

Monitoring Environmental *Aspergillus* spp. Contamination and Meteorological Factors in a Haematological Unit

M. Cavallo · S. Andreoni · M. G. Martinotti ·
M. Rinaldi · L. Fracchia

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Abstract The opportunistic pathogens belonging to the *Aspergillus* genus are present in almost all seasons of the year, and their concentration is related to meteorological conditions. The high density of *Aspergillus* spp. conidia in a haematological hospital ward may be a significant risk factor for developing invasive fungal diseases in immunocompromised patients. Aim of the present study was to evaluate the variability of airborne *Aspergillus* spp. conidia contamination in a Haematological Unit (HU) within a period of 16 months in relation with some meteorological parameters. An environmental *Aspergillus* surveillance was conducted in the HU in four rooms and their bathrooms, in the corridor and in three external sites using an agar impact sampler. During each sampling, temperature and relative humidity at each site were recorded and current wind speed and rainfall events were taken from the official weather service. *Aspergillus* spp. conidia concentration differed significantly across the sampling sites. Internal *Aspergillus* spp. loads were significantly dependent on temperature,

internal relative humidity and rain. External conidia concentrations were significantly influenced by outdoor temperature and relative humidity. A suitable indicator was introduced to evaluate the seasonal distribution of *Aspergillus* spp. conidia in the sampling sites, and a significant dependence on this indicator was observed inside the HU. Seventeen different fungal species belonging to the *Aspergillus* genus were detected during the sampling period. *Aspergillus fumigatus* was the most frequently isolated species and its distribution depended significantly on the seasonal indicator both inside and outside the hospital ward.

Keywords *Aspergillus* · Airborne contamination · Hospital · Seasonality · Neutropenic patients · Meteorological conditions · Outdoor

Introduction

The contamination of hospital environment by fungal spores is frequently implicated as the cause of aspergillosis in haematological wards or in intensive care units. The most common aetiological agent of aspergillosis is *Aspergillus fumigatus*, but *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and some other thermotolerant species of the genus are also involved in these diseases. In recent years, in fact, the

M. Cavallo · M. G. Martinotti · M. Rinaldi ·
L. Fracchia (✉)
Dipartimento di Scienze del Farmaco, Università del
Piemonte Orientale “A. Avogadro”, Largo Donegani 2,
28100 Novara, Italy
e-mail: fracchia@pharm.unipmn.it

S. Andreoni
Azienda Ospedaliero-Universitaria “Maggiore della
Carità”, Corso Mazzini 18, 28100 Novara, Italy

proportion of infections caused by non-*fumigatus Aspergillus* species has increased considerably [1, 2]. In immunocompromised patients, the inhalation of conidia may provoke pulmonary, invasive and disseminated infections associated with high mortalities.

Apart from the patient's immune status, the density of fungal conidia in the local environment may be a significant risk factor for developing invasive fungal diseases [3]. Sources of potentially pathogenic fungi can be found both outside and inside the hospital building. Fungal conidia enter hospital buildings through windows, doors or ventilation systems and sediment onto surfaces, survive in dust or grow on organic matter present in indoor environments [4, 5]. In addition, in the hospital setting, construction works that liberate large amounts of *Aspergillus* spores have been, as well, identified as source of nosocomial aspergillosis [6]. Therefore, the control of fungal air contamination in hospital wards has assumed great importance.

Fungal spores are an ever-present component of the atmosphere and are present in almost all seasons of the year [7, 8]. Nevertheless, sporulation and the dispersion of spores are closely related to variations in meteorological conditions [9, 10]. A relation between *Aspergillus* spp. spore concentration in air and seasonal changes has often been observed; however, a clear seasonal trend for airborne *Aspergillus* spp. dispersion cannot be defined, suggesting that the variation might be primarily related to the contingent meteorological conditions and the geographic characteristics of the sampling location [11–13].

