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Review

Airborne *Aspergillus fumigatus* conidia: a risk factor for aspergillosis

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ABSTRACT

Aspergillus fumigatus is an opportunistic fungal pathogen that causes invasive aspergillosis, a usually fatal infection. The disease has risen in prominence in recent years due to the increasing numbers of severely immunocompromised patients becoming infected. The fungus is ubiquitous in the environment, producing large numbers of conidia that are dispersed in the air. Humans inhale numerous conidia everyday, but infections are not seen in healthy individuals. As inhalation of conidia is the main route of infection, considerable efforts are required to prevent infection in susceptible patients. This review summarises the current knowledge on airborne concentrations of *A. fumigatus* conidia, their background levels in outdoor air and seasonal distribution patterns. New and established methods of air sampling for airborne *A. fumigatus* conidia are discussed. Common environmental sources of the fungus are reviewed, including its presence in compost heaps. Finally, the lack of stringent guidelines on the monitoring and control of airborne *A. fumigatus* concentrations in hospitals is discussed.

1. Introduction

The filamentous Ascomycete *Aspergillus fumigatus* is a fungal saprophyte that is ubiquitous throughout the world (Klich, 2002). It typically grows in soil and decaying vegetation such as compost heaps, where it plays an important role in the recycling of carbon and nitrogen (Tekaia and Latgé, 2005). It is also an opportunistic pathogen of immunocompromised hosts, one of several *Aspergillus* species that cause a range of allergic, saprophytic, and invasive diseases that are collectively termed 'aspergillosis' (Barnes and Marr, 2006; Hope *et al.*, 2008). The most severe form is invasive aspergillosis (IA), and *A. fumigatus* is responsible for 90 % of cases (Latgé, 2001). Mortality rates of 70–90 % are reported in haematopoietic stem cell transplant recipients, the most at-risk group (Denning, 1998). Despite improvements in the diagnosis and treatment of IA, the number of cases and severity of the disease have increased dramatically due to the sheer numbers of patients being subjected to increasingly powerful immunosuppressive therapies (Barnes and Marr, 2006). For example, Groll *et al.* (1996) reported a near eight-fold increase in IA cases over a 14-y period from 1978.

A. fumigatus is able to reproduce by both sexual and asexual means, producing meiotic ascospores under defined conditions and vast quantities of mitotic conidia on most substrates (Raper and Fennell, 1965; O'Gorman *et al.*, 2009). The asexual conidia extend out in long chains from the vegetative mycelium, and readily detach to become airborne (Fig. 1). Inhalation is therefore the main route of infection

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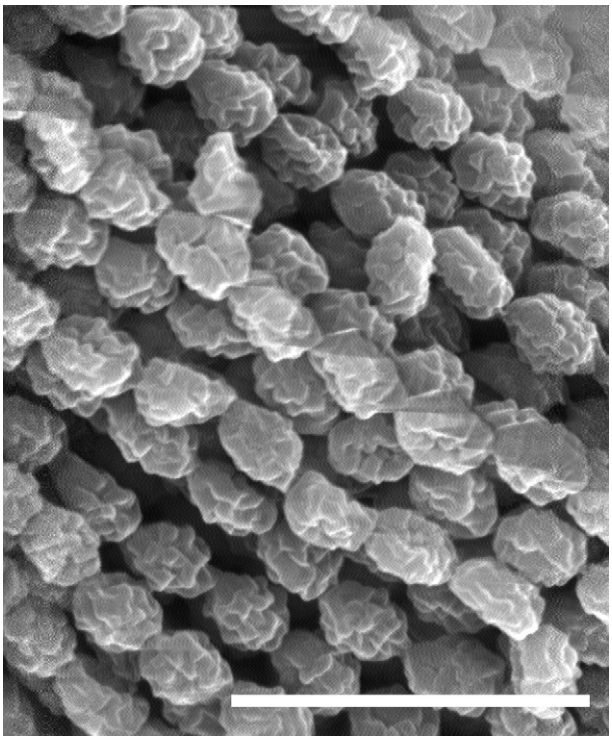

Fig. 1 – A scanning electron micrograph of long chains of echinulate *A. fumigatus* conidia (scale bar = 10 μm).

