



Review Article

The role of occupational *Aspergillus* exposure in the development of diseases

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Abstract

Aspergillus spp. have a high nutritional versatility and good growth on a large variety of construction materials. They also colonize soil or food, but decaying vegetation is their primary ecological niche. Therefore, exposure to fungi may occur at home, during hospitalization, during specific leisure activities, or at the workplace. The development of *Aspergillus* infections depends on the interplay between host susceptibility and the organism. Environments with high counts of fungal elements (conidia, hyphal fragments and others), high levels of bioaerosols, and elevated concentrations of mycotoxins or other volatile organic compounds should be considered as potential hazards, since they may present a risk to the exposed person. Rural tasks as well as work related to wood and food industries, poultries, swineries, waste handling plants, and other occupational environments involving contaminated organic material are among the ones posing higher respiratory risks to the workers. This paper presents a review of several studies related to occupational and indoor exposure to *Aspergillus*, potential health effects related to that exposure, and associated exposure assessment procedures.

Key words: *Aspergillus*, indoor exposure, fungal occupational exposure, *Aspergillus*-associated diseases, resistance, exposure assessment.

Introduction

Environments with high levels of fungal particles are found in different indoor and occupational settings, and workers are at increased risk for occupationally acquired respiratory diseases.

Fungal particles can include viable and nonviable spores or conidia, hyphal fragments, extracellular vesicles, and even submicron fragments derived from the fungal cell wall.¹ Mycotoxins and other volatile organic compounds should also be considered

as potential hazards since they can bind to particles and reach workers' respiratory systems. Exposure to environmental fungi is associated with worsening asthma symptoms, lung function, hospital admissions, and asthma-related deaths.

Important pulmonary pathology is connected with *Aspergillus*-induced allergic and asthmatic lung disease related to occupational exposure. Allergic bronchopulmonary aspergillosis (ABPA), rhinosinusitis, rhinitis, and severe asthma with fungal sensitization (SAFS) are among the diseases more frequently associated with occupational exposure to *Aspergillus*. Immunocompromised persons are at higher risk and may develop invasive infections.^{2,3}

Aspergillus conidia can be found almost everywhere and are easily disseminated in the air. The size of the conidia is species-dependent and *Aspergillus* conidia can colonize the entire respiratory tree. *Aspergillus fumigatus* is one of the species most frequently associated with respiratory symptoms due to the small size of the conidia and other virulence factors associated with this species. Nevertheless, *Aspergilli* belonging to sections *Nigri*, *Flavi*, *Circumdati*, and *Versicolores* must be considered in risk assessment of a specific occupational environment, since they include species that produce mycotoxins.⁴

Another issue of concern in specific occupational and indoor settings is the exposure to high levels of azole-resistant *Aspergillus* isolates, which may increase the risk of infections, resulting in difficult clinical management.^{5,6,7}

This paper presents a review of several studies related to *Aspergillus* occupational and indoor exposure, potential health effects related to that exposure and preventive or corrective measures against *Aspergillus* contamination and exposure in occupational environments.

Sources of fungal exposure

Indoor exposure to fungi may occur at home, during hospitalization (especially during construction work at a hospital) or at the workplace. Fungi can proliferate and disseminate within a wide number of occupational environments, where they can also be found in indoor bioaerosols, since spores and mycelium fragments can be present as single particles or complex aggregates, and adhere to dust particles. Counts and species of indoor fungi may also be influenced by outdoor air, depending on the level of ventilation.