The aims of the present work were to (1) assess the variability of airborne *Aspergillus* spp. contamination inside and outside a haematological hospital department within a period of 16 months; (2) evaluate whether a relation with different meteorological parameters could be observed; and (3) identify and study the prevalence of *Aspergillus* species isolated inside and outside the hospital department.

Methods

Air Sampling

Air sampling was carried out on sixteen different days (4 days for each season) between July 2004 and November 2005 in the Clinical Medicine

Department—Haematological Unit (HU) of the Azienda Ospedaliero-Universitaria “Maggiore della Carità” (Novara, Italy). The air sampling was performed in fifteen sites: in four rooms with neutropenic patients and in their bathrooms (R1/B1, R2/B2, R3/B3, R4/B4), in four sites in the corridor located immediately outside the rooms (C1, C2, C3, C4) and in three sites outside the hospital building, one in front of the building, one near a main road outside the hospital perimeter and one in the front courtyard (EA, EB, EC). The rooms monitored in this study were not equipped with high-efficiency particulate air (HEPA) filters, positive pressure or special devices for air sterilization. The room hosting patients in neutropenic phase were subjected to behavioural protective measures such as a ban on windows opening and on plant or flowers, entrance limited to a minimum number of people provided with masks, disposable white coats, caps and shoe coverings. Throughout our research, air samples were taken altogether in the rooms/bathrooms, in the corridor and outside the hospital building.

Air samples were collected at a height of 1.5 m above the ground level by means of an agar impact sampler SAS (Surface Air System-Super 180; PBI International, Milan, Italy), which aspirates air at a fixed speed onto a 90-mm contact plate filled with Sabouraud Dextrose Agar (Oxoid, Basingstoke, United Kingdom) added with chloramphenicol (40 mg/L). The *Aspergillus* spp. conidia concentration was evaluated by collecting, in each site, 10 air samples of 200 L each, as suggested by preliminary trials performed to determine a countable number of fungal colonies on the sampling plates. After 3–5 days of incubation at 37 °C, the *Aspergillus* spp. colonies were counted and results were expressed as colony-forming units per m³ (cfu/m³). The colony counts were corrected to account for multiple hits with the correction tables provided by International PBI. *Aspergillus* spp. were identified on the basis of both culture characteristics and morphology of conidiophores and conidia [14].

Meteorological Data

On sampling days, current temperature and humidity were detected in each site by thermohygrometer Igrotemp (PBI International, Milan, Italy); wind speed, rainfall data and mean daily temperatures were

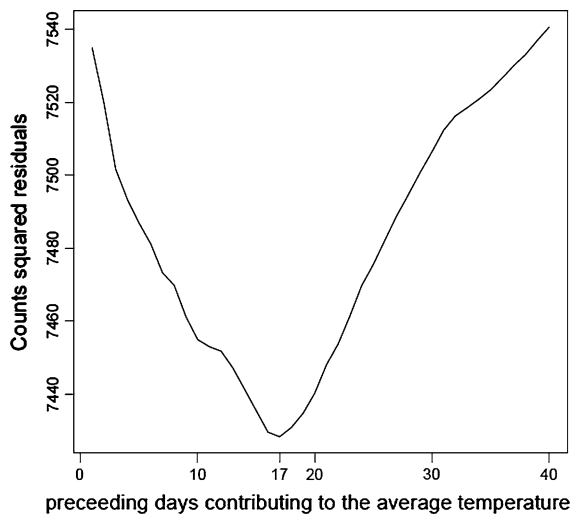


Fig. 1 Squared residuals sum of the liner model for *Aspergillus* spp. counts versus the external average temperature mediated over a period of length n (days) prior to the observations adjusted for humidity, wind speed and rain. The value of n ranges between 0 and 40. The minimum is achieved by choosing $n = 17$

obtained from the Italian Air Force weather service (<http://www.meteoam.it/>).

Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the effect of sampling sites and meteorological parameters on *Aspergillus* spp. conidia concentration. ANOVA was also used to compare *Aspergillus* spp. conidia concentration in restricted access and non-restricted access rooms. Principal component analysis (PCA) based on the detected *Aspergillus* spp. conidia concentrations during the observation period was carried out to evaluate the similarity among sites.

In order to evaluate the effect of the seasonal trend on conidia concentrations, the mean daily temperatures were averaged on a variable number n of days preceding each observation (T_n). For each n in the range 0–40, a linear model with outcome the observed counts and features T_n , humidity, wind speed and rain was considered. The sum of squared residuals of the linear models were obtained and plotted in Fig. 1. The minimum is achieved by choosing $n = 17$ days.

Statistical analysis was carried out by means of the statistical program R (R Core Team, <http://www.R-project.org/>).

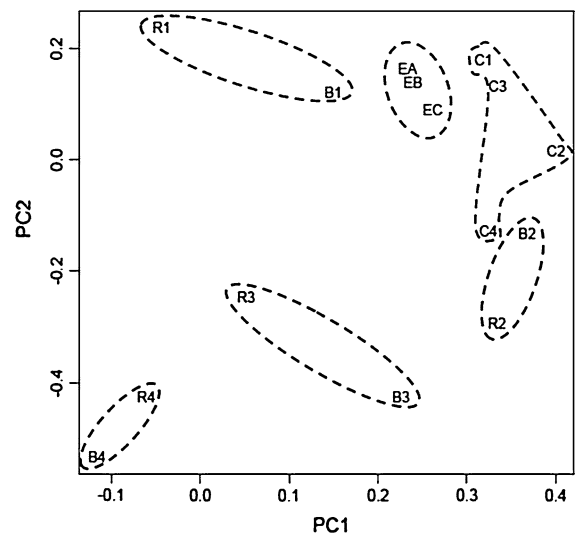


Fig. 2 Principal component analysis of *Aspergillus* spp. conidia concentrations in the different sampled sites

Results

Principal component analysis was carried out on *Aspergillus* spp. conidia concentrations considering the overall sampling period, and the first 2 Principal Components (PC1 and PC2) are reported in Fig. 2. The analysis evidenced a high similarity in relation to conidia concentration between each room and its bathroom, and the same among the corridors and among the external sites, as shown by the dashed contours. According to ANOVA, *Aspergillus* spp. conidia concentration differed significantly ($P < 0.001$) across the sites grouped in this way inside the hospital ward.

Table 1 shows the mean *Aspergillus* spp. conidia concentrations detected in each sampling day in the sites inside and outside the hospital in relation with relative humidity, temperature, wind speed and rainfall events. Mean conidia concentrations ranged between 1.1 and 14.1 cfu/m³ inside the hospital ward and 0.0 and 18.1 cfu/m³ in the external sites. In both cases, the lowest values were detected on May 17, 2005, the highest on Oct 26, 2004 (rainfall 7.0 mm). In the internal sites, *Aspergillus* spp. conidia concentrations higher than 6 cfu/m³ were detected also on Jul 27, 2004 (6.4 cfu/m³), Oct 5, 2004 (8.8 cfu/m³), May 3, 2005 (6.2 cfu/m³, rainfall 0.6 mm) and Aug 2, 2005 (11.7 cfu/m³, rainfall 43.2 mm), and in the external sites, on Oct 5, 2004 (11.5 cfu/m³), Dec 14, 2004

Table 1 *Aspergillus* spp. conidia concentrations and meteorological parameters from July 2004 to November 2005

