JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2013. Vol. **7**(1), p. 577-581

Increasing Peril of Pathogenic *Aspergillus* in Indoor Air

Subramanian Babu[1], Thangavelu Thayumanavan[2], Rathinasamy Subashkumar[3], S.V. Satish[3] and Govindhasamy Vivekanandhan[3]

[1]School of Bio Sciences and Technology, VIT University, Vellore - 632 014, India.
[2]School of Biotechnology, Dr.G.R.Damodaran College of Science, Coimbatore - 641 014, India.
[3]Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore - 641 029, India.

(Received: 05 July 2012; accepted: 05 August 2012)

Growing evidences indicate that fungi like *Aspergillus* in indoor environment can pose serious threat to public health. Many professions and lifestyles like closed-room living, expose healthy individuals to pathogenic *Aspergillus*, resulting invasive infection in some of them, which may be life-threatening. Inhalation can show the way to invasive aspergillosis in immuno-compromised individuals and respiratory allergy in healthy ones. Although we have been hindered by profound ignorance of the biology of these important environmental contaminants, evidence for indoor mould exposure and exacerbations of respiratory diseases is strong. These evidences pressurize us to typify the pathogenic species in the indoor air by molecular methods and to redesign our hygienic strategies which so far were targeting mostly the pathogenic bacteria.

Key words: Allergy, *Aspergillus*, Aspergillosis, Indoor air, Invasive, Molecular typin.

With modernization and lifestyle advancement in urban human habitats, a sizeable number of people live or stay in air-conditioned environments due to professional or personal reasons. Sick building syndrome like headache, fatigue, irritation of eye, nose, throat, dizziness and nausea due to inadequate fresh air intake is not uncommon in closed air-conditioned environments. The indoor air quality is influenced by presence of abiotic as well as biotic agents like dust particles and microbes including fungi, bacteria, viruses, mites etc. The indoor air quality thus may be a public health issue, since the health of people who live or work in these environments is affected. The microbes present may be allergenic, toxic or pathogenic. Immuno-compromised people could be at risk since they may be easily infected even by non-pathogenic organisms.

Risk of *Aspergillus* infection

Fungal spores are the major components of the respirable atmosphere throughout the world, both outdoors and indoors. Indoor spores come from either outdoor spore invasion or a specific indoor organic source. Fungi are cosmopolitan in distribution and are a serious threat to public health in indoor environments. Many fungal species that are reported to cause allergy belong to Ascomycotina, Basidiomycotina or anamorphic fungi. Although most fungi flourish optimally at high humidity, a few are actually xerophilic. Most grow optimally at 20-40ºC, and there is a wide range of specific host requirements.

Opportunistically pathogenic invasive fungal infections either systemic or sub-cutaneous mycoses, are major causes of morbidity and

* To whom all correspondence should be addressed.
Mob.: +91-9790166084
E-mail: babu.s@vit.ac.in

mortality in immuno-compromised human. However, invasive fungal infections may affect persons with intact immune system also[1]. The general public is at the risk of invasive fungal infections especially as a result of specific environmental exposures and lifestyle practices. Many professions and modern hobbies increase the exposure of humans to fungi, making specific population groups of people more vulnerable than others to fungal infections. Variety of contemporary lifestyle practices, not just only stay at air-conditioned environment but also outdoor leisure activities are believed to be associated with risk of fungal infections. Ever increasing numbers of travelers into and out of areas endemic for fungi have increased the incidence of fungal infections in non-endemic areas. Many professions, lifestyles like living in air-conditioned closed environments, poor ventilated constructions, modern buildings, hobbies etc expose healthy individuals specifically to pathogenic *Aspergillus*, resulting in invasive infections in some of them, which may be life-threatening. *Aspergillus* has been recovered from numerous environmental sources and may continue to be present as long as there are activities which disrupt it from its reservoir. The portal of entry in the majority of reported cases has been through the pulmonary tract, by inhalation of conidia. The main environmental sources included unfiltered outside air entering indoor environment, windows, backflow of contaminated air and moist environments (plumbing, leaks, rain water, air conditioning condensate etc.). And it has been reported that air conditioned closed constructions do not have much air flow and hence once contaminated, the fungi multiply and spores float permanently in the indoor air.