Airborne fungal particles found at indoor environments can be inhaled by exposed persons and cause a wide diversity of symptoms.^{1,8} In 2011, a World Health Organization (WHO) guideline documented that occupants of damp indoor environments are at risk for upper and lower respiratory symptoms, onset and exacerbation of asthma, respiratory infections, hypersensitivity pneumonitis, allergic rhinitis, eczema, and bronchitis.⁹

The link between exposure to fungi and diseases is often difficult to prove due to coexposure to other components of

bioaerosols.¹⁰ Workers that handle, disturb, or process organic material such as farmers, sawmill workers, waste collectors, mushroom processing workers, or workers that handle grain, hay or straw are likely to have been exposed to high concentrations of fungal particles; these can be as high as 10^8 colony forming units per m^3 .^{4,11} In addition to agriculture, wood and food industries, poultries and swineries, waste handling plants, and other occupational environments involving contaminated organic material have been described as having high fungal loads.^{4,12–15}

Recently, studies have assessed fungal burden in a variety of occupational environments aiming to achieve the characterization of the risk and, consequently, prevent adverse health effects. However, there is still a lack of information due to the lack of standardized methods (sampling and analyses) for assessing fungal exposure, and the lack of a consensus regarding threshold levels for fungal contaminants in indoor and occupational environments that have not been yet established.

Factors that influence the development of disease after exposure to fungi, environmental and host factors

Occupational and indoor exposure to fungi may occur by different pathways: orally, by dermal contact or puncture with contaminated materials, or by inhalation. This latter route of exposure is the most common in occupational environments. Fungal infections depend on the interplay between host susceptibility and the organism. Therefore, during, and after, exposure to fungi several adverse health effects may occur. Pulmonary exposure may have results varying from mild (runny nose, sneezing, coughing, congestion, sore throat, itchy eyes) to severe (sinus infection, asthma, ABPA, SAFS, rhinosinusitis, hypersensitivity pneumonitis, cancer or even death). The severity of the symptoms will depend on the quality of the environment, which also depends on the fungal load in the air, as well as the presence of certain fungal species or even strains. Other factors such as temperature, humidity, ventilation, and the presence of large amounts of dust and other particles will influence the microbiological quality of an indoor environment since lack of ventilation favors fungal growth and high concentration of spores.

The severity of the adverse health effects on the worker or exposed person will also depend on their immune system, genetic background, and other personal features such as sex, smoking, medication intake, or previous respiratory conditions. Tekai and Latgé¹⁶ emphasized the host and the environmental component as the major disease determinants.

There is limited invasion by aspergilli in immunocompetent individuals, but occupational environments with high numbers of conidia from certain *Aspergillus* species are found often.¹¹ This exposure may present a risk, depending on the health status of the workers. Thus, knowledge of the epidemiology of these

settings may be important given that different fungal species may cause different symptoms during colonization, infection, or sensitization and may show varying susceptibilities to multiple antifungal drugs.

Features of the *Aspergillus* genus that enables its success in occupational and indoor environments

Aspergillus spp. conidia constitute a major part of the airborne mycobiota. Several *Aspergillus* species are able to colonize immunocompromised warm-blooded hosts¹⁷ and the most common route of infection is pulmonary. *Aspergillus fumigatus* is the most frequent species implicated. Conidia of *A. fumigatus*, with a diameter of approximately 1 to 4 μm are deposited in the lower respiratory tract, whereas the larger conidia of other *Aspergillus* species, such as *A. flavus* and *A. niger*, tend to be deposited in the paranasal sinuses and upper airways.¹⁸

The genome of *Aspergillus* has limited coding of virulence factors (capsule, potent toxins).¹⁹ However, several characteristics account for the capability of *Aspergillus* to cause disease, such as its global ubiquity, stress-tolerance biology, namely, the ability to growth at high temperatures, morphology, and versatile physiology.²⁰ The small size and weight of the conidia, the fact of being produced in large numbers, and their surface characteristics (dry wall spores, desiccation-resistant), allow them to be easily dissociated, and dispersed by currents of air or water, and make *Aspergillus* a ubiquitous inhabitant of the ecosphere.²¹

