European Journal of Epidemiology **19**: 693–698, 2004.

INFECTIOUS DISEASES

Two years of a fungal aerobiocontamination survey in a Florentine haematology ward

Gabriella Pini[1], Rosa Donato[1], Elisabetta Faggi[1] & Rosa Fanci[2]
[1]*Department of Public Health;* [2]*Department of Haematology, University of Florence, Florence, Italy*

Accepted in revised form 27 October 2003

Abstract. The control of microbial air contamination in hospital wards has assumed great importance particularly for those hospital infections where an airborne infection route is hypothesised, such as aspergillosis. Invasive aspergillosis represents one of the most serious complications in immunocompromised patients. For some authors there is a direct association between this pathology and the concentrations of Aspergillus conidia in the air; in addition, reports of aspergillosis concurring during building construction have been frequent. In this study, two haematology wards were monitored for about 2 years in order to make both a qualitative and quantitative evaluation of fungal burden in the air, also in relation to major construction and demolition work taking place in the same building. Air samples were taken from the hospital rooms of neutropenic patients, in the corridors of their ward and outside the building. Total fungal concentration resulted higher outside (mean 572 Colony Forming Units/m^3 of air), lower in the corridors (147 CFU/m^3) and even lower in the rooms (50 CFU/m^3). In all the samples we found the development of at least one fungal colony. *Cladosporium* was the most frequently isolated genus (57%), in contrast to *Aspergillus* spp. (2%). The average concentration of *Cladosporium* spp. was 24 CFU/m^3 in the rooms, 78 CFU/m^3 in the corridors and 318 CFU/m^3 outside. The average concentration of *Aspergillus* spp. was 1.2 CFU/m^3 in the rooms, 3.5 CFU/m^3 in the corridors, 5.6 CFU/m^3 outside. Our observations show low concentrations of *Aspergillus fumigatus* and *A. flavus* in all the environments examined and particularly in the rooms (0.09 and 0.10 CFU/m^3 respectively); this observation could explain the absence of cases of invasive aspergillosis during the period of air monitoring in the two haematology wards.

Key words: Airborne fungi, *Aspergillus fumigatus*, Concentration, Invasive aspergillosis, Neutropenic patients

Introduction

In the last few years the control of microbial contamination levels in confined areas has assumed a role of primary importance for community health maintenance. Indeed, many experiences conducted in different environments, whether work, recreational, or health-related have been collected in order to better characterise from a qualitative and quantitative point of view the microbial contamination which, in some circumstances, can have a negative effect on man's health [1, 2].

The presence of a high percentage of microorganisms, even if not strictly pathogenic or opportunistic, can be correlated to the onset of hospital infections in those patients who, for various reasons, are in an immunodepressive state. For these subjects the quality of the environment, principally from the aspect of maintaining microbial contamination at moderate levels, is decisive even for prognostic purposes.

Hospital infections represent not only an important health problem linked to patient health, but in more advanced societies they constitute a true indicator of the quality of the hospital assistance offered [3].

This type of infection involves in more or less serious measures all countries at all levels of socioeconomic evolution. In Italy it has been estimated that, in about 8 million patients hospitalised annually, 369,000 hospital infections are found [4].

The microorganisms involved are mostly Gram negative bacteria (ex. Pseudomonadaceae), Gram positive (especially methicillin-resistant *Staphylococcus aureus*) and fungi represented mainly by the *Candida* and *Aspergillus* genera.

In hospital patients the risks of infection linked to environmental aerocontamination are mostly associated to the fungi of the *Aspergillus* genus (*A. fumigatus*, *A. terreus*, *A. flavus*), responsible for disseminated or localised infections which have often been described in hospitalised immunodepressed patients [5, 6]. These fungi are capable of reproducing on different kinds of substrata, for example decaying vegetation, ornamental plants, etc. They have also been isolated in air conditioning, humidifying and ventilation systems [2, 7, 8]. Fungal contamination inside the hospital is the result of a combination of various factors that are difficult to investigate; however, different authors have established that hospital infections caused by *Aspergillus* spp. occur with greater frequency when construction work in hospital wards are taking place or have just been completed [5, 9–11]. This has been associated with the increase of dust in the air which facilitates the spreading of the fungal particles present.

