Airborne *Aspergillus* contamination during hospital construction works: Efficacy of protective measures

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**Background:** The Dijon University Hospital in Dijon, France is involved in a large construction program with heavy truck traffic and a very dusty environment. This study aimed to assess the impact of outdoor hospital construction work on *Aspergillus* air contamination in the immediate environment of patients at high risk for aspergillosis in the presence of protective measures.

**Methods:** Prospective air and surface sampling (n = 1301) was performed in 3 hospital units over a 30-month period. Generalized estimating equations were used to test the relationship between *Aspergillus* air contamination and the different variables (construction period, air treatment system, and surface contamination).

**Results:** Positivity rates of *Aspergillus* spp varied from 21.1% before construction work to 16.9% during work for air samples (P = .07), and the associated mean fungal load varied from 1.21 colony-forming units (CFU)/m³ to 0.64 CFU/m³ (P = .04). In multivariate analysis, only the use of an air treatment system was associated with decreased airborne *Aspergillus* contamination (P < .0001). No significant difference was observed between the presence or absence of construction work and the proportion of airborne *Aspergillus* contamination (P = .91) or the *Aspergillus* fungal load (P = .10).

**Conclusions:** No influence of hospital construction work on airborne *Aspergillus* contamination was demonstrated when protective measures were taken, including reinforcement of the importance of environmental cleaning.

**Key Words:** Aspergillus; hospital; air treatment; airborne contamination; building construction; protective measures.

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*Aspergillus* is a large genus of ubiquitous filamentous fungi, representing up to 40% of hospital and home fungal contamination. 1 *Aspergillus* spores are liberated in great amounts during construction and renovation work, and many work-related aspergillosis outbreaks have been described in the literature. 2-11 As a result, specific protective measures against environmental contamination must be taken when there is a construction activity on hospital site, 12 such as keeping rooms, department doors and windows closed; reinforcing biocleaning; and using an air treatment system. High-efficiency particulate air (HEPA) filtration is known to reduce airborne *Aspergillus* contamination. 13,14 More recently, mobile air treatment decontamination units have been developed as an alternative to laminar air filtration. 15

The Dijon University hospital is currently involved in a large construction program involving heavy truck traffic and a very dusty environment. Before the start of the work, a 1-year surveillance study was conducted to establish baseline levels of airborne fungi in 3 hospital units: hematology, pediatric onco-hematology, and nephrology. A comparison of indoor fungal contamination before the construction work in adult and pediatric onco-hematology rooms equipped with mobile air treatment units and those not so equipped has been described previously. 16 Furthermore, preliminary results on airborne contamination during the work period have been published by researchers from the Dijon University Hospital, 17 but these were restricted to the adult hematology unit only.
Here we report data for both before and during the construction work, as well as data concerning the 3 hospital units involved in the surveillance program, taking into account the correlation of the data by means of a statistical model. We found this model to be more suitable than univariate analysis, because it allowed us not only to express airborne contamination after adjustment for potential confounding factors, but also to avoid fallacious associations and finally to gain power. This statistical approach was not used in previous studies.16,17

This study was designed to evaluate the impact of construction work on *Aspergillus* air contamination in the immediate environment of patients at high risk for aspergillosis by comparing *Aspergillus* airborne contamination before and during construction work, after protective measures were in place. Airborne *Aspergillus* contamination was modeled by taking into account confounding factors and the correlated nature of the data using multivariate analysis.

**SUBJECTS AND METHODS**

**Subjects**

This prospective study was conducted in an 1192-bed tertiary care teaching hospital in Dijon, France over a 30-month period. The hospital is located in Burgundy, a region of 1.7 million inhabitants in northeastern France. In 2007, the hospital facilities for adults and children included 3 intensive care units with a total of 98 beds, 11 surgery departments with 312 beds, 12 medical departments with 539 beds, and 1 obstetrics and gynecology department with 78 beds. A 165-bed continuing care and readaptation unit was adjacent to the hospital. The period before the construction work was December 7, 2004 to January 29, 2006. The construction period studied ran from January 30, 2006 to June 13, 2007.