Modern heating systems are designed to remove humidity from buildings, creating opportunities for xerophilic species of section *Aspergillus* (e.g., *A. pseudoglaucus*) to dominate indoor fungal communities.²² *Aspergillus* spp. in the genus has the ability to grow in a high range of temperatures and moisture environments. In 2017, Novak Babič reviewed the reporting of fungal contamination of ground water²³, surface water, tap water and nonmineral bottled water in European countries. Many *Aspergillus* species were found, and *A. fumigatus* was reported in all types of water from nine different countries. *A. fumigatus* conidia easily pass through many kinds of purification systems, due to the particle size and structural features, aided by natural plumbing colonization as complex biofilms. *Aspergillus* spp. have a high nutritional versatility and are able to utilize a wide spectrum of organic compounds and a variety of substances can serve as source of carbon.²⁴ These microorganisms have good growth on a wide variety of construction materials such as concrete, acrylic paints, wood-based and cellulose-based materials. It also colonizes soil or food, but decaying vegetation is its primary ecological niche. Furthermore, inorganic materials can be frequently colonized as they absorb dust and serve as good growth substrates for *Aspergillus fumigatus* and *Aspergillus versicolor*.²⁵

Occupational environments with high levels of *Aspergillus*

A recent study was published aiming to analyze the prevalence of *Aspergillus* spp. in different occupational environments, covering settings with an expected high load of fungal contamination such as wastewater treatment plants, wastewater elevation plants, waste treatment plants (e.g., incineration, composting plant, and waste sorting plants), cork industries, slaughterhouses (i.e., poultry, swine, and bovine), and two animal feeding industries, poultry pavilions, and swineries.⁴ In this study, the waste sorting plants presented the most critical fungal contamination, not only due to the high number of sampling sites that surpassed the WHO guideline (150 cfu/m³)²⁶ but also because all air samples presented a higher fungal load indoor when compared to the outdoor samples, which could mean the existence of foci of indoor fungal growth,^{4,13,27} as well as due to the presence of high amounts of dust particles where the fungal elements may be attached. This may be enhanced by the deposition of waste indoors, and also as a consequence of building materials favoring fungal growth and sporulation, helping fungal development.^{4,13,27} These are also the most critical settings regarding contamination by *Aspergillus* spp., with higher load and *Aspergillus* diversity.⁴ Of note is the increased presence of workers near the waste and in each working site in the waste sorting facilities (higher exposure to fungal contamination) compared to incineration and composting plants, where the presence of workers in the work sites is not so frequent.⁴ A variety of *Aspergillus* sections was found in the studied environments, and there were significant differences in the distribution of the sections among these environments. Since different sections show different health effects and antifungal susceptibilities, these data raise the need for preventive/protective measures to avoid specific exposure of workers and patients.

Mycotoxins in occupational environments

Mycotoxins can resist adverse environmental factors such as high or low temperatures and can persist long after the death and disintegration of the fungal species responsible for their production. This explains why mycotoxins can be present in the environment even in the absence of any visible mould.²⁸

Exposure to mycotoxins can occur by inhalation, ingestion, and dermal contact. However, the most studied exposure route is ingestion, and the effects on human health by this route are well known. The impact of mycotoxin exposure/inhalation (apart from ingestion) on health is controversial and with little evidence. In the cases of inhalation and dermal routes, available studies^{29,30} are still scarce.

As already mentioned, mycotoxins are commonly present in airborne dust³¹ and in the spores and fragments of fungal growth, both of which can act as carriers of mycotoxins to the workers' respiratory system.³² Therefore, exposure in

occupational settings occurs essentially via inhalation, particularly in the form of airborne dust.³³ This can be of relevance since some papers have demonstrated that the inhalation of some mycotoxins can be more harmful than oral exposure.³⁴ Additionally, dermal contact can also be a relevant route of exposure, in the case of workers' needs to handle and manipulate, without gloves, contaminated materials such as food and feed.³⁵

Workplaces with high fungal contamination, and with potentially higher levels of mycotoxins are: the feed/food industry, owing to raw material processing or storage;³⁶ animal husbandry, owing to feed and animal density;⁴ slaughterhouses, owing to contact with contaminated animals;⁴ the waste industry, owing to the permanent availability of nutrients;⁴ and specific food processing plants with matrices easily contaminated by fungi, such as coffee, onions, and others like sugar, grain products, spices and nuts, and corn.^{33,37,38} Additionally, animal feed processing plants may pose a risk to the workers regarding mycotoxin exposure since the authorized level of concentration in this type of commodity is 10 times higher than it is for human food. Another important aspect to understand is that even if the mycotoxin concentration in a product or material is low, handling large amounts of it in a workplace can cause an elevated airborne mycotoxin concentration at a specific moment, which then endures depending on how tasks are developed.³³

