been possible to establish a correlation between the presence of a given mycotoxin in the human diet and the incidence of a certain disease. In comparison with food and fodder, mycotoxins concentrations in the atmosphere are expected to be very low. Moreover, the simultaneous presence of several adverse and negative factors in indoor atmosphere (mycotoxins and VOCs, for instance), is not uncommon. For these reasons, it has been difficult to establish a correlation between the presence of given mycotoxins in indoor environments and health problems of their occupants (Douwes, 2009; Mendell et al., 2009; Nielsen, 2003; Verhoeff, 1993).

However, in certain situations, the evidence for this association is substantial (Rea et al., 2003). Flappan et al. (1999) reported a case of infant pulmonary haemorrhage in a home in Missouri (USA). Inspection of the house revealed serious water infiltrations in the attic and in the baby's bedroom closet. Indoor air sampling (using a volumetric spore trap and microscopic total spore counts) carried out in five different rooms revealed huge air total spore concentrations in the baby's room (higher than 10,000 spores x m^{-3}), and very high concentrations in baby's bedroom closet, in the attic and in the family room (higher than 2,000 spores x m^{-3}). *Aspergillus* and *Penicillium* were largely dominant in the air of all rooms. *Stachybotrys* was detected only in the atmosphere of the baby's bedroom. Surface samples taken from water-damaged building materials from several rooms, and dust from baby's bedroom, contained *Stachybotrys*. In contaminated building materials were detected several trichothecene molecules. This case was similar to others reported in the Cleveland area in 1993-1998, which resulted in the death of 12 infants.

Additional research employing new technologies and modern equipments (particularly mass spectrometry and/or gas chromatography) will certainly be conducted on this subject in a near future (Schuchardt & Kruse, 2009).

3. Detection of fungi in indoor environments

3.1 Volumetric and sedimentary methods

Atmosphere sampling for bioaerosols has been conducted for decades with classical monitoring that relies on collection using forced air samplers and analysis by either culture media or microscopy (Stetzenbach et al., 2004).

Quantitative microbiological methods for atmosphere analysis witnessed important developments in the 1940s-1960s.

K. R. May's cascade impactor, described in 1945 (May, 1945), was one of the first instruments that allowed the detection of fungal cells, since collected all particles with 0.6-20 µm. The cascade impactor consisted of a system of four air-jets and sampling slides in series. The slits were progressively narrower, so that the speed jet and therefore the efficacy of impaction of particles increase from slide to slide. Particles impacted on glass slides covered with an adhesive substance, and, at the end, were counted by optical microscopy. The instrument allowed discrimination of the particles by size due to the four successive stages (Burge & Solomon, 1987; Davies, 1971).

An improvement of May's device was carried out by J. M. Hirst, in 1952 (Hirst, 1952). The instrument was also a slit sampler based on impaction on an adhesive surface, but allowed monitoring during a whole day (achieved by the slow and constant displacement of the slide underneath the slit) and with strong winds and rain. The equipment was reliable for

capturing large spores. Small spores such as those of *Aspergillus* and *Penicillium* were underestimated (Davies, 1971; Martinez et al., 2004).

May and Hirst slit impactors allowed no distinction between viable and dead cells, and, very importantly, did not enabled a rigorous identification of the fungal spores, since morphological characteristics of these cells only allow an identification at a genus level, and only for a restricted group of fungi (Stetzenbach et al., 2004). These drawbacks were resolved in the slit sampler developed by Bourdillon and collaborators in the 1940s (Bourdillon et al., 1941). Using the same principle of air suction through a narrow slit, a Petri dish with culture medium was placed underneath. The dish slowly rotated during sampling, so that an annular ring trace was formed in the agar. Bacteria were collected with very high efficiency (Davies, 1971; Henningson & Ahlberg, 1994).

A great step forward was given by Andersen in 1958, with the design of a six-stage impactor, with collection of particles on culture medium (Andersen, 1958). Air sucked passed six successive aluminium plates drilled with decreasing size holes. Underneath each plate was placed a Petri dish with culture medium. The decreasing size of the holes forced air to accelerate from the upper to the lower stage. The upper stage collected the biggest particles and the lowest stage the smallest cells. Between these, increasingly smaller cells were collected. Andersen sampler allowed discrimination of the particles by size, the determination of the concentration of culturable cells, and, after observation of the colonies, the identification of the fungi at species level (Eduard & Heederik, 1998; Flannigan, 1997; Henningson & Ahlberg, 1994; Martinez et al., 2004; Stetzenbach et al., 2004).