Invasive aspergillosis represents a heavy problem in particular for those patients with a serious and lengthy neutropenia (patients who have undergone blood-marrow transplants, or with leukaemia) [12–14]; the diagnosis of this type of infection is difficult and often late [15–17] for which reason, therapy in many cases is fruitless and mortality exceeds 50% [18–20].

Our study was conducted in two haematology wards of a hospital in Florence which admits neutropenic patients undergoing chemotherapy. This research had the scope of qualitatively and quantitatively evaluating the fungal burden present in the air in this environment, with the purpose of highlighting possible risk-situations for these patients. The choice of wards was also made in consideration of the fact that on the ground floor of this building major renovation work was going on.

Materials and methods

During the years 1999–2000 we collected air samples from two haematology wards on the first floor of a Florentine hospital: from January 1999 to December 2000 the samples were taken in the women's ward; for technical reasons it was not possible to take samples in the spring of 1999. From September 1999 to December 2000 our research was extended to the men's ward.

The air samples were taken twice a month between 10:00 and 12:00 a.m. in two rooms of each ward with neutropenic patients. The rooms monitored were under a 'controlled regime' which consisted mainly in particular behavioural measures: entrance limited to a minimum number of people, a ban on opening windows and on plants or flowers, measures adopted by the medical and nursing personnel and visitors, such as masks, caps and shoe coverings. From a structural point of view the rooms monitored were not equipped with HEPA filters, positive pressure or special devices for sterilising the air. At the same time the samples were taken also in the corridors immediately outside the rooms and in two points outside of the hospital building, one in front of the entrance and the other in the rear courtyard. Throughout our research 394 air samples were taken altogether: 154 in the rooms, 154 in the corridors and 86 outside.

To take the samples we used the Surface Air Systems device (SAS, PBI International, Milan, Italy) that allows direct impaction of the air aspirated onto the microbiological culture medium. For this study Sabouraud Dextrose Agar (Difco) with Chloramphenicol 0.05%, Penicillin 500 U/l, Streptomycin 0.5% contained in 'contact' type plates was used.

After preliminary trials, it was decided to draw 390 l of air in the rooms and 240 l in the corridors during the sampling; outside the volume of air taken was between 90 and 180 l, depending on the different atmospheric conditions. After 5 days of incubation at 25°, the colonies were counted and the concentration for m^3 of air of Colony Forming Units (CFU/m^3) was calculated.

The identification of the filamentous fungi was made directly from the isolation media, on the basis of both the macroscopic (colour, dimension, aspect) and microscopic (fructification) characteristics of the colony.

For the yeasts no species identification was made.

Statistical analysis was carried out using the Microsoft Excel 2000 statistical package by variance analysis.

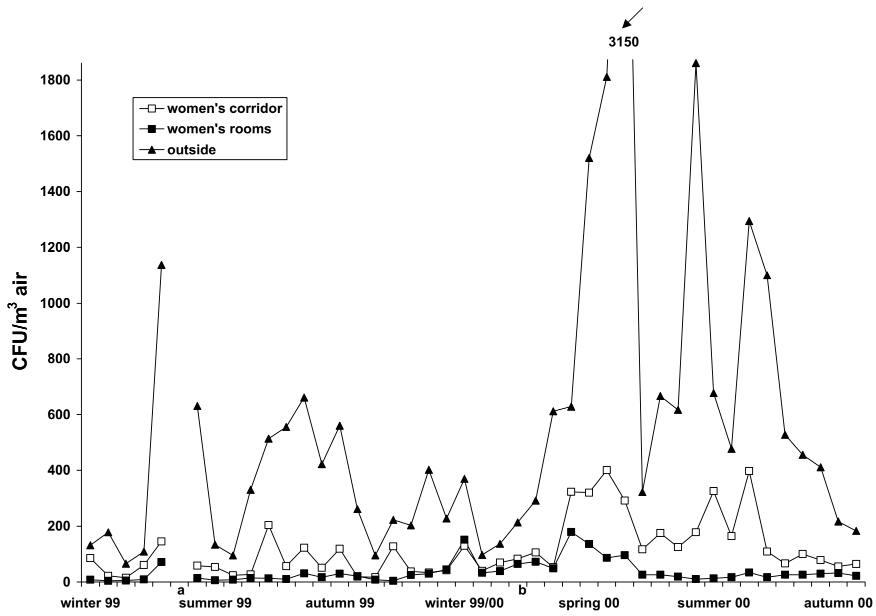
During our study and in particular in the period April–December 2000, there was major demolition and reconstruction work on the inside walls and flooring of the ground floor.

