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BMJ Open Monitoring of clinical strains and environmental fungal aerocontamination to prevent invasive aspergillosis infections in hospital during large deconstruction work: a protocol study

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ABSTRACT

Introduction Monitoring fungal aerocontamination is an essential measure to prevent severe invasive aspergillosis (IA) infections in hospitals. One central block among 32 blocks of Edouard Herriot Hospital (EHH) was entirely demolished in 2015, while care activities continued in surrounding blocks. The main objective was to undertake broad environmental monitoring and clinical surveillance of IA cases to document fungal dispersion during major deconstruction work and to assess clinical risk.

Methods and analysis A daily environmental survey of fungal loads was conducted in eight wards located near the demolition site. Air was collected inside and outside selected wards by agar impact samplers. Daily spore concentrations were monitored continuously by volumetric samplers at a flow rate of 10 L.min⁻¹. Daily temperature, wind direction and speed as well as relative humidity were recorded by the French meteorological station Meteociel. *Aspergillus fumigatus* strains stored will be genotyped by multiple-locus, variable-number, tandem-repeat analysis. Antifungal susceptibility will be assessed by E-test strips on Roswell Park Memorial Institute medium supplemented with agar. Ascertaining the adequacy of current environmental monitoring techniques in hospital is of growing importance, considering the rising impact of fungal infections and of curative antifungal costs. The present study could improve the daily management of IA risk during major deconstruction work and generate new data to ameliorate and redefine current guidelines.

Ethics and dissemination This study was approved by the clinical research and ethics committees of EHH.

INTRODUCTION

Invasive fungal infections are major threats to immunocompromised patients because of their high incidence and related mortality.¹ They occur through inhalation of airborne conidia with various consequences, such as

Strengths and limitations of this study

- This study is one of the largest ongoing prospective studies to evaluate combined approach of environmental and clinical monitoring in hospital during major deconstruction works.
- The high frequency and number of samples collected in this study during deconstruction works will allow powerful statistical analysis to evaluate the efficiency of protective measures and reduce fungal contamination.
- Non-cultivable methods monitoring outdoor *Aspergillus* aerocontamination for hospital alerts are evaluated.
- Genetic diversity and their antifungal susceptibility profiles of clinical and environmental collected isolates are determined to give complete information on *Aspergillus* spp dispersion and hopefully give new insights into improvement of environmental monitoring and of hospital guidelines during major demolition work.
- Microbiological identification will focus only on *Aspergillus* spp. because it is the leading pathogen responsible for invasive aspergillosis. Although two air sampling collectors were used in the study, no particle counter was tested.

immune-allergic reactions to invasive aspergillosis (IA), a severe opportunistic disease caused mostly by *Aspergillus fumigatus* (>80%) and, to a lesser extent, by *A. flavus*, *A. niger*, *A. terreus* and *A. nidulans*.^{2,3} IA is a very serious condition, with crude lethality ranging from 50% to 90% and greater mortality in haematological patients.^{4,5} Its epidemiology has changed in recent years, surfacing in non-haematological units, such as intensive care units (ICUs) and increasing in non-neutropenic

hosts treated with corticosteroids or lifelong immunosuppressants.⁶ The burden of patients at risk of IA is growing every year because of longer survival and improved care.⁷ Azole antifungals are commonly given to combat IA in many countries, with recent studies reporting an emerging, worldwide problem: azole drug resistance of *A. fumigatus* isolates.⁸⁻¹³

Aspergillus species are opportunistic pathogens widely distributed in the environment and easily transported by air because of their conidia size.¹³ Construction work in healthcare settings in past decades has been associated with major IA outbreaks.⁴ Construction involving periods of renovation or deconstruction increases spore release, creating high-risk situations in hospitals.^{1 14 15} In the 1980s, 22 IA cases arose over a 30-month period during building renovations at Edouard Herriot Hospital (EHH) (Lyon, France).¹⁶ A quasi-experimental study, conducted in an adult haematology unit of this hospital, underlined the need for monitoring environmental factors to prevent nosocomial IA.¹⁷ A sampling strategy for fungal monitoring in hospital was based on study results.^{14 18}

Recently, an entire block in EHH underwent deconstruction without suspension of care activities in direct proximity to it. The situation prompted the infection control team to monitor fungal dispersion and look for cases of *Aspergillus* disease. Only a few studies have been performed in this context and under similar conditions so that sampling strategies and analyses differ, indicating varying fungal loads.¹⁵⁻²¹ All these investigations have highlighted the common need for innovative approaches and tools to improve research in the field.¹⁸ Real-time methods may provide warning systems by monitoring outdoor fungal loads.²¹

The purpose of the present work was to carry out broad environmental monitoring and clinical surveillance of IA cases to better understand fungal dispersion during major deconstruction work.