Sampling date (dd/mm/yyyy)	<i>Aspergillus</i> spp. conidia concentration (cfu/m ³)		Relative humidity (%)		Mean temperature (°C)		Mean wind speed (km/h)	Rain (mm)
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor		
27/07/2004	6.4	2.2	45.9	38.0	26.4	24.3	5.1	0.0
05/10/2004	8.8	11.5	59.3	52.0	24.3	19.1	2.7	0.0
26/10/2004	14.1	18.1	62.6	79.0	24.1	15.9	10.8	7.0
14/12/2004	4.1	11.7	36.3	46.0	25.3	1.5	2.3	0.0
18/01/2005	1.1	6.7	37.6	50.0	23.3	0.0	3.0	0.0
01/02/2005	5.3	1.5	30.6	26.0	25.0	4.6	9.2	0.0
15/02/2005	1.6	1.3	27.5	26.0	25.5	5.5	13.3	0.0
15/03/2005	1.7	0.7	32.8	32.0	25.7	11.3	2.6	0.0
19/04/2005	4.5	4.0	41.5	66.0	25.6	11.1	3.3	7.4
03/05/2005	6.2	0.5	46.3	41.0	25.1	19.6	3.0	0.6
17/05/2005	1.1	0.0	47.3	42.0	23.8	15.8	6.7	0.0
31/05/2005	4.5	0.3	50.9	35.0	26.2	25.5	5.7	0.0
05/07/2005	3.2	1.2	49.4	30.0	26.3	23.1	5.6	2.4
19/07/2005	3.3	0.5	49.4	48.0	26.3	22.9	4.2	1.0
02/08/2005	11.7	4.5	51.0	62.0	26.6	22.1	10.8	43.2
29/11/2005	3.0	7.8	33.8	46.0	23.8	1.9	1.4	2.2

(11.7 cfu/m³), Jan 18, 2005 (6.7 cfu/m³) and Nov 29, 2005 (7.8 cfu/m³, rainfall 2.2 mm).

The indoor and outdoor relative humidity was variable during the aerobiological monitoring period, showing peaks corresponding to rainfall. In particular, the highest values were detected on Oct 26, 2004 (rainfall 7.0 mm) with values of 62.6 and 79.0 %, respectively, inside and outside the hospital ward. Humidity values higher than 50 % were also observed inside the hospital ward on Oct 5, 2004 (59.3 %), May 31, 2005 (50.9 %) and Aug 2, 2005 (51.0 %, rainfall 43.2 mm), in the outdoor sites on Oct 5, 2004 (52.0 %), Apr 19, 2005 (66.0 %, rainfall 7.4 mm) and Aug 2, 2005 (62.0 %, rainfall 43.2 mm). The lowest values were detected in the two samplings carried out on Feb 2005 (30.6 and 27.5 % inside and 26.0 % outside the hospital ward).

Wind speed was for most of the sampling period below 7 km/h, with the exception of the sampling on Oct 26, 2004 (10.8 km/h, rainfall 7.0 mm), of the two on Feb 2005 (9.2 and 13.3 km/h, respectively) and on Aug 2, 2005 (10.5 km/h, rainfall 43.2 mm).

Figure 3 shows the trend of mean daily temperatures detected by the local military weather service 1 month before and throughout the sampling period. It

is clear that summer is characterized by the highest temperatures, winter by the lowest temperatures, and spring and autumn by the intermediate temperatures; however, the astronomical seasons do not always correspond to the changing of meteorological conditions, temperature in particular. For this reason, in order to evaluate the seasonal distribution of conidia, we assumed the observed counts to depend—in addition to humidity, wind speed and rain—also on the mean daily temperatures averaged over a period of 17 days preceding the observations (see “Methods” section). Such averaged temperature—hereafter referred to as *seasonality*—could be considered as a continuous substitute of the astronomical season.

Aspergillus spp. conidia concentrations inside the hospital ward were found to be significantly related to seasonality ($P < 0.01$), internal relative humidity ($P < 0.001$), internal temperature ($P < 0.05$) and rain ($P < 0.001$). External conidia concentrations were significantly influenced by outdoor relative humidity ($P < 0.001$) and current temperature ($P < 0.01$).