May, Hirst, Bourdillon and Andersen samplers were based on impaction on a solid surface - the projection of particles onto the surface of a glass slide or culture medium. By the time of design of these samplers, impingement - blowing the particles into a liquid by the use of glass impingers – was also improved in order to be used in microbiological analysis. Impingement is based on the suction of the air through a narrow capillary tube, and projection of the air jet into a liquid. Particles present in the atmosphere, such as fungi, are forced to enter the liquid.

From impinger models adapted to microbiological uses, stands out the all-glass impinger AGI-30 described by Malligo & Idoine (1964) and the three-stage impinger described by K. R. May, in a paper published in 1966 (May, 1966). AGI-30 impinger was developed from the AGI-4 model - the Porton impinger. The inlet was designed to simulate the human nose. The jet nozzle was raised above the liquid in order to get an impingement surface softer than the glass bottom of the flask. The collection efficiency for bacteria was very high (Eduard & Heederik, 1998; Henningson & Ahlberg, 1994).

The multi-stage liquid impinger of May (1966), built in thick walled Pyrex glass, had three superimposed chambers. In the first two chambers, air-jets impacted vertically on to glass discs filled with sampling liquid. The third chamber was a bowl-shaped swirling impinger (Eduard & Heederik, 1998; Henningson & Ahlberg, 1994; Martinez et al., 2004).

Impingement has some advantages over impaction on solid surfaces: 1) if the concentration is too high, the liquid can be diluted; 2) affords, simultaneously, total cell counting (by microscopy) and culturable cell counting (by culturing aliquots on nutrient media); 3) different culture media can be used, at the same time, to study a given sample; 4) collection of the cells in a liquid avoids desiccation resulting from impaction on solid surfaces, especially on glass slides; 5) cell clusters, kept intact when using impaction of agar medium, are dissociated in their individual cells; 6) the particle retention efficiency is very high; 7) the

equipment is compact and inexpensive (Martinez et al., 2004; May & Harper, 1957; Stetzenbach et al., 2004). The method has however some limitations: 1) it is not appropriate for clean atmosphere, since a reduced number of cells will be present in a relatively large volume of liquid; 2) after certain time of operation, the liquid, which is under low pressure, evaporates appreciably; 3) the efficiency for collecting bacteria is higher than for spores (Eduard & Heederik, 1998).

In addition to impaction and impingement, other methods have been used in the study of fungal populations in atmospheric bioaerosols. In filtration methods, filters collect particles through impaction and interception mechanisms. Filter materials commonly used for air microbiological sampling include glass fibre filters, mixed cellulose esters, polytetrafluoroethylene, polyvinyl chloride, gelatine, and polycarbonate (Eduard & Heederik, 1998; Martinez et al., 2004). Advantages of filter sampling include the simplicity of collection and sample handling procedures, the ability to perform different analyses on the same extraction solution, and the relatively inexpensive cost. Membrane filters can be placed directly on the surface of culture medium, or washed with a liquid, and this added to culture medium. Certain filters are dissolvable in warm liquids, and the resulting suspension can be platted on agarized medium. Two disadvantages for filter sampling are the low extraction efficiency from the filter material, and the dehydration of microorganisms, which reduces their cultivability (Martinez et al., 2004).

Sedimentary sampling is generally carried out using the settle plate method. Open Petri dishes with appropriate culture medium are left open during a given time (minutes, hours or days depending on the air contamination load). After a certain period of incubation, colonies are counted and identified. Sedimentary sampling has several advantages: 1) it is simple and inexpensive; 2) allow a cumulative assessment over a prolonged exposure times - «The cfu collected on settle plates are like a photocopy, or a mirror of what was going on at a particular point, during a period of time. Long sampling periods may increase measurement significance and reproducibility» (Pasquarella et al., 2000). The method suffers however from several limitations: 1) no known volume of air is analyzed, it is therefore not quantitative; 2) the rate of deposition of cells can be affected by air turbulence; 3) small cells tend to be under-estimated (Burge & Solomon, 1987; Pasquarella et al., 2000).