METHODS

Study objectives

The objective of this study is to provide new data for future guidelines on adequate management of invasive fungal infection risk in hospital during major deconstruction work. Its intermediate objectives are to: (1) evaluate non-cultivable methods monitoring outdoor *Aspergillus* aerocontamination for hospital alerts, (2) assess the impact of meteorological parameters (MP) on *Aspergillus* aerocontamination and (3) compare the genetic diversity of clinical and environmental *A. fumigatus* isolates and ascertain their antifungal susceptibility profiles.

STUDY SITE

Built in the 1930s in Lyon, Rhône-Alpes, France, on a 0.15-km² site, EHH is a university-affiliated hospital composed of 32 independent blocks divided by tree-planted walkways and grassy areas. This 850-adult bed tertiary

institution provides care to a large panel of immunocompromised patients (solid organ transplantation, haematopoietic stem cell transplantation, immunosuppressive treatment, ICU). The central block that was demolished measured 0.006-km², representing approximately 12.2% of the area covered by hospital blocks (figure 1, online video of demolition works: <https://www.youtube.com/watch?v=Oa7xRufAnhQ>). Three deconstruction periods at EHH were scheduled between February and December 2015. The first period, between February and June 2015, consisted of gutting the building and removing asbestos from it. The floors were removed in July and August 2015. Excavation and earthwork took place between September and December 2015. Finally, concrete was poured at the end of December 2015 to allow construction of the new building comprising fully equipped areas (operating rooms, ICUs and heliport) on 2.5 ha.

STUDY DESIGN

The study was conducted in eight medical wards located around the demolition site: four ICUs, one kidney and pancreas transplantation unit and three medical wards (figure 2). All of them were possibly occupied by at-risk patients. It should be noted that EHH does not have a haematological ward any longer. In a 10-month period, about eight inside and four outside air samples were collected from each unit per week (table 1). Approximately 64 indoor and 48 outdoor air samples were obtained per week, for a total of 3885 air samples. Weekly clinical monitoring of high-risk, hospitalised patients, by infectious control practitioners, was scheduled.

PROTECTIVE MEASURES

Several preventive measures were performed: (1) doors and windows in front of the deconstruction site were maintained closed during the day and allowed for opening at night, (2) patient and medical staff movements were limited and special traffic patterns were designed, (3) masks were requested for hospitalised or non-hospitalised immunocompromised patients outside wards to limit fungal exposure, (4) adhesive decontamination carpets were installed at the entry of wards and (5) visitors, patients and medical staff were alerted about fungal exposure due to the deconstruction site. In case of high fungal exposure, after results of environmental survey, intensive biocleaning was performed.

Preventive measures were also implemented outdoor, at the deconstruction site to limit fungal dispersion. Construction site teams received information and education about IA risks. Humid environment was mandatory for all works completed by regular humidification. Furthermore, rubbles of ruined buildings are humidified and covered for evacuation. Circulation pattern for rubble evacuation is also designed. Damp cleaning of deconstruction site roads are frequently realised with, in addition, cleaning of truck wheels.