From Oct 5, 2004 to May 31, 2005, some rooms were kept under restricted access when hosting neutropenic patients. In these rooms, the average concentration of *Aspergillus* spp. was of 1.2 cfu/m³,

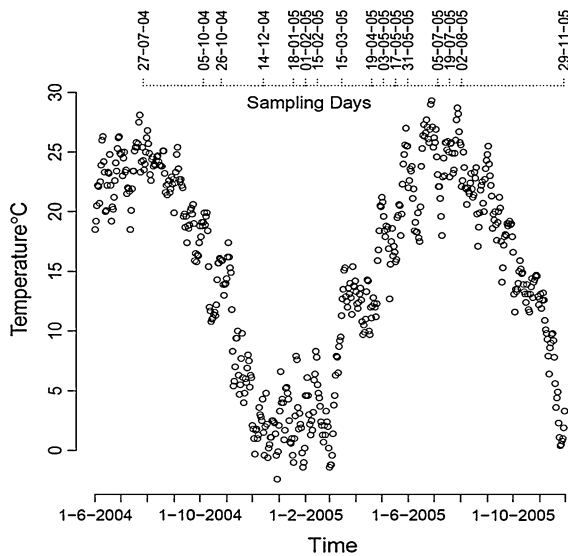


Fig. 3 Daily mean external temperatures detected by the local military weather service 1 month before and throughout the sampling period

whereas in the non-restricted access rooms, it was of 1.3 cfu/m^3 . The amount of conidia detected in the rooms was significantly dependent on seasonality ($P < 0.05$) but no differences of *Aspergillus* spp. contamination between restricted and non-restricted access rooms were observed ($P > 0.05$).

Seventeen different fungal species belonging to the *Aspergillus* genus were detected during the overall sampling period: *A. fumigatus*, *A. niger*, *A. flavus*, *A. nidulans*, *A. terreus*, *A. ustus*, *A. japonicus*, *A. flavipes*, *A. carneus*, *A. versicolor*, *A. glaucus*, *A. aculeatus*, *A. unguis*, *A. sydowii*, *A. penicilloides* and *A. deflectus* (Fig. 4). Inside the hospital ward, *A. fumigatus* was the most frequently isolated species, with a percentage of 54.7 %. The other most frequently isolated species was *A. niger* (19.7 %), followed by *A. flavus* (9.9 %) and *A. nidulans* (8.9 %). *A. terreus* was detected in lower percentage (2.7 %). ANOVA analysis indicated that *A. fumigatus*, *A. nidulans* and *A. terreus* conidia concentrations differed significantly ($P < 0.01$) between sampling sites. During the air monitoring period no cases of invasive aspergillosis (IA) were documented by clinical observations and microbiological exams.

In the external sites, *A. fumigatus* was still the most abundant species with a percentage of 46.2 %, followed by *A. niger* (27.4 %) and *A. flavus*

(11.8 %). *A. nidulans* and *A. terreus* were present in lower percentages (9.4 and 2.9 %, respectively).

It is interesting to note that, compared to the external sites, inside the hospital ward a greater variety of species belonging to the *Aspergillus* genus was detected.

Aspergillus fumigatus conidia concentration inside the hospital ward was found to be significantly dependent on seasonality ($P < 0.01$) and rain ($P < 0.001$); outside the hospital ward, it was significantly influenced by seasonality ($P < 0.01$) and outdoor temperature ($P < 0.001$). Indoor *A. niger* conidia concentration was significantly dependent on seasonality ($P < 0.01$), internal relative humidity ($P < 0.001$), internal temperature ($P < 0.05$), rain ($P < 0.05$) and wind ($P < 0.01$), whereas in the external sites, it was only dependent on the outdoor relative humidity ($P < 0.001$). *A. terreus* conidia concentration significantly depended on internal relative humidity ($P < 0.05$) inside the ward and temperature ($P < 0.01$) outside, *A. flavus* on relative humidity both inside and outside ($P < 0.001$), *A. nidulans* only on internal relative humidity ($P < 0.01$).