Results

In Figures 1 and 2 we report the course of fungal concentration during the 2 years monitored. Mean concentration is significantly higher outside the building (mean 572 CFU/m^3), compared to the corridors (147 CFU/m^3, $p < 0.001$) and to the rooms (50 CFU/m^3, $p < 0.001$). When outside concentration increases, in most cases we can observe an increase in the CFU values inside (in the corridors) which instead decrease when the outside fungal concentration decreases (Pearson correlation coefficient, $r = 0.78$, $p < 0.001$). Fungal CFU-number was significantly higher during the spring and summer both outside and in the corridors ($p = 0.009$ and 0.002 respectively) where particularly high fungal concentrations were observed during 2000. In the rooms we observed an important increase in winter and spring in comparison to the other seasons ($p < 0.001$).

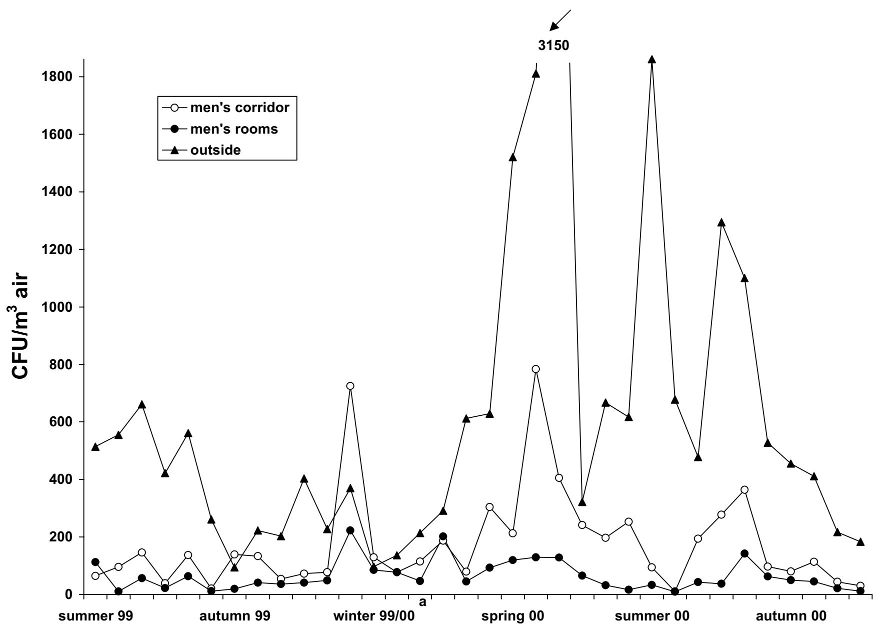
Comparing the results of summer 1999 and summer 2000 we observe an increase in fungal concentration that is significant in the corridors ($p = 0.01$) and rooms ($p = 0.02$) of the women's ward but not outside ($p = 0.07$); a comparison is not possible for the men's ward because there are no data for the summer of 1999. No significant increase was noticed in autumn. In the regime controlled rooms of the men's ward the average fungal concentration was significantly greater than in the women's ward ($p = 0.03$).