(Segal, 2009), although the conidia can also gain access through other sites including broken skin, the eyes, ears, and gastrointestinal tract (Denning, 1998). Once inhaled, their small size (2–3 μm) allows them to easily bypass the defence mechanisms of the nasal cavity and upper respiratory tract to reach the lung alveoli. While exposure to these airborne conidia is constant, human infection is confined predominantly to the immunocompromised, as the innate immune system of healthy individuals is adept at preventing infection (Latgé, 1999). Protecting immunocompromised patients from inhaling air contaminated with *A. fumigatus* conidia is therefore a very important control measure in the prevention of invasive aspergillosis. For a review of recommended control measures, see Haiduven (2009).

The conidia of *A. fumigatus* are extremely well-suited for air dispersal as their small size and hydrophobicity allows them to remain airborne for very long periods. Their echinulate (spiny) surface increases their air resistance, enhancing dispersion (Fig. 1). Melanin pigmentation in their cell walls provides some UV protection and they are thermotolerant, able to withstand a range of temperatures. Of 49 species tested by Gregory (1973), *A. fumigatus* conidia had the slowest settling velocity at 0.03 cm s^{-1}. When they eventually settle out, they contaminate all surfaces in contact with the air, and can easily become airborne again, creating mini bursts of spores (Rhame, 1991). Together with the fact that they remain viable for many months in dry conditions, it is no surprise that *A. fumigatus* has a global distribution pattern (Pringle *et al.*, 2005).

2. Air sampling methods

Culture-based methods

One of the most well established methods of identifying and quantifying viable airborne fungal spores is *via* their impaction onto a solid growth medium from a known volume of air. A portable air sampling device is used, and several different makes are available that vary in their size and rate of air intake. They aspirate air at a fixed rate up to a pre-selected volume, passing the air through a sieve plate positioned over an exposed Petri dish of agar (Fig. 2). The laminar airflow that is created forces the spores onto the agar. Following incubation for several days, fungal colonies are identified and counted and can be expressed as numbers of colony-forming units per cubic metre of air (CFU m^{-3}). If necessary, the agar can be supplemented with antibiotics (e.g. chloramphenicol) or growth-suppressants (e.g. Rose Bengal) to inhibit bacterial growth or slow down fast-growing mucoraceous moulds, respectively. Recently, the medical antifungal agent itraconazole has been successfully added to Sabouraud agar for the isolation of itraconazole-resistant *A. fumigatus* conidia from air (Vanhee *et al.*, 2010). This application will be of particular benefit for future epidemiological studies, as azole-resistant *A. fumigatus* strains are on the increase and have been isolated from different outdoor environments around Europe (Snelders *et al.*, 2009; Mortensen *et al.*, 2010).

While the detection of airborne *A. fumigatus* conidia by culturing is an inexpensive and simple method, it comes with a number of disadvantages. Long incubation periods are required and culture plates can easily become overloaded in heavily contaminated areas. A range of different media

Fig. 2 – An SAS Super 180 air sampler showing the 219-hole sieve head plate, through which air is aspirated over mycological agar in a 90 mm Petri dish.

and incubation temperatures also need to be tested, as no single set of culture conditions is suitable for all fungi. Finally, a high level of expertise is required for correct species identification, as several cryptic species have been discovered that are phenotypically similar to *A. fumigatus sensu stricto*, most notably *Aspergillus lentulus* (Hong *et al.*, 2005). As *A. lentulus* is less susceptible to certain antifungal agents than *A. fumigatus* (Staab *et al.*, 2010), it is important that these very closely related species can be differentiated in air sampling studies.

PCR-based methods

The utilisation of molecular methods for the identification of airborne fungal spores is a very attractive alternative to culture-based methods as it should be faster and less labour-intensive, whilst also offering greater sensitivity and specificity (MacNeil *et al.*, 1995). Several systems have now been developed that integrate air sampling with follow-up PCR-based identification. Questions remain as to the usefulness of these methods however, as the amount of DNA sampled cannot be converted into useful spore concentrations (Stetzenbach *et al.*, 2004).

Using *Penicillium roqueforti* spores, Williams *et al.* (2001) compared four methods of preparing spore samples collected by air sampling with three PCR identification methods, but these methods suffered from relatively high detection limits and lacked reproducibility. More recently, Bellanger *et al.* (2010) used quantitative PCR (QPCR) to detect *A. fumigatus* conidia impacted into low-melt agar, with the results available within 48 h of sampling. They simultaneously carried out culture-based air sampling for comparison, and found *A. fumigatus* in twice as many samples by QPCR than by culturing. However, as this system is unable to distinguish between the DNA from viable and non-viable conidia this result was probably an overestimation. It has been shown that propidium monoazide, a membrane-impermeant dye, successfully binds to the DNA in dead cells, inhibiting use of that DNA in any subsequent PCR reactions (Nocker *et al.*, 2007). As it is compatible with QPCR, Vesper *et al.* (2008) were able to use this dye to detect only viable conidia of *A. fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus* in both air and water samples.