In all of the noted settings, exposure to mycotoxins is frequently characterized by co-exposure to several mycotoxins. Therefore, synergic or additive effects should also be taken into account when performing a risk assessment. The analytical methods should allow looking for several mycotoxins in the same sample. This has been performed in biomonitoring approaches because multi-detection is already available.³⁹ Recent studies using these approaches have demonstrated that the exposure scenario is characterized by a co-exposure to several mycotoxins.^{40,41}

Huttunen and Korkalainen³² studied the prevalence of mycotoxins in the indoor air of several occupational environments. The concentration was measured directly from air or from dust samples with an airborne concentration of dust of 15 mg/m³.

Animal housing and food and feed industry were the settings where aflatoxin and ochratoxin A were detected above the concentration of no toxicological concern for mycotoxin exposure.

In a study performed by Viegas et al.⁴² in 34 swine and 13 poultry workers, aflatoxin B1 (AFB1) was detected in the serum of 53% and 56% of them, respectively. Since no information is available regarding the AFB1 background levels for the Portuguese population, a control group was included in this study, and none of them were positive. This suggests that the detectable levels were not due to food consumption but due to inhalation of contaminated particles from these occupational environments.

Diseases associated with *Aspergillus* occupational/indoor exposure

Unlike other allergenic sources (pollens, mites, animal dander), fungi can cause direct infections, either localized or systemic, and toxic-irritant effects due to secondary metabolites, beyond their role as allergens in severe respiratory diseases and hypersensitivity disorders.^{2,3}

There are a variety of lung diseases secondary to *A. fumigatus* and occasionally other species of *Aspergillus* in which the elements of the host response seem primarily to drive clinical manifestations. Three main categories of disease linked to host immunocompetence have been recognized: invasive, saprophytic (chronic) and allergic⁴³ (Table 1).

Allergies and other hypersensitivity responses

The prevalence of fungal sensitization presents a wide geographical variation ranging from 3% to 10% in the general population.^{44,45} Fungal colonization either in the sinuses (allergic fungal sinusitis) or the bronchi (ABPA), as well as hypersensitivity pneumonitis may induce both T- and B-cell responses.⁴⁶

The best described syndrome associated with fungal allergy is allergic bronchopulmonary aspergillosis (ABPA) usually associated with *A. fumigatus*, a ubiquitous indoor and outdoor fungus.

ABPA is an inflammatory disease that classically arises in patients with asthma (up to 13% of asthmatic patients) or cystic fibrosis, as a result of poor clearance of secretions from the airways⁴⁷. However, several reports describe patients who developed ABPA without other associated comorbidities, such as the case of a 29-year-old man, garbage collector, without any respiratory problems, who developed ABPA caused by *A. fumigatus*.⁴⁸ This disease was probably due to the continuous exposure to mouldy household waste. In another case, ABPA was diagnosed in a 19-year-old female who worked in a soy sauce factory and was continuously exposed to massive amounts of *A. oryzae*, since it is the fermentation starter for soy sauce.⁴⁹

ABPA is typically characterized by frequent asthma exacerbations, recurrent transient chest radiographic infiltrates, peripheral blood eosinophilia, and development of bronchiectasis. The presence of pulmonary infiltrates along with eosinophilia distinguishes this condition from the underlying asthma or even cystic fibrosis, but the differential diagnosis includes other syndromes known as PIE (pulmonary infiltrates with eosinophilia) syndromes. Although several clinical diagnostic criteria have been established, serological findings confirming sensitization to *A. fumigatus* are essential to confirm or exclude the disease.⁵⁰ Screening of sensitization to *A. fumigatus* can be performed by skin prick test and/or serum specific immunoglobulin E (IgE), and elevated IgE antibodies against *A. fumigatus* are considered to be the hallmark of ABPA.⁵¹ Furthermore, recombinant

