Aeromycology: An Important and Modern Tool to Recognise the Occupational Human Health Hazards

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Perspective:

In the last decades, much interest has also grown for the fungi present in indoor environments, since exposure to airborne biological agents in both the occupational and residential environments could be associated with a wide range of adverse health effects with major public health impact, including infectious diseases, acute toxic effects, allergies and cancer[4,5]. In most situations exposure occurs to complex mixtures of toxins and allergens (and chemicals) and a wide range of potential health effects have to be considered. Three major groups of diseases associated with bioaerosol exposure can be distinguished: 'infectious diseases', 'respiratory diseases' and 'cancer'. Infectious and respiratory diseases are most common; however, valid incidence or prevalence data for most diseases caused by biological agents are lacking [5,9,10]. In recent years, public attention has become increasingly focused on the very real problem of mould inside both homes and workplaces and on the very real dangers to human health posed by such mould exposure. Indoor mould is also a common cause of life threatening systemic infections in immune-compromised patients [1-6].

Almost all the citizens in an urban environment spend over 80% of weekdays inside the closed buildings or in workplaces. School children's spend their time in closed building about 6-7 hours day, college students spend 7-8 hours day, full time job workers spend 7-9 hours day, corporate spend maximum time 10-14 hours day, together with more recent indoor leisure activities such as shopping malls, cinemas, restaurants and children's play centers, result in much less time spent in open air. Moreover, fresh air has traditionally been considered more polluted and of worse quality than the air we breathe indoors [1,2,7-9]. Author has been monitoring airborne fungi in indoor and outdoor environments in the city of Nagpur for the last 22 years intermittently. The data generated from 22 years has been "useful first-hand information" for the general physicians and specialists in occupational health hazards and also to individuals who are suffering from respiratory ailments including allergy sufferers, asthmatics', who experience more frequent crises and more aggressive symptoms, but also people who are not generally allergy sufferers, but who at times complain of sporadic discomfort.

However, most adverse reactions take place during working hours/time in the work place. What is happening? Are we developing an occupational allergy? – Occupational allergy is any kind of clinical or physic-pathological event due to hypersensitivity promoted by an agent in the work place. About 100,000 fungal species have already been identified; in fact, fungi are estimated to comprise an astounding 25% of the world's biomass. The most common indoor fungi generally reported are *Aspergillus, Cladosporium, Curvularia, Alternaria, Trichoderma, Mucor, Rhizopus, yeasts, Fusarium & Penicillium* species are often being found indoors as

well. Number of environmental parameters i.e. external/internal temperature, relative humidity and the rainfall involved in symptom worsening or the appearance of symptoms in atopic and non-atopic individuals.

This article presents the analysis of the indoor and outdoor prevalence/frequency of airborne fungi and other biological particles reported from various intramural studies conducted from last 22 years. On the basis of this study base conclusion, Aeromycology is a good tool to recognize the moulds in indoor/outdoor occupational, non-occupational and industrial environments to diagnose and treat the various types of occupational human health hazards.

AN INTERACTIVE TOOL FOR THE IDENTIFICATION OF AIRBORNE FUNGI:

Much interest has also grown for the fungi present in indoor environments, since exposure to airborne biological agents in both the occupational and residential environments could be associated with a wide range of adverse health effects with major public health impact, including infectious diseases, acute toxic effects, allergies and cancer. An interactive identification tool was created [3] for airborne micro fungi at the genus and/or species level, based on morphological and physiological data, using the software FRIDA. The database comes with a set of detailed descriptions of each genus and species, a rich archive of images, a glossary of the most frequent mycological terms, and references to descriptions; in addition, culture condition requirements for identification are provided.

Although airborne fungi, producing toxins or which cause health hazards, are ubiquitous and belong to the common contamination flora, their recognition is hampered by an incomplete and often confusing literature [1]. Besides, the still poor understanding of many taxonomical groups and the high degree of pleiomorphism in response to environmental changes call for endless floristic, taxonomic and nomenclatural updating. Moreover, in most of the available books for the identification of fungi the layout follows a hierarchic approach mainly based on classification; hence this approach requires a deep theoretical and practical knowledge of mycology. Even more than for other organisms, therefore, for fungi computer-aided systems are important for the handling of data useful in their identification and as flexible as possible, not necessarily founded on traditional systematic criteria.