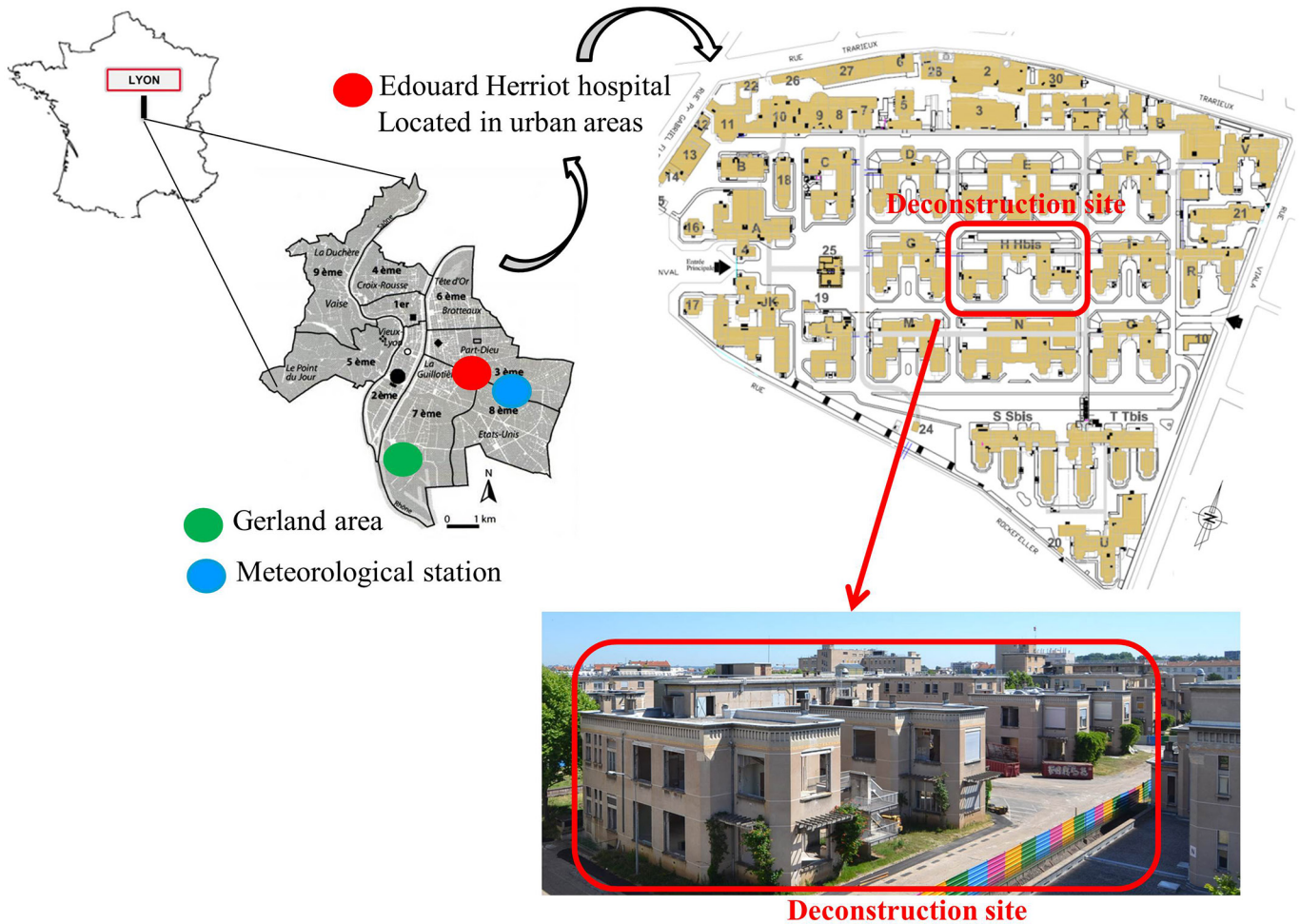


Figure 1 Location of Edouard Herriot Hospital, Lyon (France).

ENVIRONMENTAL SURVEY

The environmental survey consisted of monitoring air samples inside and outside selected wards (two blocks were monitored per day). Only moulds, in particular *Aspergillus* spp have been investigated in this study.

Sampling by the non-cultivable method

Outdoor airborne fungal spore concentrations were monitored continuously with 7-day Hirst-type spore traps (VPPS 2000; Lanzoni, Bologna, Italy) at a flow rate of 10L/min. Air drawn in by suction port was directly impacted on adhesive tape cut daily into segments.

Mean daily fungal spore concentrations were assessed for each segment by optical microscopy (AxioStar; Carl Zeiss, Göttingen, Germany). Spore counts were expressed as spores/m³/day. The Hirst-type spore trap was placed on the rooftop of a block extension consisting of a prefabricated floor located on the north side, just in front of the deconstruction site. It allowed daily monitoring of spore concentrations as total fungal load and *Aspergillaceae* fungal load (ie, *Aspergillus* spp + *Penicillium* spp). A similar Hirst-type spore trap was placed throughout the study in the Gerland area (Lyon, France), located a few kilometre south-west of EHH and served as negative control.

Sampling by the cultivable method

Air samples were collected two times a day outside and inside wards during 11 months, according to a standardised protocol (table 1). Each sample was gathered by agar impact sampler (Air Ideal; bioMérieux, Marcy l'Etoile, France) in 90mm diameter Petri dishes containing Sabouraud chloramphenicol agar. Air intake velocity of this agar impact sampler was 100L/min. Two plates were seeded at each sample site. Air sample volume was chosen according to French guidelines environmental fungal risk control.²²⁻²³ They recommend a sample volume adapted to the presumed levels of contamination in the environment. Due to the major demolition works ongoing, outdoor air was considered more contaminated by fungi than indoor. So in order to avoid overcrowding on the plates, outdoor plates were seeded for only 1 min corresponding to an air volume of 100L. Indoor samples were supposed to have an intermediate fungal contamination level due to the preventive measures applied to reduce environmental contamination inside units. Therefore, indoor plates were seeded for 2½ min, resulting in a higher air volume (250L). One of these plates was incubated for 48 hours at 37°C to grow thermotolerant *A. fumigatus* species.²⁴ The other plate was incubated