Aspergillus is capable of growth at 37ºC and can germinate and colonize the sinuses and airways unlike other fungi. *A. fumigatus* is best explored and over 60 allergens are reported from this species with a wide range of biological effects. Proteases may be important in fungal antigen penetration of the airway mucosa but could also have a broader role in permitting sensitization to other non-protease environmental allergens. Non allergic mechanisms could also act in parallel or independently as glucan is a pro-inflammatory component of fungal cell walls and fungi also produce a range of volatile mycotoxins, the importance of which we do not know with respect to respiratory disease.

Health complications due to *Aspergillus* have occurred in association with environmental disturbances including but not limited to: hospital construction, maintenance, demolition and renovation; contaminated fireproofing materials; air filters in ventilation systems, and via contaminated carpeting. In outdoor air, the concentration of fungal spores may vary considerably, depending on climate, time of day, season and site. In the closed environment, especially where there is dampness and condensation, growing fungi exploit the microclimate in ecological niches of our buildings and feed on a variety of substrates. Dry outdoor weather or freezing weather can damage fungi and reduce the spore counts on outdoor samples, but the conditions indoors may be very hospitable to fungal growth non-seasonally. Such an indoor environment, with the potential of prolonged and continuous exposure of sensitive individuals to the airborne allergic fungal spores, increases the risk of making these respiratory diseases more severe.

The bulk of literature about invasive aspergillosis involves patients with risk factors for the disease, such as prolonged neutropenia and hematopoietic stem cell transplantation. However, a broad group of patients who are admitted to intensive care units (ICUs) may also be susceptible to these infections. *Aspergillus* were also isolated from hospital environments including intensive care units (Babu and Tewari, unpublished data). *Aspergillus*-related diseases are associated with a spectrum of disorders of immunity. Allergic forms of aspergillosis, such as allergic bronchopulmonary aspergillosis, result from a poorly controlled inflammatory response to hyphae colonizing the sinopulmonary tract[2].

Health complications of *Aspergillus* infection

Invasive aspergillosis, is typically a disease of highly immuno-compromised persons and is a leading cause of infection-related death in patients with acute leukemia and recipients of allogeneic hematopoietic stem-cell transplants. There is also growing appreciation of invasive pulmonary aspergillosis in patients without classic risk factors, such as critically ill patients without documented immunodeficiency. Acute invasive aspergillosis is a rapidly progressive, frequently fatal disease that occurs in highly immuno-compromised persons. In contrast, chronic forms of pulmonary aspergillosis typically occur in patients without severe immune impairment, progress over months to years, and require prolonged antifungal therapy[3]. Aspergilloma is a fungal mass that develops in a preexisting lung cavity. Invasive aspergillosis principally involves the sino-pulmonary tranct, reflecting that inhalation is the most common route of entry of *Aspergillus* spores; other entry sites such as the gastrointestinal tract and skin occur on rare occasions. Fever, cough, and dyspnea are frequent although nonspecific findings of pulmonary aspergillosis; the lung is the most common site of invasive aspergillosis. Involvement of central nervous system is a devastating consequence of disseminated aspergillosis and may be manifested by seizures or focal neurologic signs from mass effect or stroke. *Aspergillus* species that colonize the airways in end-stage lung disease may be a source of fungal infection after single lung transplantation.