Table 1. Pulmonary diseases caused by *Aspergillus* species (adapted from Moss R. 2009).⁸¹**Invasive:** immunosuppressed host

- Angioinvasive aspergillosis
- Acute bronchopneumonia
- Pseudomembranous necrotizing tracheobronchitis
- Invasive pleural disease

Saprophytic: structurally damaged host (bronchiectasis, cavities, necrotic tissue)

- Aspergilloma (mycetoma)
- Chronic necrotizing aspergillosis
- Aspergillus* bronchitis
- Obstructing Bronchial Aspergillosis⁸²

Allergic: Immunocompetent host

- Asthma
- Allergic bronchopulmonary aspergillosis
- Hypersensitivity pneumonitis
- Bronchocentric granulomatosis
- Eosinophilic pneumonia

allergens may contribute to a more accurate diagnosis.⁴⁶ Immunoglobulin G (IgG) antibody precipitin testing from serum is useful, as positive results are found in between 69% and 90% of patients, though also in 10% of asthmatics with and without SAFS. Therefore, it must be used in conjunction with other tests.⁵² The diagnosis of ABPA does not require positive cultures for *A. fumigatus*, since the presence of serological and radiological findings are enough. Nevertheless, between 40% and 60% of patients do have positive cultures depending on the number of samples taken.⁵² However, multiple fungi can colonize the airways of patient with ABPA, and there is frequently dissociation between the colonizing and the sensitizing species. This should be taken into account in treatment, particularly regarding resistance to antifungals.⁵³

Severe asthma with fungal sensitization (SAFS) is a condition that affects asthma patients and whose development, persistence, and severity of the symptoms are triggered by fungal allergens from *Alternaria alternata*, or *A. fumigatus*, among others. The prevalence of fungal sensitization in adults with severe asthma can reach up to 70%, although it can be as high as 76% in those requiring multiple hospital admissions.⁵⁴

Allergic rhinitis (AR) is an extremely prevalent condition, associated with exposure to inhaled allergens, causing a combination of sneezing, rhinorrhea, nasal congestion, and itching. There is no conclusive evidence of a direct relationship between fungal exposure and clinical manifestations of AR. Allergic fungal rhinosinusitis (AFRS) accounts for 5%–10% of all cases of chronic rhinosinusitis in immunocompetent patients and is caused by fungal colonization of the sinus with impaired mucociliary clearance due to fungal overgrowth, mostly of *Aspergillus* species.⁵⁰ The characteristics of AFRS are similar to ABPA.

AFRS has specific diagnostic criteria that distinguish it from typical chronic sinusitis and include mucus with eosinophils

(demonstrating non-invasive fungi-allergic mucin), skin hypersensitivity to specific fungal allergens, and increased total serum IgE level, nasal polyposis, and characteristic radiographic findings.³ Computed tomography can show complete opacification of at least one paranasal sinus, atypical heterogeneity of the signal within involved sinuses, and the expansion and attenuation/erosion of the bone with displacement of adjacent anatomic compartments.⁵⁵

Another clinical manifestation associated with hypersensitivity responses is hypersensitivity pneumonitis, leading to inflammation of the alveoli within the lung caused by inhalation of large amounts of organic dust and *Aspergillus* conidia and a large variety of antigenic particles.

Sufferers are commonly exposed to the dust by their occupation or hobbies, and the name of the disease may depend on the occupation or activity developed. In the majority of the cases, *A. fumigatus* is the etiological agent of disease. In malt worker's lung disease, *A. clavatus* was identified as the main etiological agent because this species is used during the process of malt fermentation.⁵⁶