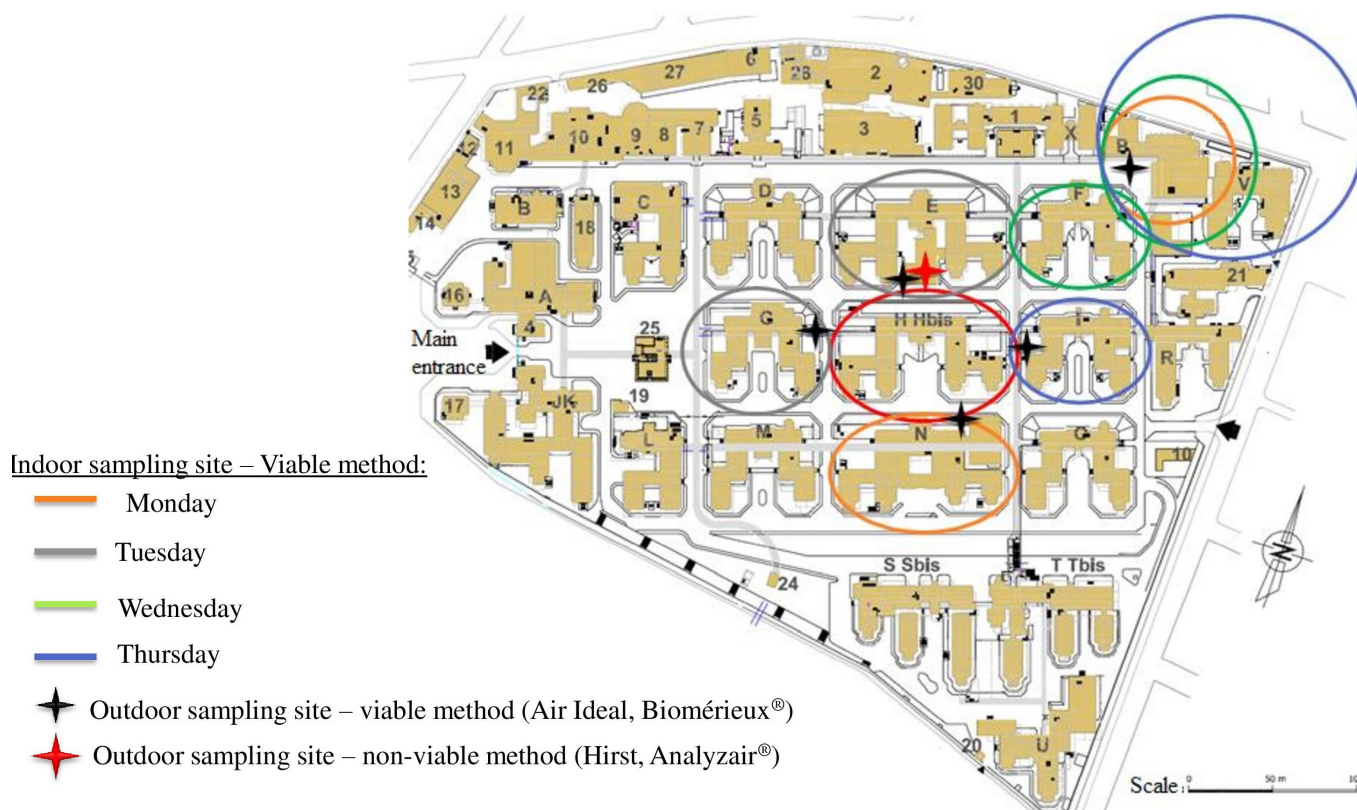


Figure 2 Detailed map of Edouard Herriot Hospital and sampling sites of environmental monitoring.

for 5 days at 30°C to allow growth of all fungi. Colonies were then counted and identified at the genus level on the basis of macroscopic and microscopic characteristics (lactophenol blue-stained preparations). The data are expressed as colony-forming units per cubic metre.

Prospective clinical survey

Patient inclusion

All hospitalised patients were surveyed prospectively at the hospital level and were eligible for inclusion. IA was classified as proven, probable and possible or excluded according to European Organisation for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) criteria.²⁵ Only cases diagnosed after hospital admission were included. Cases were categorised into three groups, according to the time between hospital admission and diagnosis: community-acquired, undetermined and nosocomial. Community-acquired cases were defined as incident cases imported from outside the hospital with apportion of clinical symptoms or positive sample in less than 2 days after admission. Undetermined cases were defined as incident cases with lag time ranging from 1 to 9 days between admission and the first IA signs without any previous negative sample. Probable nosocomial cases were defined as incident IA with lag

time between admission and symptoms onset of at least 10 days, or if there is some history of negative sample. Clinical manifestations of IA vary widely and may develop in different clinical scenarios.^{26–28}

Case detection

Case detection was based on prospective surveillance of:

- ▶ Antifungal therapies (eg, voriconazole, posaconazole, itraconazole, caspofungin and amphotericin B distributed by the hospital pharmacy) administered to patients by pharmacy informatics software.
- ▶ Mycological results positive for *Aspergillus* spp corresponding to cultures showing colony of *A. fumigatus* were investigated.
- ▶ Reporting of suspected cases by hospital clinicians and infection control practitioners.

