Prospective survey of indoor fungal contamination in hospital during a period of building construction

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Summary An 18-month survey of indoor fungal contamination was conducted in one haematology unit during a period of construction work. Air was sampled with a portable Air System Impactor and surfaces with contact Sabouraud plates. During this survey the mean concentration of viable fungi in air was 4.2 cfu/m³ and that for surfaces was 1.7 cfu/plate. At the beginning of construction work, there were increases in airborne fungal spores (from 3.0 to 9.8 cfu/m³) in the unit, but concentrations did not exceed 10 cfu/m³ during the 18-month period. The most frequently recovered airborne fungi were Penicillium spp. (27–38%), Aspergillus spp. (25%) and Bjerkandera adusta, a basidiomycete identified with molecular tools (7–12%). Blastomycetes accounted for more than 50% of the fungal flora.
on surfaces. Investigating the impact of a new air-treatment system (mobile Plasmair™ units), there were significant reductions in fungal contamination for the Plasmer™-treated rooms, and in these rooms we observed the same level of fungal load whether construction work was in progress or not. © 2007 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Fungal contamination in healthcare facilities has been the subject of numerous studies. These have shown that some hospital infections are caused by fungi, such as species of Aspergillus, Fusarium or mucorales. Indoor fungal epidemiology depends on numerous factors, including moisture, ventilation, temperature, organic matter present in building materials but also on outdoor meteorological parameters, seasonal climatic variations and construction in progress.

In hospital, during a period of construction or renovation, fungal contamination in the indoor environment may increase markedly and there is a well-recognised relationship between outbreaks of invasive filamentous fungal infections occurring in immunocompromised patients and the close proximity of construction work.

It is thus necessary to implement control strategies during hospital construction work to limit the risk of fungal contamination. Air-treatment systems such as high-efficiency particulate air filtration (HEPA) with or without laminar air flow ventilation have been shown to reduce airborne fungal contamination. This equipment is extremely expensive to install, however, and some studies have shown that cheaper portable air filtration units can rapidly reduce levels of airborne particles. Previous studies conducted by our team during a period without construction suggested that Plasmair™ units may reduce indoor fungal contamination efficiently. To our knowledge, these air filtration units have not been evaluated during a period of construction.

The Dijon University Hospital has 1250 beds providing tertiary care for Burgundy, a region of 1.7 million inhabitants in northeastern France. The hospital is involved in a large renovation programme consisting of the construction of two buildings adjacent to clinical units housing patients at high risk of fungal infection. In this context, a multidisciplinary group including mycologists, infection control, microbiologists and clinicians was set up to monitor fungal contamination inside the hospital. A survey of the indoor environment was implemented and the following parameters were studied: (i) the fluctuation of fungal load during construction work in one unit with high-risk patients; (ii) the identification and prevalence of fungi isolated in this unit; and (iii) the impact of using a specific type of portable air filtration unit (mobile Plasmer™ unit) during the period of construction.

Methods

Duration of surveillance

This study was performed over an 18-month period, from October 2005 to March 2007, in the adult haematology unit of a university hospital in Dijon. The first period of construction (phase A: a 4000 m² laboratory close to the main hospital building) began in January 2006. The second period (phase B: construction of a 592-bed hospital building on eight levels with an area of 85 000 m²) began in September 2006.

Surveillance protocol

The survey was centred on patients on admission as part of a clinical research programme described previously. Briefly, one in five randomly selected patients admitted to the adult haematology unit on specific days were included. Patients hospitalised for 72 h or less were excluded. The survey consisted in sampling air and surfaces in the patient’s room on the day of admission, once a week during the hospital stay and upon departure.

Sampling method

A laboratory technician dedicated to this programme collected environmental samples. Air samples were collected with the Air-ideal 90 mm biocollector (BioMérieux, Marcy l’Etoile, France) loaded with Sabouraud chloramphenicol plates for fungal isolation. For each sample, 0.5 m³ of air was collected within 5 min in the patient’s room. Surface samples were collected from the
Bedside table with 55 mm diameter contact Sabouraud plates (BioMérieux) and a biocontact 99 applicator (Cera Labo, Equevilly, France) using 600 g pressure for 10 s.