Some examples of common names of occupational hypersensitivity pneumonitis due to *Aspergillus* are tobacco grower's lung, cheese washer's lung, composter's lung, potato riddler's lung, or farmers' lung, where workers are exposed to many *Aspergillus* conidia and/or other fungi and actinomycetes.⁵⁶ One other example of hypersensitivity pneumonitis disease is the esparto grass lung (stipatosis). This is an occupational disease with a restricted geographic distribution, located essentially in the South of Portugal and Spain and North of Africa. It is associated with handcraft work where the workers soak and crush the fiber of the plant *Stipa tenacissima*. During that process, the workers inhale high numbers of *A. fumigatus* conidia, leading to a high variety of respiratory symptoms.⁵⁷

A subtype of hypersensitivity pneumonitis is extrinsic allergic alveolitis. Hypersensitivity pneumonitis results in repeated episodes of influenza-like symptoms, characterized by low IgE levels and few eosinophils, a Th1 response, the appearance of neutrophils in the lung early, and T cells and macrophages late, and results in a restrictive lung disease. Extrinsic allergic alveolitis results in symptoms in 4–24 hours after exposure, the presence of IgG and cellular immunity directed at *Aspergillus*, the presence of granulomas in the lung, and the absence of airway resistance. Another manifestation of allergy to *Aspergillus* is bronchocentric granulomatosis, characterized by asthma or just wheezing. There are two types of bronchocentric granulomatosis: type I, associated with eosinophilia, and seen in the young, and type II, associated with neutrophilic infiltration, and seen in the elderly. Finally, another type of allergy is mucoid impaction of the bronchi, which is a chronic condition, associated with asthma, and resulting in distal bronchiectasis.

Irritant effects caused by mould exposure

In addition to conidia and mycotoxins, there are other substances such as microbial volatile organic compounds, other fungal particles and even submicron fragments derived from the fungal cell and their wall components (such as glucans or chitins) that can be associated with a large diversity of symptoms, such as nasal congestion, airway hyper-reactivity, upper and lower respiratory symptoms, eye irritations, headache, fatigue/tiredness, joint pains, skin symptoms, flu-like symptoms, nausea, and gastrointestinal symptoms.³ There are, however, contradictory studies: some show association of those symptoms with exposure to the referred fungal elements or substances, and others show that there is no association between symptoms and exposure.

Opportunistic infections: nosocomial / community acquired aspergillosis

Invasive aspergillosis is an important cause of mortality in patients undergoing hematopoietic stem cell transplants and is also an important cause of opportunistic respiratory and disseminated infections in other types of immunocompromised patients.⁵⁸ Globally, it is estimated that more than 30 million patients are at risk of developing invasive aspergillosis due to the use of corticosteroids and other immunosuppressive therapies and that about 300000 develop the disease annually. Its diagnosis is difficult and the treatment, often late, is only partially effective. The associated mortality is 50% in the treated infection and >99% in the untreated infection.⁵⁹

Invasive aspergillosis may be acquired in hospital environments due to breaks in water or ventilation systems, construction work, or by contaminated objects or foods. This issue will be detailed further (Indoor exposure to *Aspergillus* in hospital environment section). The community acquired aspergillosis,

however, may occur when immunocompromised patients are discharged from hospital and are further exposed to high *Aspergillus* burden at their homes or during leisure or working activities.⁶⁰ Therefore, the risk of acquiring aspergillosis at work exists and during this period the risks have to be minimized at work but also at home, avoiding mouldy and dusty environments and also avoiding activities that may lead to the exposure to elevated concentrations of conidia such as gardening or composting. The usage of protective equipment is also a major recommendation for these patients. Although community-acquired aspergillosis may be frequently associated with immunocompromised subjects, there are reports of invasive aspergillosis in immunocompetent individuals due to occupational exposure to elevated amounts of *Aspergillus* conidia. That was the case of a gardener who inhaled elevated concentrations of conidia during the management of *Aspergillus* contaminated compost⁶¹ and of a plumber who was exposed to contaminated muddy water.⁶²

Exposure to *Aspergillus* in hospital environment

In a hospital environment, fungal infections may occur through direct or indirect contact with contaminated surfaces or objects, through the hands of health care workers and by ingestion or inhalation of contaminated particles or bioaerosols.⁶³ Moisture damages and related microbial growth is a common problem in all types of buildings, hospitals, and other healthcare facilities being no exception. In healthcare facilities, moisture and related mould growth may pose a health risk not only to patients but also to the workers. Husman et al.⁶⁴ described the occurrence of allergies and several respiratory symptoms, as well as elevated fungal IgG, among hospital workers.