Pasquarella et al. (2000) argued extensively about the advantages of the sedimentary methods for hospital indoor microbial analysis. A new index was defined – the Index of Microbial Air Contamination (IMA), determined with the following procedure: «A standard Petri dish 9 cm in diameter containing plate count medium is left open to air according to the 1/1/1 scheme, for 1 h, 1 m from the floor, at least 1 m away from walls or any relevant physical obstacle. After 48 h incubation at 36±1 °C the colonies are counted. The number of colonies is the IMA». IMA classes and maximum acceptable levels of IMA were defined empirically. Five classes of IMA were devised: 0–5 very good; 6–25 good; 26–50 fair; 51–75 poor; >76 very poor. Maximum acceptable values of IMA were established, related to different infection or contamination risks. These were 5, 25 and 50, in places with very high, high and medium risk, respectively. For example, hospital operation rooms, with very high risk, should have a maximum IMA value of 5, corresponding to 9 CFU x dm^{-2} x h^{-1}. The authors also provided a comparison between IMA classes and several international standards.

Two standard incubation temperatures are used: 25-27 °C for growing the great majority of species, and 35-37 °C, for human-related species such as *Aspergillus fumigatus*, *A. flavus* and

A. niger (Araujo et al., 2008a). Spores of *Aspergillus* and *Penicillium* may survive for long periods, even years, whilst the cultivability or viability of others may decline very rapidly. The use of culture-based analysis methods underestimates populations in bioaerosols owing to the detection of only those fungi that grow in culture media, while non-culturable (live or dead) organisms go undetected. As with other environments, most of atmospheric fungi appear to be in a non-culturable sate (Flannigan, 1997).

Fungi are capable of causing health effects whether they are in the culturable or non-culturable but viable state. However, these effects are expected to be very different, but are poorly known. Can a live but non-culturable fungal spore, hypha or fragment grow on the surface of our respiratory epithelium? Enumeration of total fungi by microscopy lacks identification specificity, unless accompanied by specialized staining or immunological assay. Specific antibodies with heavy metals bind only to specific microbes that are viewed under scanning electron microscopy. Epifluorescence and electron microscopy has also been used. With fluorescence microscopy, microbes are stained with fluorochromes and are viewed with fluorescent light. Fluorescein diacetate (FDA) has been used for viable fungi. Scanning electron microscopy is useful for studying fungal spore surface characteristics, but is not routinely used for microbes' identification (Martinez et al., 2004).

In addition to these methods, biochemical assays that detect fungal specific molecules such as (1,3)-β-D-glucans, chitin, and ergosterol have been to estimate total fungal bioaerosol loads. These are particularly important for the quantification of fungal fragments, which are non-culturable and difficult to recognize by microscopy (Flannigan, 1997; Martinez et al., 2004; Stetzenbach et al., 2004). As Flannigan (1997) wisely remarked, most microbiological investigations of indoor air still employ culture-based methods, but sufficient attention is seldom given to four important issues: sampler performance, temporal variability, culture media and accurate identification. Too many studies identify only to the genus level and disregard the diversity of species, their ecology and potential significance for health, especially in important genera such as *Aspergillus* and *Penicillium*.

3.2 Molecular methods

Molecular biology has been increasingly applied in the evaluation of indoor air quality in medical environments. Quantitative PCR (QPCR) has been used for the detection of *Aspergillus*, *Penicillium* and *Paecilomyces* in the atmosphere of clinical wards (Haugland et al., 2004). Using this method, a comparative study carried out during and after construction works in clinical wards showed a generalized decrease in the atmospheric concentrations of these fungi (Morrison et al., 2004). QPCR has been employed for quantification of airborne fungi in other environments, such as allergic patients' homes. Whilst some studies could not find any correlation between the results using molecular and culture-based methodologies (Meklin et al., 2004; Pietarinen et al., 2008), others described, for some fungi (*Aspergillus*, *Penicillium* and *Cladosporium*) a significant correlation, but no association in other cases (*Acremonium*, *Aureobasidium* and *Wallemia)* (Lignell et al., 2008).