Discussion

In this study, sixteen aerobiological samplings were conducted from July 2004 to November 2005 at the General Medicine ward of the University Hospital “Maggiore della Carità” (Novara, Italy) and outside the same hospital. *Aspergillus* spp. conidia levels were monitored through the sampling period and the effect of some meteorological parameters on them was analysed, taking into account, for the first time, also the internal humidity and temperature. Moreover, the large number of samples collected in this study has allowed to detect even very low amounts of propagules, thus increasing *Aspergillus* spp. level of detection.

Inside the hospital ward, *Aspergillus* spp. concentration was significantly dependent on the site of sampling, relative humidity, temperature and rain whereas in the external sites only to relative humidity and temperature. The variation of *Aspergillus* spp. concentration in external sites in relation with meteorological factors has been described in many studies. Li and Kendrick [15] correlated the conidia

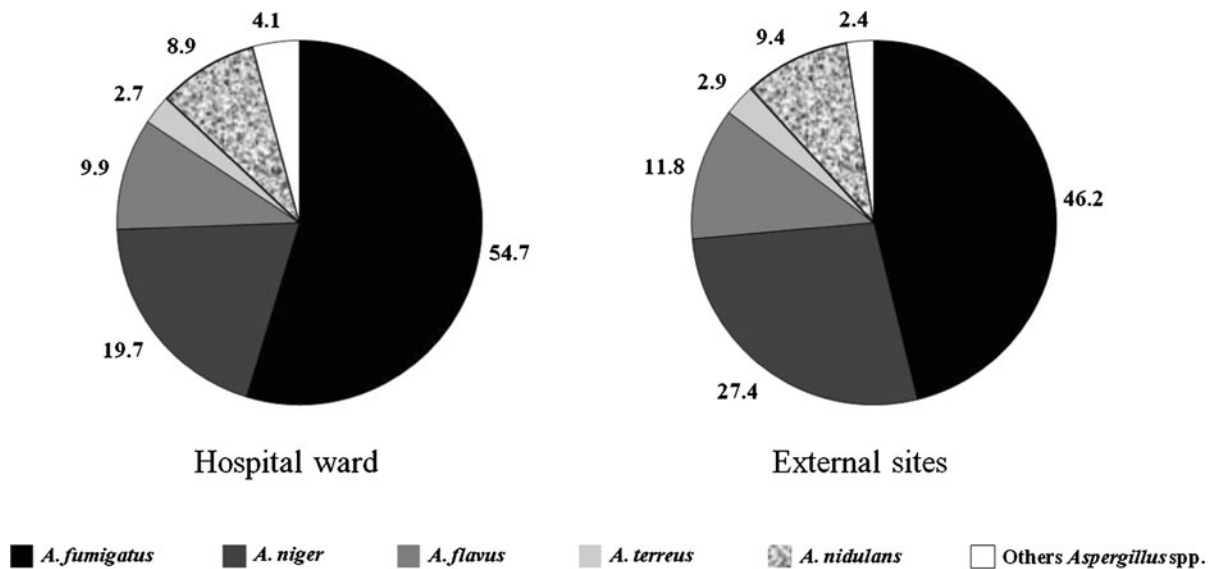


Fig. 4 Percentages of *Aspergillus* spp. in the hospital ward and in the external sites

concentration with wind speed from May to October and with temperature from November to April, and Guinea et al. [11] observed a correlation with temperature, monthly precipitation and the number of days with rainfall. Recently, Ruping et al. [3] correlated an increase in the concentration of *Aspergillus* spp. with increased humidity outside. In our survey, relative humidity and temperature seem to be the meteorological parameters with the highest influence on *Aspergillus* spp. conidia concentration both inside and outside the hospital ward.

To the best of our knowledge, no other studies evaluated the influence of meteorological factors on conidia dispersion inside a hospital ward. Surprisingly, conidia dispersion inside the ward was more subjected to the influence of environmental conditions than the external sites. Probably, the confined environment of the rooms limits the air exchange and promotes the accumulation of conidia.