Aspergillus conidia are found frequently in hospital environment namely in air, surfaces and water and ventilation systems. Sabino et al. carried out a study in a central hospital in order to perform the characterization of the hospital environment in regard to the predominant fungal species but also to perform a directed search of isolates belonging to *Aspergillus* genus.^{65,66} According to the data obtained, from 101 air and 99 surfaces samples, *Aspergillus* was the most frequently recovered fungal genus (20%), followed by *Cladosporium* (19%), and *Penicillium* (17%). Among the 75 *Aspergillus* isolates analyzed, a high percentage of cryptic species (i.e., not *sensu stricto*) was found (59%). When susceptibility testing was performed on these hospital environmental cryptic species, those belonging to the sections *Nigri*, *Circumdati* and *Versicolores* showed low susceptibility to the antifungals tested (amphotericin B, itraconazole, voriconazole, posaconazole). Although aspergillosis caused by cryptic species remain less frequent than infections caused by “*sensu stricto*” isolates, the high prevalence of cryptic species in the hospital environment may change the “usual” antifungal susceptibility profile, increasing the number of resistant strains.

Exposure to *Aspergillus* antifungal resistant isolates

A primary (intrinsic) resistance is found naturally among certain *Aspergillus* species without prior exposure to the drug. These species have coded in their genome molecular mechanisms that enable them to grow in presence of those antifungals. An example is *A. fumigatus*, which shows resistance to fluconazole or of some *Aspergillus* cryptic species such as *A. calidoustus*, showing resistance to the triazoles. A secondary resistance, also defined as acquired resistance, arises when initially susceptible microorganisms develop resistance after exposure of the organism to the drug. Across all of Europe, the United States, Asian countries, and Latin America, studies indicate that azole-resistant *Aspergillus fumigatus* isolates have been found in different environments.^{5,6} The causes may be associated with the release of antifungals from agriculture, animal feeding, aquaculture, and also from hospital sources.^{6,7} In agriculture, for example, triazole pesticides (also called DMI-sterol demethylation inhibitors) are used for crop protection and to preserve materials from fungal decay.⁷ They have a very similar chemical structure to the ones used in clinical practice. Therefore, there are concerns that azole resistance could become a global public health threat, since fungal spores can disperse easily by circulating air flows from crops and inhaled.⁷

The exposure to specific environmental settings with high *Aspergillus* burden together with species (or strains) less susceptible to the antifungals will enhance the chances of patients/exposed workers acquiring infections difficult to treat. In a study performed by Sabino et al.,⁶⁸ the antifungal susceptibilities of clinical and environmental *Aspergillus* isolates were compared. The environmental sources selected were based on the criteria of probable higher *Aspergillus* contamination or level of exposure (poulties, swineries, beach sand) but also based on the relevance to human health (hospital environment). Clear differences among the susceptibilities of clinical and environmental isolates were observed. Furthermore, four isolates with minimum inhibitory concentrations higher than 8 µg/ml to both itraconazole and posaconazole were found. Curiously, those isolates came from different settings (one isolate from beach sand, one isolate from poultry, and two isolates from swineries). None of those resistant isolates were *A. fumigatus*, and they belonged to sections *Versicolores*, *Nidulantes*, and *Aspergillus*. Some species from these sections may be associated with human infection⁶⁹ and the obtained data raise the need for awareness to the resistant *Aspergillus* isolates, especially those from environmental sources.