A consistent observation has been reported in all studies - molecular methods' sensitivity is considerably higher in comparison with traditional approaches based on culture methods (sometimes of several orders of magnitude). However, some fungi grow in culture but are not detected by molecular methods, and others do not grow in agarized media but are identified by molecular methods (Pietarinen et al., 2008). An example of differential results yielded by these two types of methods was recently presented by Bellanger et al. (2009) in a

study of allergic (with asthma, allergic rhinitis or conjunctivitis) patients' houses. *Aspergillus versicolor* grew well in culture media but went undetected by molecular methods. Molecular methods may not be able to detect all fungal species, particularly when large amounts of DNA of other fungi are present, or in presence of certain PCR inhibitors (those inhibitors can be present in the environment or be added through sample management). However, a wise selection of primers can detect many fungal species. Nevertheless, standardization of most procedures, such as extraction methodology, the selection of primers that should be employed for QPCR and amplification conditions, are still needed in order molecular methods be widely used and the results allow further comparisons.

3.3 Airborne fungal diversity and detection of rare taxa

Global assessment of the genetic diversity in a given environment has been studied using several molecular techniques. The metagenome description of the microbial communities in Sargasso Sea is still not concluded but a vast amount of new data was obtained (Venter et al., 2004). Metagenome fingerprinting techniques, such as automated ribosomal intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphism (TRFLP) and denaturating gradient gel electrophoresis (DGGE), have been employed worldwide for measuring fungal species richness in communities. However, these methods may not reflect the actual microbial diversity, as they tend to identify only the dominant members of the community (Bent et al., 2007). ARISA is a high-resolution, highly reproducible, automated technique that uses the variability in the length of the intervening transcribed spacer regions (ITS) of rRNA genes in order to separate several samples into operational taxonomic units (OTUs). ARISA allows the characterization and distinction of fungal communities and has been employed to distinguish fungal soil communities from distinct cities and countries (Ranjard et al., 2001). The other two methods (TRFLP and DGGE) employ restriction enzymes or specific primers and non-automated gel electrophoresis for identification of microbial OTUs. TRFLP allowed a good characterization of fungal communities isolated from air samples collected from an urban area of Seoul (Korea) and soil samples in UK (Lee et al., 2010; Schütte et al., 2008).

Recently reported fungal metagenomic studies found that *Ascomycota* (*Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, and *Sordariomycetes*) and *Basidiomycota* (*Agaricomycetes)* were the most represented Divisions in outdoor atmospheres (Fröhlich-Nowoisky et al., 2009; Lee et al., 2010). Reports on fungal metagenome of indoor environments have been included in studies screening complete microbial communities (Angenent et al., 2005), but these are still very incomplete. The construction of metagenomic libraries is nowadays possible, although technically demanding and economically expensive. The future will bring new technologies and cheaper alternatives for sequencing large number of OTUs and these will allow knowledge of the composition of complete communities. The study of hospital metagenome can allow physicians, researchers and other medical staff a full knowledge of the microbial communities present inside medical wards. This is expected to give information on the presence of certain fungi in highly-restricted areas where critical patients are admitted, and therefore to have a considerable impact on public health.

Metagenomic strategies are not only an important tool for characterization of the genetic diversity in whole communities, but also represent an indispensable approach for detection of rare fungal taxa (by revealing new OTUs). Molecular methods such as QPCR are based on a previous selection of primers, and these are limited to *a priori* chosen taxa. These methods

cannot be used to find new taxa (Fröhlich-Nowoisky et al., 2009; Lee et al., 2010). The detection of rare taxa in the environment still remains a critical issue for the global understanding of the value and importance of each and all microorganisms. However, precautious should be taken against a simplistic interpretation of molecular data, since the presence of genetic material, even in good and preserved condition, is no guarantee that the organism is active (or alive) in the environment. In terms of interaction between the fungus and the human body, such active condition is probably indispensable for infection.