Another novelty introduced in this study is the statistical computation of a time period preceding the observations (17 days) that could more likely represent the seasonal effect of external temperature, on *Aspergillus* conidia concentration, taking into account also humidity, wind speed and rain data collected on the sampling days. This effect has been defined as *seasonality*.

A significant dependence of *Aspergillus* spp. and *A. niger* counts with seasonality was observed inside the

hospital ward, and a dependence of *A. fumigatus* was observed both inside and outside.

Many studies tried to correlate airborne fungi dispersion with the astronomical season. Guinea et al. [11] collected, in outdoor environment, the highest loads of *Aspergillus* spores in autumn. This can be due to the availability of nutrient-rich decaying plant debris originating from fallen leaves, which promote the growth of the fungus. Research conducted by Augustowska and Dutkiewicz [12] in a pneumology ward revealed higher concentrations of *Aspergillus* spp. in winter and spring, whereas Satour et al. [13] detected, in a haematology department, higher levels in summer. Other studies conducted in various hematology wards have not described significant correlation of *Aspergillus* spp. conidia with the seasons [16–19]. In general, thus, a clear seasonal trend for airborne fungi dispersion cannot be defined, suggesting that the seasonal variations might be primarily related to the microclimatic conditions of the sampling location and to the contingent meteorological conditions (humidity, temperature, UV radiation, wind speed and direction, pollutants), as well as to the presence of vegetation in the nearby which can be source of fungal growth [20–22].

The amount of *Aspergillus* spp. conidia in the restricted access rooms was not significantly different to that observed in the non-restricted access rooms, suggesting that the behavioural protective measures

were not effective in decreasing the contamination of *Aspergillus* spp. The Centers for Disease Control and Prevention [23, 24] as well as the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) [25] and the Italian Academy of Medicine [26] recommend air filtration using HEPA filters in protective environment areas. However, Meneguetti et al. [27], in a recent review, have questioned whether HEPA filters are really necessary, concluding that invasive fungal infection in hemato-oncology patients is an outcome related also to many other risk factors, including the degree of immunosuppression, comorbidities, use of anti-fungal prophylaxis and the microbiological quality of the tap water in the units.

In our study, high levels of *Aspergillus* spp. conidia were occasionally detected in the hospital rooms and corridor (data not shown). At present, there is no consensus on a threshold limit for conidia concentration at which a significant risk of *Aspergillus* infection occurs [27–29]. *Aspergillus* air counts of less than 5 cfu/m³ in protective isolation suites are recommendable, although counts of less than 0.1–1 cfu/m³ are desirable [25, 30]. Peaks of air contamination by *Aspergillus* ≥ 2 cfu/m³ in conventional patient rooms (not equipped with HEPA absolute filters) had a determining role in the occurrence of IA [31]. In our study, cases of patients with IA were never recorded during the sampling period, although the recommendable threshold limit of 5 cfu/m³ was sometimes exceeded in the hospital ward.

Over 50 % of *Aspergillus* isolated in the ward was represented by *A. fumigatus*, the principal species involved in IA, and in the external sites, the situation was similar. As noted by Pini et al. [32], this thermophilic species can be more easily detected in environmental samples by using an incubation temperature of 37 °C. At temperatures below 30 °C *A. fumigatus* could be inhibited by non-thermophilic fast-growing fungal species such as *Cladosporium* and *Penicillium*, very abundant in the air, resulting in an underestimation of potentially more dangerous *Aspergillus* species.

In conclusion, according to our observations, *Aspergillus* spp. conidia dispersion was significantly influenced by meteorological conditions (i.e. relative humidity, rainfall and wind speed), and a clear seasonality of fungal dispersion could be observed. The increase in relative humidity and rainfall phenomena can be, thus, good indicators of the increase in

Aspergillus spp. concentration in the air. Hence, airborne *Aspergillus* spp. monitoring has a great importance, especially in hospital rooms hosting immunocompromised patients not equipped with modern systems for the isolation from the external environment.

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