Alternative strategies

Vanhee *et al.* (2009a) have developed a novel system combining flow cytometry and epifluorescence microscopy to count viable airborne spores. Air is impacted onto polyvinyl alcohol (PVA) plates and following dissolution, samples of the resulting suspensions are fluorescently labelled. A solid-phase cytometer is then used and the fluorescent spores are distinguished from other particles using specialist software, with the results later verified by microscopy. The technique has since been successfully applied to detect airborne *A. fumigatus* conidia (Vanhee *et al.*, 2009b). It is extremely well-suited for environmental monitoring of airborne microbes as it yields results within a few hours of sampling and has a highly dynamic detection range. It is theoretically capable of detecting one cell per filtered volume up to *ca* 10,000 cells per membrane filter, with the possibility of sample dilution for higher loads (Vanhee *et al.*, 2009a).

3. 'Background' levels *A. fumigatus* conidia in outdoor air

Despite the danger posed by *A. fumigatus* conidia to human health there have been very few year round studies to determine ambient 'background' levels of airborne *A. fumigatus* conidia in either indoor or outdoor environments. While not the dominant species of the airborne mycoflora, *A. fumigatus* is one of the most abundant (Mullins *et al.*, 1976). Lacey (1996) estimated that we are constantly exposed to between 1 and 100 *A. fumigatus* conidia m^{-3} air throughout the year.

Seasonal variation

Many authors have reported strong seasonal variations in *A. fumigatus* spore concentrations, with levels generally increasing during the autumn and winter months. Mullins *et al.* (1976) attributes winter increases of *A. fumigatus* counts to the increased availability of nutrient-rich organic matter from fallen leaves. Hudson (1969) reported a winter rise in *A. fumigatus* counts in his study of aspergilli in the outdoor air of Cambridge. Later in 1973 he again reported a winter rise, with annual *A. fumigatus* concentrations in the range 0.3–13 conidia m^{-3} (Hudson, 1973). In the Washington, DC area *A. fumigatus* concentrations were reported to increase in the summer and autumn, with a median recovery of 1 CFU m^{-3} air (Jones and Cookson, 1983). A 12-m comparative study of the outdoor air in Cardiff, Wales and St Louis, MO, USA, recorded average *A. fumigatus* concentrations of 11.3 conidia m^{-3} air in Cardiff and 13.5 conidia m^{-3} air in St Louis, with the highest concentrations during winter at both sites (Mullins *et al.*, 1984). Solomon *et al.* (1978) studied the outdoor air outside a university hospital in Michigan and found mean and median *A. fumigatus* concentrations of 6.25 CFU m^{-3} air and 1.75 CFU m^{-3} air respectively, however there was only a limited summer increase. Guinea *et al.* (2006) collected air samples from urban and rural environments across the province of Madrid in Spain and found that *A. fumigatus* spore concentrations never exceeded 70 CFU m^{-3} air, with the highest counts in the autumn. A 1-y study at four outdoor locations around Dublin, Ireland reported that while *A. fumigatus* conidia were generally present at concentrations under 10 CFU m^{-3}, sporadic high counts of up to 400 CFU m^{-3} were occasionally measured (O'Gorman and Fuller, 2008). Unusually however, O'Gorman and Fuller (2008) found no clear seasonal distribution of airborne *A. fumigatus* conidia. Similarly, Goodley *et al.* (1994) also found no seasonal trend in *A. fumigatus* concentrations in the air outside a London hospital.

Compost heaps

As it is a thermotolerant fungus (Cooney and Emerson, 1964), *A. fumigatus* is typically found in self-heating compost heaps, where it has an essential role in the degradation of plant material (Tekaia and Latgé, 2005). Colonisation of the compost is typically very high, with Millner *et al.* (1994) reporting 10^6–10^7 CFU *A. fumigatus* per gram of dry weight compost. Large-scale industrial composting releases extremely high, albeit transient, concentrations of *A. fumigatus* conidia (Fig. 3).