At least one fungal colony developed in every sample. *Cladosporium* was the most frequently iso-



a = For technical reasons it was not possible to take samples in the spring of 1999.
b = From April to December 2000, on the ground floor, important renovation work was goingon.

Figure 1. Fungal concentration (CFU/m^3) in the woman's ward and outside the hospital.



a = From April to December2000, on the ground floor, important renovation work was going on.

Figure 2. Fungal concentration (CFU/m^3) in the man's ward and outside the hospital.

lated genus (57%), followed by *Penicillium* (10%), *Alternaria* and other Dematiaceae (4%), *Aspergillus* (2%), *Paecilomyces* (2%), yeasts (2%) (Figure 3).

Considering the three sampling sites separately, the average concentration of *Cladosporium* was 24 CFU/m^3 in the rooms, 78 CFU/m^3 in the corridors and 318 CFU/m^3 outside. The average concentration of *Penicillium* was 7 CFU/m^3 in the rooms, 19 CFU/m^3 in the corridors and 37 CFU/m^3 outside. It is interesting to note that *Paecilomyces* has a higher mean CFU/m^3 in the corridors (4 CFU/m^3) than outside (2.3 CFU/m^3) and in the rooms (1.9 CFU/m^3), in contrast to the other fungal genera whose average of CFU/m^3 diminishes coming inside from outside the hospital and finally is minimum in the rooms (Table 1).

Among the species of the genus *Aspergillus* we isolated: *A. flavus* (6%) *A. fumigatus* (10%), *A. niger* (24%), *A. ochraceus* (4%), and other species of *Aspergillus* (56%) (Figure 4).

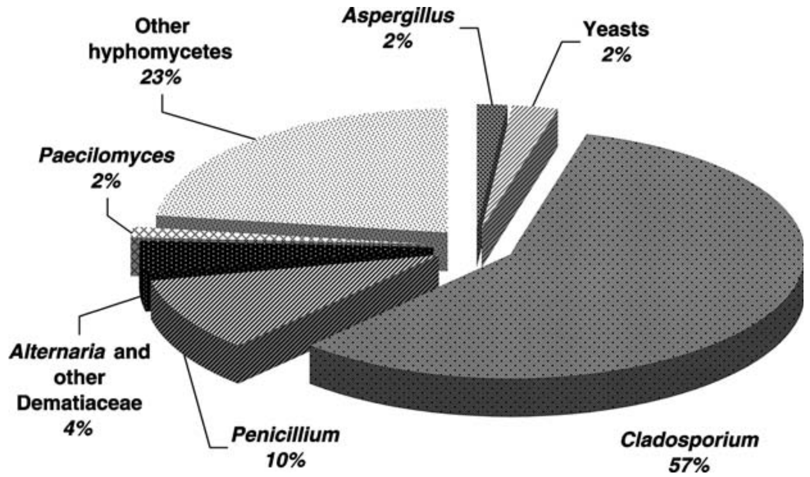


Figure 3. Distribution of mycotic genera collected from air samples in the haematology wards and outside the hospital.

Table 1. Mean concentration (CFU/m^3) of some fungal genera collected from different sampling sites

Sites of sampling	*Cladosporium*	*Penicillium*	Yeasts	*Paecilomyces*	*Aspergillus*	*Alternaria.* and other Dematiaceae	Other Hyphomycetes
Rooms	24.0	7.2	3.4	1.9	1.2	1.0	8.5
Corridors	78.0	19.0	3.8	4.0	3.5	4.1	26.5
Outside	318.1	36.8	8.7	2.3	5.6	26.9	130.2

A. fumigatus and *A. flavus*, which represent the species mainly responsible for cases of invasive aspergillosis, were found sporadically in the rooms (3% of the samples for each species), in the corridors (5 and 3% respectively) and outside the building (7 and 5%). The average concentration of *A. fumigatus* was 0.09 CFU/m^3 in the rooms, 0.21 CFU/m^3 in the corridors, 0.88 CFU/m^3 outside; this species was never isolated in spring. *A. flavus* was isolated in all seasons with a mean concentration of 0.1 CFU/m^3 in the rooms, 0.2 CFU/m^3 in the corridors, 0.39 CFU/m^3 outside (Table 2).