All suspected cases were investigated by two infection control practitioners (one resident and one physician). External validation was requested in case of uncertain diagnosis by standardised chart, allowing the collection of demographic characteristics, disease history, clinical features, mycological, biological and radiological data, antifungal therapy and disease outcome. If an increased incidence of IA was detected, infections control specialists and mycologists would be solicited to review IA cases.

Table 1 Description of manual air sampling sites monitored at Edouard Herriot Hospital

Sampling	Medical unit				ICU				Medical unit				Kidney and pancreas transplantation unit				ICU			
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon		
	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch		
Monday	Outdoor	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch		
	Indoor	Room+ corridor	Room+ corridor	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room	Corridor+ treatment room		
Tuesday	Outdoor	-	-	-	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch		
	Indoor	-	-	-	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors	2 Corridors		
Wednesday	Outdoor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Indoor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Thursday	Outdoor	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch	Building porch		
	Indoor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor	Room+ corridor		
Friday	Outdoor	Additional sampling, if needed																		
	Indoor	Additional sampling, if needed																		

ICU, intensive care unit.

A. fumigatus collection

During the study, a maximum of four *A. fumigatus* colonies per day among all *A. fumigatus* environmental cultures incubated at 37°C were arbitrarily isolated, subcultured on Sabouraud dextrose agar and incubated at 45°C in order to select *A. fumigatus* before being frozen. All *A. fumigatus* clinical isolates of interest also were frozen at -20°C. In total, 400 *A. fumigatus* isolates, corresponding to maximal laboratory capacity and budget, were constituted arbitrarily.

Molecular identification of *Aspergillus* isolates











All *A. fumigatus* isolates stored will be identified retrospectively at the species level by sequencing β -tubulin gene (*benA*, using Bt2a/BT2b primers). Isolates will be subcultured on Sabouraud dextrose chloramphenicol agar for 48 hours at 37°C. A piece of approximately 1 cm² of culture will be cut and transferred to microtubes for DNA extraction with QIAamp DNA blood mini kits (Qiagen) according to the manufacturer's instructions. All isolates will be identified by partial sequencing of β -tubulin gene (*benA*, using Bt2a/BT2b primers). Sequence alignments will be analysed by Chromas Lite, V.2.01 (<http://technelysium.com.au>) and compared with genome sequences in GenBank and MycoBank. The results will be considered acceptable if homologies with other entries in the databases used for comparison are >99%.

Genotyping of *A. fumigatus* isolates

Isolates will be genotyped by multiple-locus variable-number tandem-repeat analysis (MLVA) based on

selected variable-number tandem-repeat (VNTR) polymorphism. The MLVA protocol for *A. fumigatus* genotyping targeting 10 markers will be adapted from Thierry *et al* for multiplexing and capillary electrophoresis (CE).²⁹ One primer couple will be modified to provide shorter amplicons while ensuring the absence of overlaps across VNTR loci. Primers targeting new VNTR flanking regions have been designed by Primer3Plus software. MLVA primers for 10 loci and the fluorescent dyes in CE are enumerated in table 2. MLVA PCRs were performed in two multiplexes in a final volume of 50 μ L containing: 1–5 ng of DNA, 1X Multiplex PCR Master Mix (Qiagen) and 0.2 μ M of each flanking primer. The initial denaturation step at 95°C for 10 min was followed by 35 cycles, consisting of denaturation at 95°C for 30 s, primer annealing at 58°C for 40 s and elongation at 72°C for 10 min. The final extension step was set at 60°C for 10 min. PCR products were diluted 1:50 in deionised water, and 1 μ L of the diluted sample was added to 18.5 μ L formamide and 0.1 μ L of GeneScan 500 LIZ dye size standard (ThermoFisher; Life Technologies, Courtaboeuf, France). All samples were denatured for 5 min at 95°C, then cooled to 4°C before being subjected to CE in a 3130 XL DNA analyser (Applied Biosystems, Courtaboeuf, France) with 3130 POP7 polymer (Applied Biosystems). Each VNTR locus was identified by colour and size in electropherograms by GeneScan analysis (Applied Biosystems). Fragment sizes were converted to repeat numbers based on the formula: number of repeats (bp)=fragment size (bp)—flanking