Fig. 3 – The release of *A. fumigatus* conidia and other bioaerosols during the grinding of organic waste at an industrial composting facility. Credit: Ezoom/Dreamstime.com.

Agitation activities such as the screening, shredding, and turning of compost release spore concentrations in the range 10^4–10^7 CFU m^{-3} air (Recer *et al.*, 2001; Wheeler *et al.*, 2001).

The handling of large quantities of compost by industrial workers exposes them to very high concentrations of *A. fumigatus* on a daily basis, which typically overloads their immune systems leading to diseases such as ABPA and hypersensitivity pneumonitis (Bünger *et al.*, 2007). The same is also true for workers at landfill and sewage sludge treatment sites (Millner *et al.*, 1980; Lis *et al.*, 2010). However, the potential health risks of such high *A. fumigatus* spore concentrations for immunocompromised individuals living in areas adjacent to composting facilities is not so clear (Sánchez-Monedero and Stentiford, 2003). Based on the work of Wheeler *et al.* (2001), the UK Environment Agency (2009) states that total emissions of bacterial and fungal spores from compost facilities that do not exceed 10^3 CFU m^{-3} are within "acceptable levels". Most studies have revealed that these high concentrations do not to persist in open air, rapidly diminishing with increasing distance from the compost. Taha *et al.* (2006) found that airborne *A. fumigatus* levels generated by turning deplete rapidly to background concentrations after 100 m. The UK Environment Agency recommends a 250 m set-back distance or 'buffer zone' from a composting facility site boundary to the nearest dwelling to remove the potential for increased exposure to *A. fumigatus* conidia (Environment Agency, 2009).

4. Reservoirs of *A. fumigatus* conidia

Haiduven (2009) defines a 'reservoir' as "a place where an infectious agent can survive but may or may not multiply". There are a large number of reservoirs of *A. fumigatus* conidia, many of which are common to both residential and hospital settings. It is important to be aware of all potential reservoirs as the US Centers for Disease Control and Prevention (CDC) recommends that all possible sources of fungal spores should be removed from the environment of high-risk patients (CDC, 2003). Dust is a major reservoir, and large collections of dust harbouring *A. fumigatus* conidia can usually be found in the attics, basements, and infrequently cleaned places of houses. Fresh and dried flowers and the soil of ornamental plants are other common sources (Staib *et al.*, 1978). Certain foods have been shown to harbour *A. fumigatus* conidia, including tea, biscuits, fruits, and spices, particularly pepper (Bouakline *et al.*, 2000; De Bock *et al.*, 1989). The smoking of tobacco and marijuana has also been shown to release large quantities of contaminating *A. fumigatus* conidia (Verweij *et al.*, 2000). Everyday human activities such as gardening (e.g. grass cutting, digging, potting plants, and raking leaves) and household cleaning (e.g. vacuuming, dusting) also aerosolise *A. fumigatus* conidia (Rhame *et al.*, 1984; Millner *et al.*, 1994).

Additional reservoirs of *A. fumigatus* conidia within hospitals include false ceilings and horizontal window blinds that accumulate dust. *A. fumigatus* also readily grows within damp insulation and fireproofing material and on the filters used in hospital air handling systems, which in turn enables further dissemination of its conidia (Barnes and Marr, 2006). Hospital supplies that have been reported as contaminated with *A. fumigatus* conidia include intravenous catheters, intravenous arm boards, and bedding (McCarty *et al.*, 1986; Rowen *et al.*, 1992; van Burik *et al.*, 1998). Dressings such as gauze, elastic bandages, and adhesive tape are also thought to act as direct transmission sources (Granstein *et al.*, 1980). Finally, *A. fumigatus* conidia present in water have been shown to become aerosolized during patient showering, providing yet another source of airborne conidia (Anaissie *et al.*, 2002). The relative impact and importance of these different sources is unknown and most likely varies for each patient, depending on their amount of exposure and severity of immunosuppression.

5. Threshold *A. fumigatus* concentrations

Any increase in airborne *A. fumigatus* concentration increases the risk of aspergillosis infection in immunocompromised patients (Latgé, 1999). This was clearly illustrated in a study by Arnow *et al.* (1991), who showed that mean increases in *A. fumigatus* and *A. flavus* concentrations to >1 CFU m^{-3} air were sufficient to increase the incidence of nosocomial IA from 0.3 % to 1.2 %, a four-fold increase. However, a subsequent sustained reduction in airborne *Aspergillus* concentrations (through active measures) resulted in a decrease in the numbers of new IA cases back to 0.3 %.