Martellos[3] created an interactive tool for the identification of food- and airborne micro fungi at a genus and/or species level. This computer-aided tool can provide access to and simplify the study of fungi by various kinds of users: mycologists, but also those concerned with environmental hygiene (i.e. microbiologists employed in food or pharmaceutical industries), those seeking to create interactive floras, those concerned with the management, planning and conservation of natural resources, and teachers at each educational level.

1. Being printed on paper, their content is frozen and hence nomenclatural taxonomic changes and the discovery of new species render them rapidly

outdated. Computerized systems, on the contrary, can be updated and corrected in real time.

2. Traditional keys are rigid. They contain a huge amount of information which is fixed into the format and the logical structure selected by the author. Computerized tools permit to reduce the set of organisms using different combinations of morphological, physiological, ecological, distributional characters .e. special habitats, mycotoxin production and physiological features (temperature, water activity, pH…).
3. Databases are accumulative. A small database can be the starting point for future expansions.
4. Outputs can be edited in several different formats, from simple texts to illustrated books. In conclusion, our key, especially if integrated with existing systems based on physiological and molecular criteria, could promote the identification of this important group of organisms even by unskilled persons who lack specific mycological expertise.

Bioaerosol sampling and theory

Indoor/Outdoor Aero mycology

- Outdoor spores enter readily
- Many fungi can also amplify indoors – anytime moisture is available fungi can grow on many
- indoor substrates
- Many indoor areas similar to natural environment (i.e. carpet dust similar to soil)
- Cladosporium, Penicillium, Aspergillus, are the most common genera indoors
- Recent concern with fungi that can form mycotoxins – Stachybotrys, Aspergillus spp.

Air Sampling Collection Methods and Analytical Methods Are Not Readily Interchangeable

- The choice of sampling method is often wrongly governed entirely by convenience. Instead, sampling method should be selected to suit the hypothesis.
- Sampling method:
- Determines what is collected
- Dictates analytical method
- Analysis method:
- Influences sample characterization
- Enumeration
- Identification

Bioaerosol Samplers

- Wide variety of bioaerosol sampling instruments available
- New methods and instruments are continually being developed
- No single method appropriate for all Bioaerosols

Analytic Methods

1. Microscopy
2. Culture
3. Biochemistry
4. Molecular Biology

1. Microscopy

- Identification of total spores (both culturable and non-culturable) along with
- pollen and other particles
- Identification to species-level is usually not possible
- Identification of morphologically similar spores not possible

2. Culture

- Only culturable bacteria and fungi
- "Culturable" means both "viable" (alive) and able to grow on the medium provided
- Excludes taxa that cannot grow on the medium provided or under the particular
- incubation conditions
- Permits species-level identification

3. Biochemistry

- Detection of specific compounds
- May not be specific for a genus or species
- Assay examples:
  - Ergosterol (unique fungal membrane sterol)
  - beta-(1,3)-D-glucan
  - Mycotoxins
  - Allergens

4. Molecular Biology

- Detection of specific genetic elements
- Highly specific and sensitive
- Currently restricted to a few organisms more about these methods later, but first:

Principal Collection Methods

- Gravity
- Impaction
- Filtration
- Impingement
- Cyclonic separation

Gravity

- Simplest but least accurate method of collecting airborne biological samples
- Coated microscope slide or open agar-filled Petri dish exposed to air
- Non-volumetric, cannot be used to determine atmospheric concentrations
- Affected by particle size, morphology and air movement
- Biased collection of large particles

Impaction

- Separate particles from the air stream by using inertia of particles
- Particles are collected on a solid plate or the surface of an agar growth medium
- Most common bio aerosols sampling method used
- Instruments available for both culturable sampling and total spore sampling is of two kinds: 1) jet-to-plate; 2) centrifugal (Hi-Air sampler Mark-II).