Aspergillus occupational exposure assessment

As in any other bioburden assessment, sampling to assess occupational exposure to *Aspergillus* spp. is performed to achieve different goals, such as verifying and quantifying the presence of airborne *Aspergillus* particles or material contamination, identifying contamination sources, monitoring the effectiveness

of corrective or control measures, and assessing human exposure.⁷⁰ Exposure assessment to microorganisms, however, and specifically to *Aspergillus*, remains a challenge to every exposure assessor.⁷¹ Occupational exposure to *Aspergillus* burden can be estimated using a variety of different sampling and analyses approaches despite each occupational environment being unique and requiring adjustments in methodology.^{4,72} To overcome these difficulties, different active and passive methods for sampling should be employed, which results in a more accurate occupational exposure assessment to *Aspergillus*.^{70,71} Active air sampling techniques of impaction, impinger and filtration have been extensively reported in the literature.^{70,73} Passive methods (surface swabs, electrostatic dust cloth, litter, feed, raw materials, etc.), adapted to the setting to be assessed, should also be used. Passive methods allow the collection for a longer period of time (weeks to several months), while air samples can only reflect the load over a shorter period of time (usually minutes).⁷⁴ Besides the sampling methods, different strategies also can be applied in culture based-methods or molecular tools. As such, the use of different media to achieve *Aspergillus* burden characterization result in a wider assessment scenario of this risk factor.^{75,76} Although quantitative polymerase chain reaction (qPCR)-based methods overcome the culture based-methods limitations, such as the ability of a given fungal species to be cultivated on nutrient media, it does not differentiate viable from the non-viable particles; this is not very suitable when microorganisms, such as *Aspergillus* spp., are studied in assessment of occupational environment.⁷⁷ Thus, it is increasingly accepted that culture-based and culture-independent methods are complementary,^{77,78} which is also true for *Aspergillus* burden in occupational exposure assessments. Toxigenic isolates belonging to specific *Aspergillus* taxonomic sections (i.e., *Flavi*, *Nigri*, *Circumdati*, and *Versicolores*), and *Aspergillus* section *Fumigati* should be targeted with molecular tools to follow the surveillance recommendations from several occupational health entities,^{26,79} as they are frequently associated with respiratory symptoms.

There is a need for strategies to avoid occupational *Aspergillus* exposure. This may include engineering, sampling and control, as well as personal protective equipment. As suggested in a previous study, where *Aspergillus* burden was assessed in several settings,⁴ a common protocol providing guidance for occupational exposure purposes, to help industrial hygienists to achieve a proper assessment of *Aspergillus* spp. exposure in highly contaminated settings, should be widely implemented. The same study suggested a protocol that was revised and improved in further studies,^{71,74} covering the sampling and analyses methods to be adopted.

Several features should be highlighted in this protocol: a multi-approach in the sampling methods will enrich data findings, enabling a more refined risk characterization;⁷⁰ the use concurrently of culture-based methods to obtain information about the infection potential of the fungal burden present;

comparing quantitative information with guidelines are crucial; and molecular tools to target specific species indicators of harmful fungal burden and to overcome some culture-based methods constraints.⁷¹

Studies that have assessed occupational environments have predominantly used culture based-methods of assessment, including culture or microscopic identification of airborne spores. In addition, results of preliminary culture-independent analyses suggest that fungal identification may be underestimated using culture based-methods, and the results of next-generation sequencing (NGS) surveys possibly detect additional fungi that could influence the respiratory health of a worker.⁸⁰ However, we cannot neglect the fact that NGS is a qualitative method, and to get a better understanding of the mycobiota exposure, the concentration must be assessed using culture-based methods and molecular biology methods such as qPCR.¹³ In fact, all molecular methods also have disadvantages such as, for example, the effectiveness of DNA extraction. The use of culture together with molecular approaches seems to be the best way to overcome those limitations.

Implementation of these methodologies in exposure assessment and occupational health studies will generate data that can be used to derive occupational exposure limits (OEL) and identify the most suitable indicators of harmful fungal burden in each occupational environment.

Recognizing possible exposure in indoor and occupational settings is fundamental because it will allow appropriate health interventions such as the implementation of exposure monitoring programs, the application of suitable preventive and protective measures, and the implementation of an adequate health surveillance programs for workers who are potentially exposed.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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