Table 2 MLVA primers and fluorescence dyes used in each multiplex reaction

	VNTR	Fluorochromes	Primer sequences (5'-3')	Allele size range (bp)
Multiplex 1 CE 1	Asp 167	F: ATTO565- R:	TGAGATGGTTAACTTACGTAGCGC CGCTCCCACCGTTACCAAC	374-422 
	Asp 330	F: ATTO550- R:	ATCTGGTCGCGAAATTCCTCT TCTTCGGCCTTTTCATCCC	152-218 
	Asp 345	F: Yakima Yellow- R:	TCTCCAACCCTTCGGACG GCCGGAAGAGCATGAAGACA	191-246 
	Asp 443	F: ATTO565- R:	AAGCTTCGTCTGGCGAAGAG GCACGTGTACGGTGTTCCTG	154-280 
	Asp 446	F: 6-FAM- R:	CGATCATGTTTGCCTGAGGA CCGACAGCATCGAGCAACTA	197-260 
Multiplex 2 CE 2	Asp 20	F: 6-FAM- R:	GGGAAGAGAGGAACCGATCC CGCAGTGGGCAGTTTGAAT	182-222 
	Asp 165	F: Yakima Yellow- R:	TGATGGGCCGAGTCG GCACCTGCTTGTGCGATTCTG	154-214 
	Asp 202	F: ATTO565- R:	AGGATCACTGCCCTCAACCC CCGAAATCCGCGGGA	200-296 
	Asp 204bis	F: ATTO565- R:	ATTGGGAAGAGACGGGGTAT GTCCTCACTTTTGCCTTGGT	134-178 
	Asp 252	F: ATTO550- R:	CAGATTGGAGACACGAAGCG ACCACGGATTGCCAAGGA	176-224 

6-FAM, 6-carboxyfluorescein; CE, capillary electrophoresis; F, forward; MLVA, multiple-locus variable-number tandem-repeat analysis; R, reverse; VNTR, variable-number tandem repeat.

regions (bp)/repeat size (bp). Absent PCR products were designated an allele number, for example, '-1'. Phylogenetic relationships between isolates will be studied, generating a minimum spanning tree with PHYLOViZ V.2.0.³⁰

Antifungal susceptibility testing

Briefly, the antifungal susceptibility of stored *A. fumigatus* isolates to itraconazole, voriconazole and amphotericin B will be analysed by Etest on RPMI medium supplemented with 2% glucose, according to the manufacturer's instructions. A conidial suspension adjusted at 0.5 McFarland will be inoculated on RPMI 1640 agar plates (bioMérieux). Etest strips (bioMérieux) will then be applied, and the plates incubated for 48 hours at 37°C. Minimal inhibitory concentrations (MICs) of amphotericin B, itraconazole, voriconazole, posaconazole and will be tested after 24 hours and 48 hours incubation, respectively, depending on growth rate.³¹ They will be estimated visually as no-growth endpoints, where the edge of the inhibition ellipse intersects the side of the Etest strips.

METEOROLOGICAL SURVEILLANCE

Meteorological conditions monitored were temperature (degree celsius), relative humidity (percentage), wind direction and speed (kilometre per hour). They were recorded every 2 hours by Meteociel, the French regional meteorological station at Bron, 5 km from EHH (figure 1).

DATA ANALYSIS

Data quality control

Location of and information on sampling sites were recorded on paper and electronic database. To avoid typing errors, a computer model was created to clean up the electronic database. Every detected error was discussed by the infection control team and screened to assess its reliability. *A. fumigatus* isolates stored for data collection were correctly identified in a strain bank. Antifungal treatments, delivered by the hospital pharmacy, and microbiological data were extracted every week from infection control practitioners. Deconstruction work meetings involving clinicians, infection control practitioners and engineers, held every month, to ensure conformity with and respect of French deconstruction work guidelines.

Statistical analysis

Descriptive statistics, such as means, SD (or medians and quartiles), numbers and percentages, have been obtained for indoor and outdoor fungal loads. Incidence rates will be calculated as the ratio of detected IA cases over the population at risk during the study period. Generalised linear models (appropriate for outcomes) will examine correlations between MPs and fungal contamination. To compare results obtained with cultivable and non-cultivable methods, Pearson correlation coefficients

or Spearman's rank correlation coefficients (r) will be applied according to data normality. Time series, by the non-cultivable method, autocorrelation and cross-correlation across sites (EHH and Gerland), including meteorological factors at different time scales and time lag, will be studied. $P < 0.05$ values will be considered to be statistically significant. Statistical analyses will be performed with R language (V.3.0.2).

DISCUSSION

Clinical and environmental monitoring was reinforced during deconstruction work, according to standard French recommendations.³² No specific prophylaxis strategy was implemented at our hospital for immunocompromised patients. Only patients with acute leukaemia were susceptible to received prophylaxis therapy according to their condition. Outdoor and indoor environmental monitoring of contamination was implemented at EHH to prevent high-risk airborne fungal situations.