4. Air quality and fungal infections

4.1 Hospital indoor air quality and incidence of fungal infections

Many outdoor activities such as gardening, hunting, or camping, and few sports such as caving or cave diving, are associated with increased environmental exposure to pathogenic fungi and increased risk of invasive fungal diseases (IFDs) (Sipsas & Kontoyiannis, 2008). Inhalation of conidia and direct inoculation through minor skin lesions are the most common mechanisms for developing fungal disease. Some human practices, such as smoking tobacco or marijuana, use of illicit intravenous drugs, body piercing or tattooing, pet ownership, and travelling to endemic areas, are associated with an increased risk of IFD. Endemic mycoses usually occur in limited geographic areas and individuals outside the fungal ecological niche are not at risk of infection.

Immunocompromised patients are also at the highest risk for development of fungal infections in hospitals. The complete list of fungemia risk factors is vast, but the most relevant factors are: submission of patients to immunosuppressive treatments (such as chemotherapy, or corticosteroids therapy); neutropenia (<500 polymorphonuclear cells x ml^{-1}); treatment with antimicrobial agents; submission to bone marrow or solid organ transplants; previous colonization with fungal agents; presence of indwelling catheters; extensive surgery or burns; need of parenteral nutrition; assisted ventilation or haemodialysis; malnutrition; prolonged hospitalization particularly at intensive care units (De La Rosa et al., 2002; Fridkin & Jarvis, 1996). The most important fungal agents responsible for infection in these patients are *Candida*, *Aspergillus*, and several zygomycetes. In addition, emerging fungal pathogens (*Fusarium* sp., *Scedosporium* sp., *Thichosporon* sp. and *Malassezia* sp.) are becoming also a threat to these patients. Risk patients need to be protected from fungal pathogens, particularly by isolation in highly-restricted units, as most of these agents are airborne pathogens.

Aspergillus fumigatus is the main responsible for airborne infections in immunocompromised patients and one of the most common airborne moulds found indoors at clinical wards. The ability of the fungus to colonize and resist indoors, even under unfavourable conditions, and to germinate and grow faster under human internal milieu conditions (Araujo & Rodrigues, 2004) makes *A. fumigatus* one of the most serious fungal agents worldwide. The fungus is responsible for high mortality rates. Invasive aspergillosis generally involves inhalation of conidia or hyphae and further growth in human internal milieu. Vonberg & Gatsmeier (2006) reviewed all cases of invasive aspergillosis and concluded that the fungus is able to cause disease in environments with less than 1 CFU x m^{-3} of air. They recommended that risk patients should not be exposed to the fungus and concluded that prevention from all routes is critical. Patients staying long periods at clinical units with high degree and long duration of immunosuppression are at the highest risk for developing

invasive aspergillosis. In haematological patients, the incidence of fungal infections is higher in the group of patients suffering of acute leukaemia and aplastic anaemia malignancies (Araujo et al., 2008b; Pagano et al., 2006). In nosocomial aspergillosis, transmission occurs generally through the air, but the involvement of water (Warris et al., 2003), plants (Lass-Flörl et al., 2000), furniture (Menotti et al., 2005) or even person-to-person contact (Pegues et al., 2002) have been confirmed by molecular studies. In fungal infections caused by yeasts and other moulds, molecular studies have found a connection between the isolates collected from patients and from environmental samples (Cortez et al., 2008; Lupetti et al., 2002; Vos et al., 2006).

Some studies have reported a decrease in the incidence of fungal infections in clinical units following a decrease in levels of airborne fungi (Alberti et al., 2001; Araujo et al., 2008b; Berthelot et al., 2006), but this improvement was not consistently found in all hospitals. One of the first studies reporting the absence of *A. fumigatus* infections in clinical environments with less than 0.1 CFU x m^{-3} was performed by Sherertz et al. (1987). No environmental breakpoints have been yet defined in order to completely prevent fungal infections in clinical units, nor it is well defined the frequency for collection of air samples and monitoring indoor air quality. Several measures have been described for protecting patients and decreasing the risk for acquisition of IFDs. Air filtration systems are the most used in clinical units, mostly the high efficiency particulate air (HEPA) filters, and they will be discussed in detail in the next topic.