**Study design**

The survey investigated patients on admission in 3 critical areas hosting immunocompromised patients at increased risk for aspergillosis: adult hematology, pediatric onco-hematology, and nephrology departments (postgraft hospitalization). Patients were included if they came directly from home or a day care hospital and were hospitalized for at least 72 hours. Because of the large number of adult hematology patients, only those admitted on 1 randomized day per week were included. Nasal swabs for fungal cultures were collected on entry and once a week until departure to assess patient colonization.

**Air-handling systems in study areas**

Together, the adult hematology, pediatric onco-hematology, and nephrology units had a total of 47 beds. HEPA filtration (class 100, 10,000, or 100,000, according to American Federal Standard FS 209D) was present in 15 rooms, 5 of which had unidirectional flow and positive air pressure; 15 other rooms were equipped with Plasmer mobile air treatment decontamination units. Instead of mechanically filtering the air, these units use a novel technology, based on nonthermal-plasma reactors adapted from a system originally developed for use in space stations. The units destroy airborne organisms through a 3-step process: (1) exposure to high electric fields, (2) subsequent bombardment with positive and negative ions, and (3) electrostatic nanofiltration. Organic compounds are finally oxidized in molecular components, and clean air is released.18 The remaining rooms had no air treatment at all, neither positive air pressure nor unidirectional flow.

**Materials**

Environmental sampling was carried out in the patient rooms by a laboratory technician dedicated to this study. Air sampling was conducted with the Air Ideal 90-mm biocollector (BioMérieux, Marcy l’Étoile, France), which draws air through a sampling grid with 265 holes over a 90-mm-diameter petri dish filled with Sabouraud chloramphenicol plates (BD, Le Pont de Claix, France). The Air Ideal biocollector operates according to the impaction principle recommended by International Organization for Standardization Standardization Draft International Standard 14698-1, with an air intake of 100 L/min and an impact speed of 20 m/s, capturing particles from 0.3 to 10 μm in diameter. According to recommendations from the French Consensus Conference,12 the biocollector collected 0.5 m³ in 5 minutes in the patient room near the head of the bed. The number of colonies recovered on the air sample plates was adjusted for multiple impact; a positive hole correction was used to determine the likely number of fungi passing through the orifices of the grid. This correction was calculated using Feller’s law.19 The concentration of airborne fungi was expressed as the number of colony-forming units per cubic meter of air (CFU/m³).

Surface sampling was conducted on bedside tables with 55-mm-diameter contact Sabouraud plates (Bio-Mérieux) using a biocontact applicator (Cera Labo, Equevilly France) with a 10-second 600-g pressure. Samples were collected at the time of patient admission, on discharge, and once a week during the period of hospitalization.

**Methods**

**Mycological methods.** Air and surface samples were processed in the hospital’s clinical mycology laboratory. The Sabouraud plates were incubated at 30°C.
and were examined daily for fungal growth by a trained hospital microbiologist. To optimize the production of fruit bodies, a limited number of strains were cultivated on malt-agar 3% (AES, Bruz, France). Negative plates were incubated for up to 15 days to detect slow-growing fungi.

Nasal swabs were suspended in 1 mL of sterile 0.9% NaCl, and a 100-μL fraction was seeded on Sabouraud–dextrose agar–containing antibiotics. Plates were incubated at 30 °C and were examined daily for fungal growth and quantification of colonies. Negative plates were incubated for up to 10 days. Fungal species were identified on the basis of their morphological characteristics when the development of the colonies was sufficient.20

Outcome assessment

A patient was considered colonized if nasal swabs retrieved Aspergillus. The dependent variable Aspergillus airborne contamination was expressed both qualitatively (Aspergillus airborne contamination vs no Aspergillus airborne contamination) and quantitatively (mean number of Aspergillus spores in the air sample, expressed in CFU/m3). The mean number of Aspergillus spores was calculated by dividing the number of CFUs by the total number of samples collected.