Fungi are increasingly being recognized as important inhalant allergens also. Among the fungi, *Aspergillus* is linked to asthma in more ways than one. Its spores are inhaled by one and all but in the healthy normal individual, they seldom have any effect due to the immune-scavenging functions of cells like macrophages in the mucus of respiratory tract. However, in asthmatic subjects, the fungal spores are trapped in the thick and viscid secretions that are usually present in the airways. *Aspergillus* have a spore size (~5um) within the respirable range, unlike other fungi which have much larger spores. This supports the observations that *Aspergillu*s are major biotic agents among the indoor mould allergens. The clinical spectrum of *Aspergillus*-associated hypersensitivity respiratory disorders include *Aspergillus* induced asthma, allergic bronchopulmonary aspergillosis and allergic *Aspergillus* sinusitis[4]. *Aspergillus* induced asthma is slowly receiving the recognition that it deserves. The association between the mould *Aspergillus* and asthma makes it imperative to know the frequency of sensitization to *Aspergillus* in asthmatic subjects in each geographical region.

Diversity typing of indoor *Aspergillus*

The true extent of microbial diversity in indoor environments still remain underestimated. Genomic technologies have brought considerable advances to microbiology, allowing the detailed analysis of populations and complex microbial environments, as well as the comparison of multiple genomes. Air borne metagenome studies performed in indoor environments show extensive number of new bacterial and fungal types but the overall microbial diversity of air borne samples may be far below the estimated diversity for soil and water samples. Micro-variation events seem very frequent in indoor environments and *A. fumigatus* air borne populations tend to evolve fast with a large amount of new genotypes closely related to the ones typed before.

Remarkably, we still do not understand what actually constitutes airborne mould in the indoor environment. The public and the physicians consider indoor *Aspergillus* as that black rind in the shower, or where there is water penetration onto walls and ceiling often in the low income households. However, there may be other important sources of *Aspergillus*. Direct inhalation from bedding may be an important exposure in the context of allergy or infection in, for example, immuno-suppressed patients. Air conditioned rooms could be reservoirs of fungi and may cause allergic problems or infections in healthy or immuno-compromised individuals living in these environments. Most of the A/Cs are shut off for 6-12 h per day. This may add to relative humidity and may favour fungal growth. A/C filters were rarely cleaned, thus they may act as reservoirs of fungi. There may also be source materials such as used papers, used carpets, window screens, couches etc.

Guidelines for the concentration of indoor moulds have been published by a number of governmental and nonpublic entities world wide, but to date, none of these guidelines are based on scientific data regarding the effects on human health or any specific disease[5]. To reduce the exposure to *Aspergillus* from environmental factors the major interventions could be protective environment and infection control risk assessment. New techniques of risk assessment of indoor air pollution by fungi include exposure assessment using nasal samplers and silent ion charged plates together with multiplex technology for measuring multiple allergens simultaneously[6]. Infection control risk assessment is a multidisciplinary process focusing on reducing risk from infection throughout facility planning, design, and construction (including renovation) activities. It considers the environment, infectious agents, human factors and the impact on human health in this process.

Despite the presence of thousands of genotypes from the environment potentially inhaled by human, only one or two strains are reported with invasive and or disseminated infections. It leads to the interpretation that, infection is random and the strains that predominate are the first to grow at a time when the patient is the most susceptible. Based on the previous reports on the typing of environmental and clinical strains of *Aspergillus* which doesn't show any clustering, it is obvious that every environmental strain should be considered a potential pathogen for any appropriate host at risk of infection. If every isolate of *Aspergillus* is potentially pathogenic, then detection of one or two genotypes per patient would more likely reflect the competition among isolates for infection or colonization at one point in time, rather than the existence of isolates with different levels of virulence. Strain typing of *Aspergillus* has many potential applications such as outbreak analysis and environmental monitoring, patient monitoring and treatment follow-up, local and global epidemiology, database construction, strain identification and many more.