Sampler Performance

- Collection efficiency described by d50 cutpoint
  - Diameter of particles that are collected by impaction and pass through the sampler in equal number
  - Refers to mechanical collection efficiency
  - Dimension refers to µm AED
- Physical characteristics of the inlet and the airflow rate influence d50 cutpoint
- Generally accepted that larger particles are collected with nearly 100% efficiency

Impaction Samplers Total Spore Samplers

- Outdoor Samplers (aeroallergens, pollen, etc.)
  - Burkard (Hirst) Spore Trap
  - Rotorod Sampler
  - Samplair MK-3 Sampler
- Indoor Samplers (IAQ investigations)
  - Burkard Continuous Recording Air Sampler
  - Samplair MK-3 Sampler
  - Burkard Personal Volumetric Sampler
  - Air-O-Cell & Allergenco-D Samplers

Spore trap microscopy

- Minimum configuration
- Bright field at 600 X or Nomarski DIC at 500 X
- Desirable configuration
- Bright field at 1000 X or Nomarski DIC at 600 X

Resolving power is more important than magnification

Inpingement

- Separate particles from the air stream by using inertia of particles
- Forces deposition into liquid collection medium (usually a dilute buffer)
- Aggregates of cells can be broken apart
- Allows for several possible analytic applications: culture, microscopy,
- biochemistry, immunoassays, PCR

Impingement samplers (impingers)

- AGI-30 Coriolus Burkard Multi-stage

Cyclones

- Separate particles from the air stream by using inertia of particles in a vortex
- Often used in hygiene to remove large particles so small particles can be collected
  - large particles → "grit pot", discarded
  - small particles are collected on a membrane
- Biological sampling analyses the grit too
- Very good for DNA-based analyses

 37 mm filter

NIOSH 2-stage biocyclone

BioSampler d50 values for some samplers

- Burkard Personal Sampler 2.52 µm
- Air-O-Cell Cassette 2.30 µm
- BioSampler Impinger 0.30 µm
- Andersen Single Stage 0.65 µm
- RCS Standard ~5 µm
- AGI-30 Impinger 0.30 µm

References:

1. **B. Simon-Nobbe, U. Denk, V. Pöll, R. Rid and M. Breitenbach**, "The Spectrum of Fungal Allergy" Int. Arch. Allergy Immunol., vol. 145, pp. 58–86, 2008.
2. **J. Brett, J. Green, E. R. Tovey, J. K. Sercombe, F. M. Blachere, D. H. Beezhold and D Schmechel,** "Airborne fungal fragments and allergenicity" Medical Mycology, vol. 44, pp. 245-255, 2006.
3. **S. Martellos,** "Multi-authored interactive identification keys: The FRIDA (Friendly IdentificAtion) package", Taxon, vol. 59 (3), pp. 922-929, 2010.
4. **P. Carinanos, P. Alcazar, C. Galan, R. Navarro, E. Dominguez.** Aerobiology as a tool to help in episodes of occupational allergy in work places. J. Invest Allergol Clin Immunol 2004; Vol. 14(4): 300-308.
5. **Lacey, J., 1991.** Aerobiology and Health: The Role of Airborne Fungal Spores in Respiratory Disease. In: D.L. Hawks worth Ed., Frontiers in Mycology. C.A.B. International, Kew, UK. pp. 157-185.
6. **Hugues Beguin., 1995.** Mould biodiversity in homes II. Analysis of mattress dust. Aerobiologia 11, pp. 3-10.
7. **Dales R.E., Miller D., McMullen E., 1997.** Indoor air quality and health: validity and determinants of reported home dampness and moulds. Int. J. Epidemiology. 26: pp. 120-125.
8. **Ronald E Gots; Nancy J. Layton and Suellen W. Pirages., 2003.** Indoor health: Background levels of fungi. American Industrial Hygiene Association Journal 64: pp. 427-438.
9. **Hedayati, M.T. Pasqualotto, A.C., Warn, P.A., Bowyer, P. and Denning, D.W., 2007.** Aspergillus flavus: human pathogen, allergen and mycotoxins producer. Microbiology, 153, 1677-1692.
10. **Alan P. Knutsen, Bush, Demain, Denning; 2012.** Fungi and allergic lower respiratory tract diseases. J.Allergy Clin Immunol., Vol.129, No. 2, pp. 280-290.