4.2 Air filtration systems

As most indoor fungi came primarily from outdoors, it cannot be discarded the impact that an increase in outdoor fungi may have in increasing the risk of fungal diseases in clinical units. Some studies have tried to correlate outdoor fungal concentration and incidence of fungal diseases. However, other variables interfere with this relationship: the exact amount of fungi that in fact reach indoor air in clinical units; ventilation rates; protective measures present in wards (particularly the presence of air filtration systems); fungal colonization indoors; other routes for fungal access besides air; patient immune response; administration of prophylactic antifungal treatments. Nevertheless, some studies reported an association between outdoor fungi and incidence of indoor infections (Bouza et al., 2002; Radin et al., 1983; Srinivasan et al. 2002). The relationship between outdoor and indoor airborne fungi is much easier to find and has been observed by several researchers (Brenier-Pinchart et al., 2009; Curtis et al., 2005; Dassonville et al., 2008; Falvey & Streifel, 2007; Pini et al., 2004).

Air filters like F7-F9 retain around 90 % of the particles, while HEPA filters H13 retain 99.97 % of the particles with more than 0.3 µm. The installation of HEPA filters is commonly associated with positive pressure (>2.5 Pa) and air flow rates higher than 12 exchanges of air per hour (Sehulster et al., 2003). The complete HEPA filtration system is usually based upon a pre-filter G2-G4 (made of synthetic fibres, such as polyester, or glass fibre; with initial efficiency of around 70 %), a fine filter F5-F9 (several types are commercially available such as bag filters, rigid pocket, or cardboard filters), and a HEPA filter H10-H14. Pre-filters should be replaced often as they retain most part of airborne particles. The impact of filtration systems such as F8 or F9, and the presence of negative air flow, has been less studied than HEPA filters. F8 and F9 filtration systems may reduce significantly fungal air levels, but, as expected, are not as efficient as HEPA filters (Araujo et al., 2008a). Negative

air flow rates are presently forbidden in wards near renovation and construction sites (Sehulster et al., 2003).

Indoor levels of *Aspergillus* sp. can be greatly reduced by air filtration systems, such as the HEPA system, and this can result in a concomitant decrease in the incidence of invasive aspergillosis (Alberti et al., 2001; Araujo et al., 2008b; Vonberg & Gatsmeier, 2006). A significant reduction of *Candida* infections has been described after the installation of HEPA filters (Araujo et al., 2008b; Boswell & Fox, 2006). A systematic review focusing on the influence of HEPA filters in wards with immunosuppressed patients was recently reported by Eckmanns et al. (2006) who concluded that HEPA filters could be occasionally beneficial for patients. However, a significant decrease in fungal-related mortality rates was not found for HEPA protected areas.

All over the world, studies have been carried out in order to evaluate indoor air quality in medical facilities. The fungal airborne values in clinical wards without air filtration system are usually between 50 and 500 CFU x m^{-3} (Alberti et al., 2001; Brenier-Pinchart et al., 2009; Cooper et al., 2003; Curtis et al., 2005; Dassonville et al., 2008; Falvey & Streifel, 2007; Panagopoulou et al., 2002; Pini et al., 2004; Sautour et al., 2009). In clinical wards with air filtration, airborne fungal values are much lower ranging from 0 to 50 CFU x m^{-3}. Multiple factors may affect indoor concentrations, namely construction works, people's access to ward, the presence of additional protective barriers, and the implementation of water filtration (Anaissie et al., 2003; Araujo et al., 2008a; Carreras, 2006; Clark & de Calcina-Goff, 2009). In general, the major challenge in clinical wards is to prevent the entrance of fungi that are ubiquitous outside. By keeping lower fungal concentrations in areas around units with risk patients, it is also possible to find better air quality in clinical units.

Some outbreaks have been described following failures in air-filtration systems or by the presence of contaminated air-handling systems (Lutz et al., 2003; Muñoz et al., 2004). *A. fumigatus* was responsible for the fungal infections, in most cases. By repairing the air-filtration system and replacing old filters, it was possible to recover the unit and clinical department. The installation of portable HEPA filters may also be used in places where fixed HEPA filters are not possible to install. This strategy has been successfully described in some hospitals (Abdul Salam et al., 2010; Boswell & Fox, 2006; Engelhart et al., 2003). Verdenelli et al. (2003) have described that filters treated with antimicrobials displayed markedly less microbial colonization than untreated filters, resulting in less problems in the maintenance of filtration systems. However, this alternative is disputable, since it may enhance microbial resistance in clinical environments. The application of new materials for air filtration systems, such as silver- or cooper-impregnated materials, may represent an alternative to be tested (Clark & de Calcina-Goff, 2009).