When confluent growth occurred, the CFU/m3 could not be assessed precisely. Nevertheless, these samples were taken into account in our analysis when a qualitative procedure was used (Aspergillus airborne contamination vs no Aspergillus airborne contamination). When a quantitative procedure was used (mean number of Aspergillus spores), invaded samples were not taken into account.

Statistical analysis

The statistical unit studied was the sample. The comparison of percentages between the period before construction work versus the construction period was done using the χ² test or Fisher’s exact test. A paired Wilcoxon rank-sum test was used for continuous variables. Quantitative variables were expressed as mean ± standard deviation.

Generalized estimating equations (GEEs) with a robust variance estimator were used to test the relationship between Aspergillus air contamination and the different variables, assuming an exchangeable structure for the correlation matrix. Both binary (contamination: yes/no) and count (mean number, expressed in CFU/m3 for air samples and in CFU/plate for surface samples) variables were modeled by GEE regression. For count variables, a negative binomial distribution was used. The GEE results were expressed as coefficient and 95% confidence interval (CI) and associated P value. All analyses were systematically adjusted on the hospital unit (hematology, pediatric onco-hematology, or nephrology).

The model variables included the construction period, the air treatment system, Aspergillus surface contamination, other filamentous fungi surface contamination, and yeast surface contamination. All variables with a P value < .20 were introduced in multivariate analysis. The final significance level was P < .05. All statistical analyses were performed using Stata 8.0 (StataCorp, College Station, TX).

RESULTS

Patient colonization

The study cohort comprised 345 patients. Five patients were colonized with Aspergillus, and the first nasal swab collected on the day of admission was positive in 3 of them.

Environmental sampling

Univariate results. A total of 1301 environmental samples were collected: 694 in the hematology unit, 132 in the nephrology unit, and 475 in the pediatric onco-hematology unit. Aspergillus was identified in 258 air samples, with A. fumigatus being the most common species retrieved (53.1%).

Before the construction period, 21.1% of the air samples and 2.1% of the surface samples were contaminated with Aspergillus, compared with 16.9% of the air samples and 1.0% of the surface samples during the construction work. These differences were not statistically significant, however (P = .07 and .09, respectively). The construction period was associated with greater use of air treatment systems such as Plasmair. Only 11.5% of the rooms had no air treatment. The proportion of air samples contaminated with Aspergillus did not differ significantly between the 2 periods for any air treatment system modality (Table 1). The mean load of Aspergillus in air samples varied from 1.21 ± 4.18 CFU/m3 before construction work to

| Table 1. Comparison of the proportion of Aspergillus-contaminated air samples according to air treatment system and the periods before and during work, univariate analysis |
|---------------------------------|----------------|--------------|----------|--------|
| **Air treatment system** | **Before work** | **During work** | **P** |
| None | 58/93 | 53/95 | 55.8 | .36 |
| HEPA filtration | 0/134 | 0 | 2/234 | 0.8 | .54 |
| Plasmair | 42/248 | 16.9 | 85/497 | 17.1 | .95 |
| Aspergillus airborne contamination | 100/475 | 21.1 | 140/826 | 16.9 | .07 |

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Table 2. Proportion of airborne Aspergillus contamination using the GEE model

<table>
<thead>
<tr>
<th>Airborne Aspergillus contamination (yes/no)</th>
<th>Bivariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>Construction works (reference: no works)</td>
<td>-0.21</td>
<td>-0.47 to 0.05</td>
</tr>
<tr>
<td>Air treatment systems (reference: no air treatment system)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPA filtration</td>
<td>-5.64</td>
<td>-7.04 to -4.23</td>
</tr>
<tr>
<td>Plasmair &lt;1000 m³/hour</td>
<td>-1.85</td>
<td>-2.26 to -1.44</td>
</tr>
<tr>
<td>Plasmair ≥1000 m³/hour</td>
<td>-2.98</td>
<td>-4.07 to -1.89</td>
</tr>
<tr>
<td>Aspergillus contamination surface (reference: no contamination)</td>
<td>-0.03</td>
<td>-0.96 to 0.90</td>
</tr>
<tr>
<td>Other filamentous fungi surface contamination (reference: no contamination)</td>
<td>0.37</td>
<td>0.005 to 0.74</td>
</tr>
<tr>
<td>Yeast surface contamination (reference: no contamination)</td>
<td>0.26</td>
<td>-0.13 to 0.66</td>
</tr>
</tbody>
</table>