Mycological methods

Samples were processed in the clinical mycology laboratory of the hospital. Sabouraud plates from air and surface samples were incubated at 30 °C and were examined daily for fungal growth. This allowed immediate quantification of the colonies as colony-forming units per cubic metre for air samples and colony-forming units per plate for surface samples. Fungal species were identified on the basis of their morphological characteristics when growth was sufficient.16

Molecular tools

A limited number of strains were identified with molecular tools by sequencing the internal transcribed spacer 1 (ITS1) regions of the ribosomal DNA gene using primer pairs and amplification conditions as described elsewhere.17,18 DNA extraction was processed using MasterpureTM Yeast DNA purification kit (Epicentre Biotechnologies) according to the manufacturer’s instructions.

Air handling systems

During the study period, 15 rooms of the adult haematology unit were protected with HEPA filtration (class 100 or 10 000), 11 rooms were treated with Plasmer™ and two rooms had no air treatment. Plasmer™ (Airinspace) is a European Community (EC) marked mobile air-decontamination unit in which organisms are destroyed through a three-step process that includes exposure to high electric fields and electrostatic nanofiltration.15

Statistical methods

Positivity rates of fungal cultures (treated vs non-treated rooms) were tested for significance by the Chi-squared test or Fisher’s exact test, where appropriate. Bartlett’s test was used for mean fungal loads of air and surface samples. All statistical analyses were performed using STATA software (Statacorp, 2005, TX, USA).

Results

The study used environmental data collected in non-protected rooms and in rooms treated with Plasmer™. Rooms protected by HEPA filtration were considered as a special environment and were not included. During the 18-month surveillance period, a total of 377 paired air and surface samples were collected in adult haematology. Positivity rates of fungal cultures were 66.6% and 27.3% for air and surface samples, respectively. The mean concentration of viable fungi in air was 4.2 cfu/m³ and for surfaces was 1.7 cfu/plate.

Figure 1 shows the monthly fluctuation of internal fungal load in the adult haematology unit during the period from October 2005 to March 2007. From October to December 2005 before the start of construction, the fungal load was low, ranging from 3.0 to 5.3 cfu/m³ in the air and from 1 to 1.5 cfu/plate for surface samples. In January and February 2006 during the first period of construction (phase A), an increase in the fungal load was observed in the unit (9.5 to 9.8 cfu/m³) after which the values returned to baseline at about 5 cfu/m³ in April. High air loads were also recorded during summer 2006 (8.2 cfu/m³ in July) before returning to low rates after September 2006. No peak in fungal contamination was observed during the second period (phase B) of construction.

Table I shows the overall frequencies and the corresponding loads of the fungi isolated in the clinical unit. The identified fungi included Aspergillus, Penicillium, Alternaria, Bjerkandera, dematiaceous, mucorales, yeasts and other filamentous fungi. The non-sporulating fungi showed no spores after three weeks of incubation. The main species of Aspergillus were A. fumigatus (45%), A. niger (15%) and A. glaucus (12%).

With respect to air samples, Penicillium spp. (27%) and Aspergillus spp. (25%) were the two most common fungi recovered (Figure 2), followed by Bjerkandera spp. (12%) and Alternaria spp. (7%).

A considerable number of blastomycetes were isolated from surface samples (Table I), accounting for 52% of the fungal flora detected in the haematology unit (Figure 2). Penicillium spp. (19%) and Alternaria spp. (3%) were less likely to be identified than in air samples and Aspergillus spp. in less than 2% of surface fungi.

Positivity rates and fungal loads of air and surface samples were compared in Plasmer™-treated and non-treated rooms during the period of construction (January 2006 to March 2007). Table II shows the positivity and mean fungal load during the period of construction. Highly significant differences between treated or untreated rooms were observed in terms of positivity rates of fungal cultures from both air (P < 0.0001) and surface samples (P = 0.007). The mean fungal load for positive samples in air also differed significantly between
treated and non-treated rooms \((P = 0.0054)\), whereas fungal loads of surface samples showed no significant differences between Plasmer™-treated and untreated rooms.

### Discussion

During the first period of construction in January and February 2006 (phase A), increased levels of airborne fungi were recorded in the adult haematology unit, close to the construction works. This fungal contamination was significantly higher \((P < 0.05)\) compared to the level of contamination observed one year before, during the same period without construction (January and February 2005, data not shown). The increase in fungal load was mainly related to an increase in *Penicillium* and *Aspergillus* conidia (peaks of contamination up to 40 cfu/m³), attributed to wood-cutting operations, earth removal and digging, which produced copious amounts of dust. These results contrast with other studies in which a dramatic increase in fungal counts during building activities was observed. For example, in one study in Hospital Maranón (Madrid, Spain), the mean count of fungal spores in internal air during the four weeks after a period of demolition was 23.3 cfu/m³ with peaks up to 80 cfu/m³.\(^{10}\) In another survey conducted in a haematology unit at the Hôtel-Dieu Hospital of Paris (France), the mean count of *Aspergillus* conidia in air samples increased considerably during construction (4 cfu/m³ prior to renovation to 24.7 cfu/m³).\(^8\) In our case, the mean count of *Aspergillus* conidia for this first period of construction was below 10 cfu/m³ in the haematology unit.