During the air monitoring period in the two haematology wards we observed no cases of invasive aspergillosis documented by clinical observations and microbiological exams.

Discussion and conclusions

The air monitoring in the haematology ward, conducted for about 2 consecutive years, was oriented to highlighting the airborne propagules; so the culture conditions were not optimal for all the species and

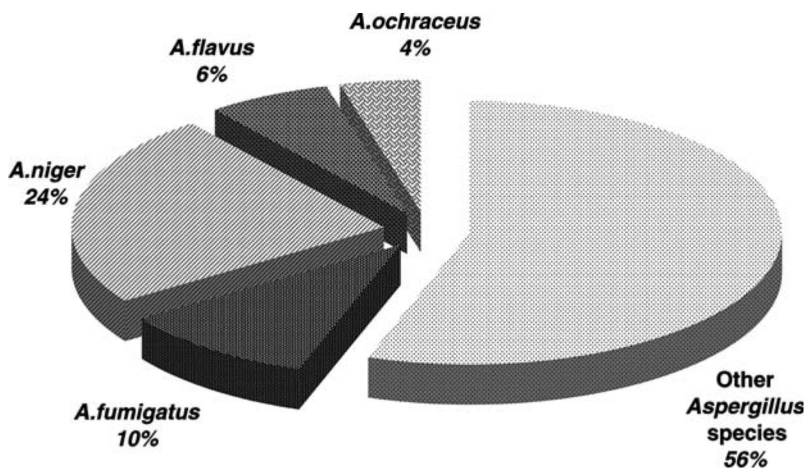


Figure 4. Distribution of *Aspergillus* species collected from air samples in the haematology wards and outside the hospital.

Table 2. Mean concentration (CFU/m^3) of *Aspergillus* species collected from different sampling sites

Places of sampling	*A. fumigatus*	*A. flavus*	*A. niger*	*A. ochraceus*	Other *Aspergillus* species
Rooms	0.09	0.10	0.18	0.03	0.87
Corridors	0.21	0.20	1.03	0.12	1.95
Outside	0.88	0.39	1.23	0.30	2.79

there might have been problems of competition in the development on the plate. This may have influenced the data relative to the air samples taken outside where the average mycotic concentration was 572 CFU/m^3, corresponding to a number that varied between 45 and 85 colonies per plate; however in the rooms there was never a strong concentration (average 50 CFU/m^3 corresponding to about 20 colonies per plate).

The data obtained during this study showed that the total CFU-numbers tend to increase from closed to open areas in the haematology ward, until a maximum outside the building. The low number of propagules found in the closed rooms of the ward may be the result of the level of environmental isolation (entrance limited to a minimum number of people, a ban on opening windows, etc.). However we must not forget that the increase in values of fungal concentration outside almost constantly means an increase, even if modest, in the ward rooms. This result reports therefore that outdoor concentration can directly influence indoor concentration.

While many authors [21, 22] report an increase in CFU concentration in summer, the observations on the influence of climatic conditions related to the seasons are contradictory for the *A. fumigatus* species: Daniau et al. [21] and Goodley et al. [23] do not report seasonal variations, Mullins et al. [24] indicates winter as the season with the highest concentration of conidia while for Jones and Cookson [25] it is summer-autumn. In our observations the CFU concentration was significantly higher in spring and summer both outside and in the corridors while in the rooms we observed a significant increase in winter and spring in comparison to the other seasons. From our data it is difficult to evaluate if climatic conditions had a true influence on the results obtained since it is important to remember that, in the spring of 2000 renovation work starting. Indeed, many studies refer to an increase in the concentration of Aspergillus [8, 26] and an increase in cases of invasive aspergillosis in relation to important construction or demolition work [5, 9, 11]. Nolard [10] reports observations on air monitoring during the demolition of a hospital wing and concludes that the increase in fungal spore concentration during this work is sudden and short-lived. Instead, there is a long-lasting increase in dust in closed environments [10]. Our results seem to agree with these observations, in fact, the samplings taken in the corridors and the rooms show an increase of CFU concentration in summer 2000 in comparison to the same period of the previous year; this increase was not found outside where, instead, we can notice an unusual peak of 3150 CFU/m^3 in the last sampling of spring 2000 and another slighter peak (1861 CFU/m^3) during the fourth sample in the summer. These peaks might coincide with major demolition in the downstairs ward undergoing restructuring. In the corridors and rooms the increase in total fungal concentration is more constant and lasts longer.