The present study addresses several questions. Its main objective is to detect clinical cases as soon as possible to adequately manage invasive fungal infection risk in hospital during major deconstruction work and report new findings for future guidelines. Are national recommendations on protective measures in high-risk units efficient in shielding patients from *A. fumigatus* aerocontamination? Totally, 3885 air samples were collected between 23 February 2015 and 17 December 2015. Air specimens, assessed by the cultivable method, were grouped into 2141 indoor and 1744 outdoor samples. A total of 296 days of recording were undertaken with the non-cultivable method. The high frequency and number of samples collected in this study during the three deconstruction periods will allow powerful statistical analysis to evaluate the efficiency of protective measures and reduce fungal contamination.

One of its objectives is to scrutinise the performance of non-cultivable methods of outdoor *Aspergillus* aerocontamination monitoring, such as Hirst-type spore traps, and to ascertain the impact of MP on *Aspergillus* aerocontamination. One preliminary study demonstrated potential interest in monitoring outdoor contamination.²¹ Complete data on airborne fungal concentration will be analysed by cultivable and non-cultivable sampling methods.^{33 34} Non-cultivable methods allow the sampling of numerous spores, useful to carry out surveys but have limitation in fungal spore's identification only possible at the genus level.³⁵ Cultivable method can identify spores at the species level but is time-consuming and depends on the substrate plated and culture condition applied.³⁶ Cultivable methods generally allow the identification at the species level. Cultivable method is required to perform genotyping and evaluation of antifungal susceptibility of the isolates. Cultivable and non-cultivable methods will be compared, which results could improve current prevention guidelines by providing early warning systems to hospitals.