Despite this vulnerability, only two sets of guidelines recommend a threshold airborne *A. fumigatus* spore concentration above which the risk for aspergillosis infection increases. This is due in part to the requirement for substantial laboratory support to regularly monitor airborne spore concentrations and the technical limitations of air sampling. These limitations include the absence of standard protocols (e.g. agreement on suitable replication, sampling locations, incubation temperature) and the variability and sensitivity of the air sampler used (CDC, 2003).

In Ireland, the Health Protection Surveillance Centre (HPSC) advises that an intensive evaluation be carried out to identify and remove the source of fungal contamination in hospitals if the total fungal spore count exceeds 1 CFU m^{-3}

air on several occasions (NDSC, 2002). The Spanish Society of Infectious Diseases and Clinical Microbiology states that concentrations of fungal spores under 25 CFU m^{-3} are acceptable in unprotected air, while a limit of 0.5 CFU m^{-3} should be maintained in protected areas (Ruiz-Camps *et al.*, 2011). In the UK, various NHS foundation trusts have brought out guidelines for their hospitals, but these vary substantially in their recommendations. For example, the Tameside Hospital NHS Foundation Trust (2010) does not recommend the use of air sampling, even during building works, stating that control measures are more effective. Conversely, the Royal Liverpool Children's NHS Trust (2004) sanction air sampling and the use of settle plates if deemed necessary by their Consultant Microbiologist. They also carry out routine air sampling during the summer months to monitor levels of *A. fumigatus*. The US CDC (2003) also recommends that air sampling is carried out in hospitals, both periodically to accurately determine indoor air quality and the efficacy of their control measures as well as during periods of building work. While all of these guidelines agree that airborne concentrations of *A. fumigatus* should be kept to a minimum, there is a lack of published data proving a connection between aspergillosis infection rates and airborne *Aspergillus* levels. More research is needed to examine this relationship, particularly long-term surveillance studies of background spore levels, with and without stringent control measures in place.

6. IA outbreaks and building work

There have been many reports of IA outbreaks during periods of building work in and around hospitals (Krasinski *et al.*, 1985; Weems *et al.*, 1987; Loo *et al.*, 1996). This is thought to be because renovation, excavation and construction work is known to contaminate the air with short-term concentrated bursts of *A. fumigatus* conidia (Streifel *et al.*, 1983). Common building activities that disturb *A. fumigatus* reservoirs include the digging and movement of soil during earthworks and the removal of old fireproofing and insulating material during renovations (Haiduven, 2009).

It has not always been possible to connect these outbreaks of IA to increases in airborne *A. fumigatus* levels in the patient's surroundings (VandenBergh *et al.*, 1999). For example, during major construction work inside and outside a London hospital Goodley *et al.* (1994) found no increases in either indoor or outdoor airborne *A. fumigatus* concentrations, nor were there any aspergillosis cases during the period. Pini *et al.* (2003) measured concentrations of *A. fumigatus* in the air of two hospital wards during a period of construction in the same building and reported low concentrations of *A. fumigatus* in the wards, particularly in the rooms. The lack of IA cases during this time was attributed to the low spore counts. Similarly, Cooper *et al.* (2003) found that the rate of nosocomial IA did not increase when a median spore count of 0 CFU m^{-3} air was maintained in the rooms of neutropenic patients during a construction period. Nosocomial IA outbreaks in the United States (Hospenthal *et al.*, 1998) and the Netherlands (Leenders *et al.*, 1999) could also not be explained by increases in airborne *A. fumigatus* concentrations. However, there is large variability in patient exposure levels to airborne concentrations of *A. fumigatus*, due in part to the many 'reservoirs' of *A. fumigatus* conidia, as discussed in Section 4. This was clearly illustrated by Vonberg and Gastmeier (2006), who reviewed 24 nosocomial IA outbreaks and found that *A. fumigatus* concentrations ranged between 0 and 100 CFU m^{-3} air in patient care areas during the outbreaks.

7. Conclusions

While some degree of exposure to the conidia of *A. fumigatus* is unavoidable, hospitals face an increasing need to monitor and control the exposure of their immunosuppressed patients to *A. fumigatus*. The importance of environmental monitoring for airborne *A. fumigatus* conidia should not be underestimated as regular air sampling can determine whether control measures are effective or need to be improved.

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