In conclusion, regular and appropriate maintenance of air filtration systems is decisive for keeping excellent air quality in medical units. New engineering-made materials are expected in a near future and these hopefully will bring improvements in the air quality in hospital environments.

4.3 Recommendations for highly-protected medical environments

For highly-protected wards, a list of recommendations was issued in 2003 by the Center for Disease Control and Prevention (CDC) and Healthcare Infection Control Practices Advisory Committee (HICPAC) (Sehulster et al., 2003) and is currently available for free consulting (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5210a1.htm). In addition to the

recommendations already described in topic 4.2, routine hand hygiene taken by staff, patients, and visitors can prevent infections in health-care facilities (Carreras, 2006). Alcohol-based antiseptics (60-95 % alcohol) are recommended for hand washing. Similar procedures should be followed by people in contact with food, objects and other materials accepted in clinical units where risk patients are admitted. All objects admitted at units with risk patients should be kept clean and disinfected.

Wards are advised to be private (for single patients) and should be cleaned at least once a day and the high-touch surfaces cleaned much more frequently (Sehulster et al., 2003). Special attention should be given to bathrooms (carefully cleaned before patient's shower). Adequate temperature (22-24 ºC) and humidity (30-60 %) should be maintained.

Other routes, like water, objects, beds (and pillows), plants or food, were shown to represent reservoirs of fungal agents and may be able to transfer conidia or hyphae to patients (Anaissie et al., 2003; Bouakline et al., 2000; Lass-Flörl et al., 2000; Potera, 2001; Warris et al., 2003; Woodcock et al., 2006). Hospital fabrics and plastics also act as reservoirs of medically important fungi. Some materials may influence the length of fungal survival (Neely & Orloff, 2001). Sodium hypochlorite is commonly used for cleaning walls and surfaces in wards, but other chemicals presenting fungicidal activity against most yeasts and moulds can be used as disinfectants (Araujo et al., 2006; Wilson et al., 2004). Chlorine is frequently recommended for routine treatment of the water and its recirculation in distribution systems is important (Sehulster et al., 2003). Alternatively, hot water can be maintained at temperatures ≥51 ºC and cold water at <20 ºC (a periodical increase to temperatures ≥66 ºC is recommended in order to eliminate any microbial contamination). In order to prevent infections transmitted by contaminated foods, neutropenic patients are advised to consume low-microbial-content diets (Carreras, 2006; Remington & Schimpff, 1981). Abstention from pepper and other spices, tea, seeds, fruits and vegetables, which usually contain fungal conidia or hyphae (Bouakline et al., 2000), can restrict considerably patients' life and well being. Heating or irradiation (ultraviolet, gamma, microwave) can reduce or eradicate completely fungi present in water, food and some materials (Araujo et al., 2006; Gangneux et al., 2004).

Some cases of invasive aspergillosis have been associated with marijuana consumption. Therefore, smoking should be avoided by immunosuppressed patients (Verweij et al., 2000). Patients should remain isolated as long as their immune system is compromised and all treatments or diagnostic procedures should be conducted into the protected unit or ward. In occasions of leaving isolated wards, patients must wear facial mask (Raad et al., 2002).

Human movement may also be associated with an increase of indoor microbial contamination and the number of visitors should be restricted (Clark & de Calcina-Goff, 2009; Sehulster et al., 2003). Patients, visitors and unit staff should be continuous alerted to the procedures followed in restricted-environments. The implementation of educational programmes can result in a reduction of infections in clinical units (Jain et al., 2006).

Assessment of fungal genetic diversity may represent a useful tool for detecting the eventual presence of specific clonal populations in a clinical setting. *A. fumigatus* populations have been described as highly dynamic indoors, since new populations were found in just a few months (Araujo et al., 2010). Due to the high dispersion capability of moulds in indoor environments, more attention should be given to strains with increased pathogenic potential or reduced susceptibility to antifungal drugs. More attention will be given in coming years

to molecular epidemiological studies as they are becoming much cheaper and consistently more accepted and validated.