Table I shows the spectrum of fungi cultured in the clinical unit. A variety of agents were identified and *Aspergillus* spp. and *Penicillium* spp. were by far the most frequently encountered genera. The prevalence of these two genera has been previously observed in Dijon and other hospitals and preliminary studies indicate that fungal epidemiology inside hospital buildings is different from that outside.\(^{15,19}\) This suggests that most of the indoor contamination concerns micromycetes that have a particular ability to adapt to the environment inside hospitals.

Concerning percentages of fungi isolated in the air (Figure 2), the most prevalent fungi detected after *Penicillium* and *Aspergillus* were *Bjerkandera* and *Alternaria* spp. Comparison of these data with previous surveys of the hospital environment shows that airborne *Cladosporium* spp. (1.2% in adult haematology unit) were considerably lower compared to the 16% observed from the haematology hospital of Grenoble, France, or the 19.8% from one Asian hospital.\(^2,19\) In contrast, *Bjerkandera* was the third most frequent genus in the air in the haematology unit of Dijon hospital (12%). The presence of solely vegetative mycelium producing arthroconidia made identification difficult and it was initially identified in our laboratory as an *Arthrographis* species. The molecular identification was found to match perfectly with GenBank sequence AY089741.1 reported as *Bjerkandera adusta*.\(^20\)
This fungus is an anamorphic stadium of white-rot *Basidiomycetes* spp. which produce hyaline hyphae, poorly branched with narrower hyphae and rectangular arthroconidia (Figure 3). The occurrence of this fungus in clinical samples was reported, but to our knowledge there are no published data reporting such levels of airborne *Bjerkandera* in hospital environments. There are probably ecological niches in hospitals that allow this fungus to survive. As yet, no case report has been described in medical mycology.

Some studies have shown differences in the frequency of fungal species isolated in air and surface samples. Approximately 50% of the surfaces examined yielded a considerable number of yeasts and non-sporulating fungi (Figure 2). The greatest difference between air and surface samples relates to the detection of *Aspergillus* spp. This genus accounted for ~25% of fungi isolated in air and <2% of fungi isolated from surfaces. A previous study showed a difference in the frequency of *Aspergillus* spp. between air and surface samples, suggesting different reservoirs or different adherence and settlement capacities for these fungi. These results cannot be explained by the methods used in our study, which have been recommended and used by others. It is possible that a more thorough daily cleaning of surfaces in clinical units modifies the profile of the fungal flora.

**Figure 2** Percentages of fungi isolated in air and surfaces during the study period.
In situations where construction activity is planned to take place, the use of mobile air filtration devices could reduce the risk of fungal contamination, as shown by previous studies. In rooms equipped with Plasmer™, the current study showed levels of fungal load that were the same whether construction work was in progress or not (4.3 cfu/m³ during construction vs 4.9 cfu/m³ in the absence of construction, in the haematology unit). We also confirmed that mobile Plasmer™ units significantly reduced indoor fungal contamination during construction compared with rooms without air filtration unit (Table II). There is no agreement on a threshold of spore concentration at which a significant risk of invasive fungal infection occurs and such a threshold needs to be defined. We were able to maintain a spore concentration of below 5 cfu/m³ in a protective environment during a period of construction work.

This study emphasises the benefits of environmental surveillance for airborne contamination to help prevent outbreaks of nosocomial mycosis related to construction work. It appears that use of mobile air filtration devices may be helpful in reducing fungal spore load during renovation works.

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#### Conflict of interest statement

None declared.

#### Funding sources

None declared.

#### References


### Table II

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<th></th>
<th>Air</th>
<th>Surface</th>
<th>Total</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Fungal load (cfu/m³)</td>
<td>Positive</td>
</tr>
<tr>
<td>Plasmer™ treatment</td>
<td>186 (66.7%)</td>
<td>4.3</td>
<td>76 (27.2%)</td>
</tr>
<tr>
<td>No air treatment</td>
<td>26 (100%)</td>
<td>14.3</td>
<td>14 (53.8%)</td>
</tr>
</tbody>
</table>

#### Figure 3

*BJERKANDERA ADUSTA*: hyphae and arthroconidia.