In the regime controlled rooms of the men's ward we found much higher average CFU concentrations than in the women's ward. Considering that the precautions taken by the health personnel in the two wards were the same and the number of patients was similar, the motive for this result seems difficult to interpret. The isolation of species such as *A. fumi gatus* and *A. flavus* was sporadic in all the environments examined so it was not possible to evaluate the seasonality and influence of the construction work on the concentrations of these species. Even the CFU/m^3 number for positive samples was very low except for the third outside sampling in the autumn of 2000 when we found 28 CFU/m^3 of *A. fumigatus* in a total CFU-number slightly lower than the average; inside on the same day, one sample taken from the corridor in the women's ward was positive.

The correlation between the grade of fungal aerobiocontamination and cases of invasive aspergillosis is admitted by different authors [10, 27]. Arnow et al. [7] in 6 years of surveillance for hospital aspergillosis found that an increase in the average of *A. fumigatus* and *A. flavus* concentrations from <0.2 to >1.1 CFU/m^3 in the air was accompanied by an increase in incidence of invasive aspergillosis from 0.3 to 1.2% in immunocompromised patients.

Our observations show low concentrations of *A. fumigatus* and *A. flavus* in all the environments examined. In the rooms the concentrations (0.09 and 0.10 CFU/m^3 respectively) are similar to that found by Arnow in non-epidemic periods, consequently the absence of cases of invasive aspergillosis in the period of air monitoring in the two haematology wards could be directly correlated to the low Aspergillus concentration found.

The monitoring of airborne fungi can have great importance in perspective of the prevention of hospital infections. In fact, it allows us to verify the efficiency of the prevention measures taken; it can account for occasional and incidental factors which may influence the risk increase linked to the exposition to a higher mycotic concentration such as particular meteorological situations or important construction or reconstruction work.

If prevention measures (sealing doors and windows and behavioural measures) are insufficient, adequate air filtration devices must be used [26].

Acknowledgements

We would like to thank Mr Cesare Berardi and Mr Roberto Citernesi for their technical support and the Department of Haematology at the Polyclinic of Careggi for their collaboration, in particular Mr Bartolacci.