Molecular tools in assessment of pathogenicity

Traditional methods of fungal identification include culture and microscopic analysis. However, these methods are laborious, time-consuming and require a competent mycological expertise. In addition, some fungal species are non culturable or are unable to produce classical structures under laboratory conditions that are necessary for identification. To circumvent the cultivation limits, several molecular techniques have been proposed: specific assays using single-step PCR, nested PCR and PCR followed by Southern blotting and probing, ribosomal DNA (rRNA gene) sequencing, rRNA gene restriction analysis. Techniques based on mass spectrometry and flow cytometry have also been described. Molecular methods currently used to study microbial communities include broad-range PCR, using primers that target highly-conserved regions of genes allowing the simultaneous amplification of DNA from large groups of microbes present in one sample in a single-step. The different amplicons are then subsequently separated with sequence specific separation tools such as single strand conformation polymorphism, temperature gradient gel electrophoresis, temporal temperature gradient gel electrophoresis and denaturing gradient gel electrophoresis[7]. Very many methods have been developed for *Aspergillus* strain typing but mostly directed towards *A. fumigatus*. The techniques include PCR based polymorphism such as microsatellite length polymorphisms (MLP), short tandem repeats (STR) and amplified fragment length polymorphism (AFLP) analysis, based on non-coding repetitive sequences in combination with restriction fragment length polymorphisms (RFLP). In addition, multilocus sequence typing (MLST), coding tandem repeats and retrotransposan insertion-site context (RISC) typing have also been reported. In the post genomics era, the availability of whole genome sequences of *Aspergillus* has had a great impact on our options to develop novel and state-of-art molecular typing methods. Highly targeted approaches can be developed for typing *Aspergillus* at molecular with higher chances of being successful than before.

Air sampling needs standardization since the fungal spore concentration may vary with time and human activity. Poor recovery of colony forming units has been observed by many air sampling experiments so far. It would be useful if the initial stage of an environmental survey were to include the ability to quickly screen and roughly estimate fungi in the environment, because this would allow one to rapidly determine the seriousness of fungal infestation. PCR techniques based on rRNA genes have been used widely in developing sensitive systems for the detection of micro-organisms. Although some universal primers targeted to the small ribosomal subunit gene have been described, these systems are not suitable for environmental monitoring because of cross amplification and or lack of coverage of common indoor fungi. A convenient fungus-specific detection system which used PCR to amplify DNA from the common fungi without any cross-amplification with DNA from bacteria and other eukaryotes like pollen in the indoor air. Increasing reports on the misidentified isolates, clearly indicate the need for more accurate and accessible methods to identify a fungal isolate at species level. In the light of available genomic data, targeted approaches allow strain typing methods to be directed at specific parts of fungal genome.

CONCLUSION

Environmental monitoring of microorganisms to detect potential sources of pathogens for preventive public health and epidemiological purposes does require a rapid, sensitive and reliable approach which can detect pathogenic microbes whether they are culturable or non-culturable. It is important to know the molecular diversity and pathogenicity of fungi present in the indoor air and their effects so that public health strategies can be developed. Future epidemiological and clinical studies should address these issues, as the allergic *Aspergillus* represent an increasing saddle on public health.

REFERENCES

1. Sipsas, N.V., Kontoyiannis, D.P. Occupation, lifestyle, diet and invasive fungal infections. *Infection,* 2008; **36**: 515-525.
2. Segal, B.H. Aspergillosis. *N. Engl. J. Med.,* 2009*;* **360**: 1870-1884.
3. Sambatakou, H., Dupont, B., Lode, H., Denning, D.W. Voriconazole treatment for subacute invasive and chronic pulmonary aspergillosis. *Am. J. Med.,* 2006;6: **119**: 527.
4. Shah, A. Allergic bronchopulmonary aspergillosis. *Indian J. Chest. Dis. Allied Sci.,* 1998; **40**: 41-54.
5. Terr, A.I. Are indoor molds causing a new disease? *J. Allergy Clin. Immunol.,* 2004; **113**: 221-226.
6. Chapman, M.D. Challenges associated with indoor moulds: health effects, immune response and exposure assessment. *Med. Mycol.,* 2006; **44**: S29-32.
7. Nieguitsila, A., Deville, M., Jamal, T., Halos, L., Berthelemy, M., Chermette, R., Latouche, S., Arne, P., Guillot, J. Evaluation of fungal aerosols using temporal temperature gradient electrophoresis and comparison with culture. *J. Microbiol. Methods*, 2007*;***70**: 86-95.