4.4 Prevention during hospital construction and renovation works

Construction and renovation of departments or hospitals have been carefully followed by medical administrations since these interventions can result in an increase of infections in clinical units. Inadequate ventilation and proximity to renovation and construction sites have been repeatedly implicated in the epidemiology of IFDs, mostly invasive aspergillosis (Cooper et al., 2003; Engelhart et al., 2003; Muñoz et al., 2004). Most hospitals surrounding construction sites are usually exposed to higher levels of airborne particles and additional protective measures should be followed. If large renovation works take place in units admitting risk patients, the patients should be transferred far from construction sites.

Airborne fungal levels are significantly higher when clinical units are subjected or close to construction or building demolition (Bouza et al., 2002; Cooper et al., 2003; Srinivasan et al., 2002). The adoption of all protected measures described in chapters 4.2 and 4.3 allows an efficient and protective environment to patients (Bouza et al., 2002; Cooper et al., 2003; Srinivasan et al., 2002). Additional attention should be given to the presence of barriers that limit the access of fungi and other particles to wards (doors that should be kept closed as long as possible, as well as tightly-closed windows). Different access routes for workmen, staff, patients and visitors have also been suggested (Cooper et al., 2003). Portable filtrations systems located in strategic places along the access to medical units can also be used (Abdul Salam et al., 2010; Engelhart et al., 2003). These systems are useful alternatives in emergencies following a complete breakdown of the fixed air filtration system (Sehulster et al., 2003). The use of facial masks by patients in close contact to risk environments can prevent IFDs (Raad et al., 2002).

Routine mycological assessment of the air and water should be carried out in hospitals, especially in areas where immunosuppressed patients are treated, aiming to detect anomalous situations and post alert warnings. These must be followed by a rapid intervention in order to avoid possible nosocomial infections. Such policies, in addition to educational programmes, are expected to result in a control and reduction of nosocomial infections and a promotion of patient's protection and well-being.

5. Conclusion and future perspectives

It is probably true to say that moulds cannot be completely eliminated from indoor environments. Normal buildings contain a diversity of materials and substrates that allow growth and sporulation of many species of fungi. Some strategies can be used to reduce indoor fungal load in wards receiving high-risk patients, namely by adding air filters and a positive air flow rate, by the presence of an anteroom, the use of protective clothes and of hair and shoe covers, the implementation of regular water filtration and the regular cleaning of walls and surfaces. The development of new engineering-made materials for air filtration systems may represent an alternative to be tested in a near future.

In hospitals or other institutions admitting immunosuppressed individuals, environmental reservoirs, namely air and water, should be routinely evaluated for the presence of fungi. Assessment of indoor fungal genetic diversity may represent a useful tool for studying the eventual presence of specific clones in clinical wards. Airborne fungal populations can

evolve fast, making difficult the study of the molecular epidemiology of fungal agents, but the use of intensive airborne sampling and genotype characterization can help to fulfil this desideratum. The era for characterization of hospital metagenome has been launched and fungal communities will certainly give us unexpected surprises and new perspectives regarding the quality of life for patients and staff at medical units.

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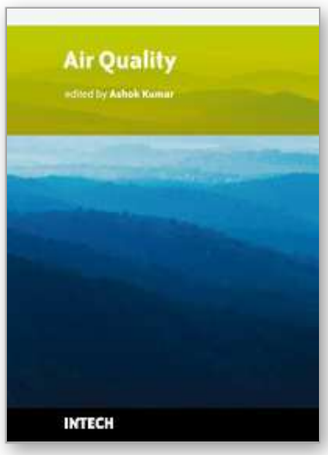

Air Quality

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Air pollution is about five decades or so old field and continues to be a global concern. Therefore, the governments around the world are involved in managing air quality in their countries for the welfare of their citizens. The management of air pollution involves understanding air pollution sources, monitoring of contaminants, modeling air quality, performing laboratory experiments, the use of satellite images for quantifying air quality levels, indoor air pollution, and elimination of contaminants through control. Research activities are being performed on every aspect of air pollution throughout the world, in order to respond to public concerns. The book is grouped in five different sections. Some topics are more detailed than others. The readers should be aware that multi-authored books have difficulty maintaining consistency. A reader will find, however, that each chapter is intellectually stimulating. Our goal was to provide current information and present a reasonable analysis of air quality data compiled by knowledgeable professionals in the field of air pollution.

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