References

1. Reynolds SJ, Black DW, Borin SS, et al. Indoor environmental quality in six commercial office buildings in the midwest United States. Appl Occup Environ Hyg 2001; 16: 1065–1077.
2. Parat S, Perdrix A, Baconnier P. Relationships between air conditioning, airborne microorganisms and health. Bull Acad Natl Med 1999; 183: 327–342.
3. Brusaferro S. Infezioni ospedaliere: modelli epidemiologici e strategie di prevenzione. Ann Ig 2001; 13(Suppl 2): 3–19.
4. Privitera G, Panceri ML, Castaldi S, Auxilia F. Il costo delle infezioni ospedaliere in Italia. Ann Ig 1998; 10(Suppl 2): 273.
5. Arnow PM, Andersen RL, Mainous PD, Smith EJ. Pulmonary aspergillosis during hospital renovation. Am Rev Respir Dis 1978; 118: 49–53.
6. Germaud P, Haloun A. Invasive nosocomial aspergillosis. Rev Pneumol Clin 2001; 57: 157–163.
7. Arnow PM, Sadigh M, Costas C, Weil D, Chudy R. Endemic and epidemic aspergillosis associated with in-hospital replication of *Aspergillus* organisms. J Inf Dis 1991; 164: 998–1002.
8. Nolard-Tintigner N, Snoeck R, Leleux A, Beguin H, Moonens F, Meunier-Carpentier F. Mise en evidence d'*Aspergillus fumigatus* lors de travaux de construction et de rénovation en milieu hospitalier. Bull Soc Fr Mycol Méd 1985; 14: 93–98.
9. Oren I, Haddad N, Finkelstein R, Rowe JM. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: Before and after chemoprophylaxis and institution of HEPA filters. Am J Hematol 2001; 66: 257–262.
10. Nolard N. Les liens entre les risques d'aspergillose et la contamination de l'environnement. Path Biol 1994; 42: 706–710.
11. Barnes RA, Rogers TR. Control of an outbreak of nosocomial aspergillosis by laminar air-flow isolation. J Hosp Infect 1989; 14: 89–94.
12. Bodey G, Bueltmann B, Duguid W, et al. Fungal infections in cancer patients: An international autopsy survey. Eur J Clin Microbiol Infect Dis 1992; 11: 99–109.
13. Sherertz RJ, Belani A, Kramer BS, et al. Impact of air filtration on nosocomial *Aspergillus* infections. Unique risk of bone marrow transplant recipients. Am J Med 1987; 83: 709–718.
14. Rogers TR. Infections in hematologic malignancy. Infect Control 1986; 7(Suppl 2): 140–143.
15. Aisner J, Wiernik PH, Schimpff SC. Treatment of invasive aspergillosis: Relation of early diagnosis and treatment to response. Ann Intern Med 1977; 86: 539–543.
16. Klont RR, Meis JFGM, Verweij PE. Critical assessment of issues in the diagnosis of invasive aspergillosis. Clin Microbiol Infect 2001; 7(Suppl 2): 32–37.
17. Jantuen E, Piilonen A, Volin L, et al. Diagnostic aspects of invasive *Aspergillus* infections in allogenic BMT recipients. Bone Marrow Trasplant 2000; 25: 867–871.
18. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: Systematic review of the literature. Clin Infect Dis 2001; 32: 358–366.
19. White MH, Anaissie EJ, Kusne S, et al. Amphotericin B colloidal dispersion vs. amphotericin B as therapy for invasive aspergillosis. Clin Infect Dis 1997; 24: 635–642.
20. Denning DW. Therapeutic outcome in invasive aspergillosis. Clin Infect Dis 1996; 23: 608–615.
21. Daniau C, Kauffmann-Lacroix C, Castel O. L'aérobiocontamination fongique en milieu hospitalier. J Mycol Méd 1998; 8: 139–146.
22. Leenders A, Van Belkum A, Behrendt M, Luijendijk AD, Verbrugh H. Density and molecular epidemiology of *Aspergillus* in air and relationship to outbreaks of *Aspergillus* infection. J Clin Microbiol 1999; 37: 1752–1757.
23. Goodley JM, Clayton YM, Hay RJ. Environmental sampling for aspergilli during building construction on a hospital site. J Hosp Infect 1994; 26: 27–35.
24. Mullins J, Hutcheson PS, Slavin RG. *Aspergillus fumigatus* spore concentration in outside air: Cardiff and St Louis compared. Clin Allerg 1984; 14: 351–354.
25. Jones BL, Cookson JT. Natural atmospheric microbial conditions in a typical suburban area. Appl Environ Microbiol 1983; 45: 919–934.
26. Mahieu LM, De Dooy JJ, Van Laer FA, Jansens H, Ieven MM. A prospective study on factors influencing *Aspergillus* spore load in the air during renovation works in a neonatal intensive care unit. J Hosp Infect 2000; 45: 191–197.
27. Alberti C, Bouakline A, Ribaud P, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in Haematology patients. J Hosp Infect 2001; 48: 198–206.

Address for correspondence: Gabriella Pini, Dipartimento di Sanità Pubblica, Viale Morgagni, 48, 50134 Florence, Italy
Phone: + 39-0553262443; Fax: + 39-0553262446
E-mail: gpini@unifi.it