Table 3. Quantity of Aspergillus air samples contaminated using the GEE model

<table>
<thead>
<tr>
<th>Quantity of Aspergillus air samples, CFU/m³</th>
<th>Bivariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>Construction works (reference: no works)</td>
<td>-0.59</td>
<td>-0.98 to -0.20</td>
</tr>
<tr>
<td>Air treatment systems (reference: no air treatment system)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEPA filtration</td>
<td>-5.72</td>
<td>-7.20 to -4.24</td>
</tr>
<tr>
<td>Plasmair &lt;1000 m³/hour</td>
<td>-2.10</td>
<td>-2.48 to -1.73</td>
</tr>
<tr>
<td>Plasmair ≥1000 m³/hour</td>
<td>-3.19</td>
<td>-4.15 to -2.22</td>
</tr>
<tr>
<td>Aspergillus contamination surface, CFU/plate (reference: no contamination)</td>
<td>0.26</td>
<td>-0.005 to 0.52</td>
</tr>
<tr>
<td>Other filamentous fungi surface contamination, CFU/plate (reference: no contamination)</td>
<td>0.07</td>
<td>0.01 to 0.12</td>
</tr>
<tr>
<td>Yeast surface contamination, CFU/plate (reference: no contamination)</td>
<td>-0.02</td>
<td>-0.04 to 0.006</td>
</tr>
</tbody>
</table>

0.64 ± 2.60 CFU/m³ during work (P = .04), whereas the mean load of Aspergillus in surface samples varied from 0.04 ± 0.55 CFU/plate before work to 0.01 ± 0.1 CFU/plate during works (P = .09).

Multivariate results

Positivity rates of Aspergillus spp in air samples. In bivariate analysis with GEE model regression, airborne Aspergillus contamination was significantly lower in patient rooms equipped with an air treatment system and higher when surfaces were contaminated by Aspergillus or other filamentous fungi (Table 2). In multivariate regression, only air treatment system was significantly associated with decreased airborne Aspergillus contamination (P < .0001). No significant difference was observed between the proportion of airborne Aspergillus contamination and the presence or absence of construction work (P = .91).

Fungal loads of Aspergillus spp in air samples. In bivariate analysis, the quantity of Aspergillus in air was significantly lower in patient rooms equipped with an air treatment system and higher in the presence of greater quantities of other filamentous fungi on surfaces. In multivariate analysis, only the presence of an air treatment system significantly decreased the Aspergillus load in air samples (P < .0001) (Table 3).

DISCUSSION

In the present study, 1.5% of the patients were colonized with Aspergillus, lower than the 6% colonization rate reported by Goodley et al. Moreover, most of our colonized patients were already colonized on admission to the hospital. Consequently, it was not possible to study the Aspergillus colonization of our patients according to environmental contamination. However, the aim of the present study was to evaluate the impact of construction work on Aspergillus air contamination in the immediate environment of patients at high risk for aspergillosis.

In our study, positivity for Aspergillus spp in air samples varied from 21.1% before construction work to 16.9% during work, and the associated mean fungal load varied from 1.22 to 0.64 CFU/m³. The positivity rates
were lower than those reported by Cornet et al.\textsuperscript{13} The differences between their study and ours can be explained by the areas of sample collection. In the Cornet et al study, samples were collected from patient rooms, nursing stations, and corridors, and results were reported collectively; however, airborne contamination likely was higher in corridors than in patient rooms. This may have led to overestimation of the positivity rate.