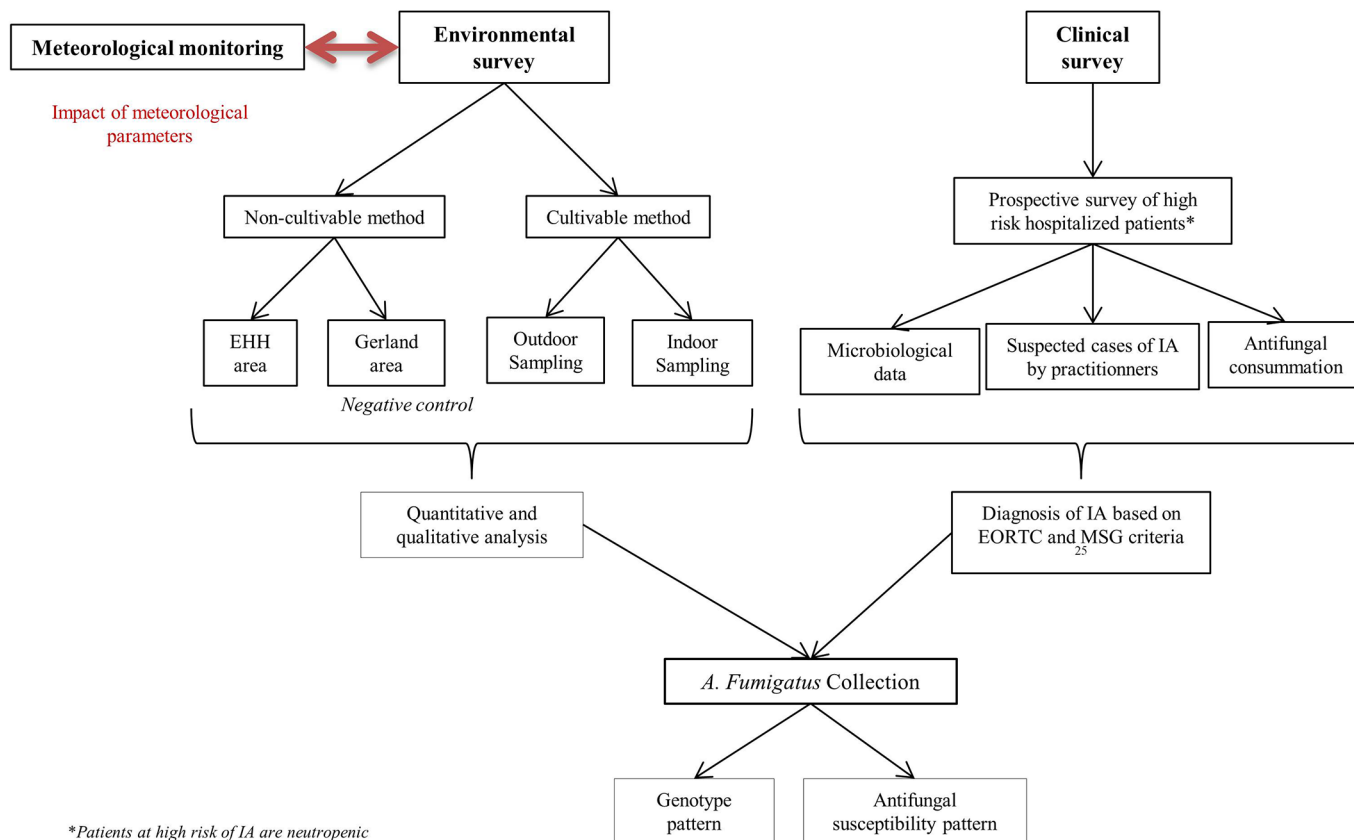


Figure 3 Flow chart of protocol study.

Part of the study analysis evaluated the impact of MP on airborne fungal contamination. The relationship between MPs and fungal spore concentrations has already been scrutinised.^{37 38} However, no study has investigated the role of meteorological factors in outdoor and indoor air contamination during construction works. A better understanding of MP impact on airborne fungi is needed to see if it could help to assess high-risk situations during construction work in hospital.

In addition, the present study compares the genetic diversity of clinical and environmental *A. fumigatus* isolates and profiles their antifungal susceptibility patterns. The aim is to describe *A. fumigatus* diversity over deconstruction periods along with genetic correlations between environmental and clinical strains collected. Correlation between environmental contamination and the occurrence of IA is hardly ever established due to limitations of both environmental sampling and genotyping.^{39 40} Only a few authors have demonstrated links between clinical and environmental genotypes, and only a small number of environmental strains were included in the analysis, limiting the possibility of finding matches.^{41 42}

The present work provides considerable data on potential IA outbreaks and determines genetic patterns among *A. fumigatus* isolates (figure 3). Principally two methods of genotyping have emerged for study of medically important fungi: multilocus sequence typing and method bases on short tandem repeats.⁴³ Comparison of several *A. fumigatus* typing techniques has suggested

that methods based on short tandem repeats combine high discriminatory power, unambiguous interpretation and significant interlaboratory reproducibility.^{44 45} MLVA based on 10 VNTR markers will be tested in this study: it has already permitted to evaluate *A. fumigatus* dispersion and diversity in the environment.²⁹ The MLVA-10 protocol normally employs singleplex PCRs and agarose gel electrophoresis (AE).²⁹ While traditional AE is relatively cheap, it is also time-consuming. In this study, a multiplex PCR system with multicolour CE for the MLVA-10 panel was designed and provided 97% typeability of isolates, good reproducibility and high discriminatory power. The main advantage of this new MLVA is the high throughput that is facilitated by multiplex PCR and CE.

The present study evaluates the antifungal susceptibility pattern of environmental and clinical isolates collected. The past decade has seen worldwide reports of azole drug resistance, with prevalence depending on country.^{11 46–48} Recently, Choukri *et al*¹² observed that the overall prevalence of azole resistance in France was about 1.8% in unselected clinical *A. fumigatus* isolates. Resistance has also been encountered in the environment, but possible linkage between clinical resistance and environmental usage of azole fungicides is still unclear.^{49 50} Environmental acquisition and dispersion of resistance have been reinforced by IA attributed to azole-resistant *A. fumigatus* isolates in patients who have never ever been subjected to azole therapy.⁵¹ Determination of the prevalence of azole

resistance may contribute to better management of IA cases.

Azole resistance and genetic patterns can provide experimental data to evaluate correlations between clinical and environmental isolates and clearly understand *A. fumigatus* dispersion during large demolition programme.

This work had some limitations. Adjustment of MP could change the data analysis and conclusions. As IA incubation time is unknown or variable, ranging from a few days to 3 months, its diagnosis may involve some bias.^{52–56} Precise definition by EORTC/MSG was respected to identify IA cases and reduce misclassification.²⁵ This protocol could not be implemented during a baseline period before construction work began. Microbiological identification will focus only on *Aspergillus* spp because it is the leading pathogen responsible for IA.³ To improve study outcomes, data on airborne fungal colonisation in hospital need to be analysed in association with indoor air-handling systems. Although two air sampling collectors were used in the study, no particle counter was tested. Klassen *et al* have underlined the existence of possible genetic differentiation and variable recombination rates of *A. fumigatus* which could prevent correct analysis of genotyping result.⁵⁷

A combined approach could deliver meaningful conclusions on *Aspergillus* spp dispersion and hopefully give new insights into improvement of environmental monitoring and of hospital guidelines during major demolition work. This study fits in with larger, ongoing exposome research based on increased knowledge of connections between environmental exposure and health.⁵⁸

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