The mean fungal load was much lower than those published previously.\textsuperscript{16,17} In most previous studies, fungal load was calculated by dividing the number of CFUs by the number of positive samples.\textsuperscript{15,16,17} However, in the present study, fungal load was calculated by dividing the number of CFUs by the total number of collected samples, both positive and negative samples. This led to an underestimation of the mean fungal load. We chose this approach because non-contaminated samples provide valuable information and better reflect the global situation. What is important is that we used the same methodology in the 2 periods that we studied, allowing reliable comparisons. Most of the previous studies examining the influence of hospital construction or renovation work on airborne Aspergillus contamination did not involve multivariate analysis.\textsuperscript{15,21-24} To the best of our knowledge, the present study is the first to use a multivariate model taking into account the correlation between air and surface sampling and repeated measurements in the same place.

We found that the presence of an air treatment system (HEPA or Plasmair) was associated with decreased frequency and quantity of airborne Aspergillus contamination. Our results are consistent with the results of previous studies on the impact of air treatment systems on airborne contamination control.\textsuperscript{12,16,17}

Surprisingly, once adjusted for other factors, construction work was not significantly associated with increased airborne Aspergillus contamination, regardless of the type of air treatment system used. Thus, even in rooms with no air treatment, airborne contamination was not increased during construction activities. This finding may reflect the efficacy of protective measures more than the impact of construction work on airborne contamination. Indeed, many invasive aspergillosis outbreaks during construction periods are described in the literature.\textsuperscript{2-11} Consequently, we recommended protective measures advocated by the National Disease Surveillance Center\textsuperscript{25} during the study period, to limit airborne contamination as much as possible. The recommendations, such as keeping windows and doors closed in both clinical units and patient rooms, were made to clinical units and applied during the construction period. To reduce dust, contractors and workmen were instructed to wet the construction site.

Moreover, biocleaning might be a confounding factor. Indeed, the present study focused solely on care units that accommodate patients at high risk for invasive aspergillosis and where biocleaning had been consequently highly reinforced during the construction period, completed when necessary by wet-brooming. This hypothesis is supported by the low proportion of contaminated surface samples compared with air samples, whereas surface contamination would have been expected to reflect air contamination.

All of these protective measures might explain the lower Aspergillus contamination during construction. In 2003, Cooper et al\textsuperscript{22} studied the influence of construction work on fungal air contamination after the implementation of protective measures. No significant increase in fungal airborne contamination was observed during construction work. Our results are consistent with theirs. The originality of our study is that it takes into account the air treatment system as well as other covariates in 2 multivariate analyses. Moreover, no factors other than protective measures can explain our results. Indeed, there were no minor internal building work or maintenance work, and we observed no changes in ward entrance routes during the study period. Furthermore, unlike what was observed outdoors, the presence of Aspergillus spp remained stable in the clinical units.\textsuperscript{26} thus, our results cannot be explained by seasonal variations.

Fungi environmental surveillance relies on air and surface sampling. Environmental sampling was performed following standardized techniques. The application of Sabouraud plates on surfaces was reproducible because the pressure and time contact were constant, and because it was done by a technician trained in this technique. Our microbiological studies did not allow us to quantify air or surface contamination with sufficient precision, however, because a CFU can be composed of a variable number of microorganisms. This risk exists especially when readings of plates are tardy; colonies may be very developed and may be superposed, making quantitative evaluation difficult. We limited this risk by examining Sabouraud plates from 48 hours onward after collection. Despite these precautions, we had to exclude 10 invaded samples, collected before the construction period, from the quantification of Aspergillus airborne contamination. Such a small proportion of samples (8%) should not affect the validity of the results, however.

In summary, Aspergillus can be responsible for invasive pulmonary aspergillosis in immunocompromised patients, with a vital prognosis frequently engaged. Construction work represents a major environmental risk factor, necessitating protective measures that can be easily implemented. The use of air treatment systems was associated with decreased Aspergillus air contamination. In the literature, construction work is linked to increased airborne Aspergillus contamination;
however, we found that implementing preventive measures during the construction period limited contamination. Our results may reflect the efficiency of protective measures taken, and they reinforce the importance of environmental cleaning.

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References