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Original Research

Fungal Contamination of Air in the Department of Haematology

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Abstract

The aim of this study was to test the air in a single hospital department for fungal contamination. The department included three fully protected rooms with laminar air flow, comprising a bone marrow transplant unit (BMTU) and eleven naturally ventilated patient rooms of a haematology unit (HAEMU). Air samples were taken with an IDEAL air sampler (bioMerieux) on Sabouraud dextrose agar plates. The concentration of fungi in the air of the HEPA-filtered rooms of the BMTU ranged from 0-75 CFU/m^3. *Penicillium* and *Cladosporium* were dominant among the fungal biota in the whole department. Of aspergilli, *A. fumigatus* was prevalent and seasonal increases in the frequencies of *A. clavatus* and *A. niger* isolation were observed. The detection of potentially pathogenic species of *Aspergillus* and *Mucor* in the BMTU and an increased concentration of *Aspergillus* in the HAEMU (up to 200 CFU/m^3) instigated the introduction of additional preventive measure besides routine disinfection, namely an exchange of the HEPA filters in the BMTU and the installation of equipment based on multifunctional ion technology in the HAEMU. In a subsequent examination, a diminished number of fungi in the air was observed. During the study, 2 cases of proven and 3 of probable aspergillosis (according to EORT criteria) were noted. There was no link observed between the higher concentration of *Aspergillus* detected in the hospital air and the development of the infection. The authors conclude that hospital air examination can be helpful in indicating problems with hospital air facilities, enabling the introduction of procedures improving air quality and subsequently diminishing the risk of nosocomial mycoses.

Keywords: *Aspergillus*, aspergillosis, hospital air examination, haematology unit

Introduction

Patients with blood neoplasms constitute a group at high risk for invasive mycoses, of which invasive aspergillosis is the most frequent. Infections are usually located in the lower respiratory tract and nasal sinuses. Fungal spores are ubiquitously distributed in both outdoor and indoor environments and can easily enter the respiratory tract via inhalation. The development of mycoses depends on the number of conidia to which patients are exposed and the efficiency of the host defence, especially the activity of alveolar macrophages and neutrophils. Impaired immunological response, including periods of severe neutropenia, is a typical disorder observed in

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Table 1. Fungal contamination of the air in the BMTU.

Date of sampling	Range and mean ±SD of the CFU/m^3 found in three rooms:						
	Aspergillus	*Penicillium*	*Cladosporium* and *Alternaria*	*Mucoraceae*	Other*	Yeasts**	Total
November 2006	0-5 2.5±3.5	0-10 5±7.1	0-10 5±7.1	0	0-10 5±7.1	0	15-20 17.5±3.5
August 2007	10-20 15±7.1	5-45 25±28.3	0	0	0	0	25-55 40±21.2
November 2007	15-60 30 ± 26.0	0	0	15-15 15±0	0	0	30-75 45 ± 26.0
April 2008	0	0-5 1.6±2.9	0-5 1.6±2.9	0	5-25 11.6±11.5	0	10-25 15±8.7
June 2008	0	5-25 15±10	5-15 10±5	0	5-15 10±5	0-5 1.6±2.9	25-60 38.3±18.9
October 2008	0	0	0	0	0	0	0

*filamentous fungi excluding *Aspergillus, Penicillium, Cladosporium, Alternaria,* and *Mucoraceae* representatives
**strains from the taxa: *Rhodotorula, Cryptococcus laurentii, Candida parapsilosis,* and *Candida* spp.

patients with blood neoplasms and cytotoxic and immunosuppressive chemo- and corticosteroid therapies supporting that health state. Immunological response disorders are regarded as the main reason for frequent invasive aspergillosis in this group of patients. Despite the new antifungal drugs introduced in the last decade, mortality from invasive mycoses is high and ranges from 50-80% [1-3].

The protection of high-risk patients against the development of mycoses is a task of prime importance. There are two solutions: the first based on antifungal therapy (preemptive, prophylactic, or empiric) and the second relying on placing the patients in a fungus-free environment [1-4]. The preventive procedures established for hospital units with high-risk patients are meant to eliminate or significantly diminish fungal contamination in hospital environments. These procedures recommend air filtration with high-efficiency particulate air (HEPA) filter systems, laminar air-flow systems, positive air-pressure rooms, and high air exchange rates. The effectiveness of such procedures has been well documented [5, 6]. However, full antimicrobial air protection cannot encompass the whole hospital and is restricted to select rooms occupied by patients with the highest risk (mostly those treated with mega-chemotherapy and those with prolonged neutropenia). An important consequence of this procedure may be a change in the incidence of aspergillosis, which may become a rare infection in high-risk patients placed in fungus-protected rooms, but a more frequent one in patients at lower risk exposed to airborne fungi.

The aim of this study was to determine fungal air contamination in a single hospital department that included fully protected rooms with laminar air flow as well as naturally ventilated rooms in which the air quality was later improved by the installation of equipment based on multifunctional ion technology (MFI).

Material and Methods

Hospital

The hospital department was located on the first floor of a 100-year-old building. The department consisted of three patient rooms of the bone marrow transplantation unit (BMTU) and 11 patient rooms of the haematology ward (HAEMU). The BMTU rooms and 3 of the 11 HAEMU rooms were one-bed rooms, whereas there were 2 to 4 beds in the remaining patient rooms. The BMTU rooms were equipped with a HEPA filtration system with laminar flow and no opened windows. The HAEMU rooms were naturally ventilated, but then a Genano 310 Medical Air Cleaning System (Genano Ltd., Finland) was installed in September 2007. The HAEMU also possessed two treatment rooms intended for small surgery and injections, which were also included in the study. These rooms were naturally ventilated and disinfected daily with 2- to 5-h UV radiation.

Examination of Hospital Air

Hospital air was examined eight times (Tables 1 and 2). Air samples were taken from the patient rooms and the two treatment rooms using an IDEAL air sampler (bioMerieux). Fifty litres of air were taken on Sabouraud dextrose agar plates with chloramphenicole (four plates in each room). Each examination was performed early in the morning with the doors and windows closed and the patients present in the room. The samples were incubated at 28°C for up to 10 days. To exclude the possibility of counting daughter mould colonies, all the plates were read after 2 days of incubation and the number of colonies was updated to include slowly growing strains. The number of colony-forming units (CFU) per cubic meter of air was calculated on the basis of

Table 2. Fungal contamination of the air in HAEMU.

Date of sampling	Range and mean ±SD of the CFU/m^3 found in 11 rooms:						
	Aspergillus	*Penicillium*	*Cladosporium* and *Alternaria*	*Mucoraceae*	Other*	Yeasts**	Total
November 2006	0-35 16.9±11.3	10-65 32.65±21.95	0-25 10.9±10.8	0-5 0.6±2.0	6.6-70 23.7±21.5	0-10 2.7±3.4	40-153 87.1±35.6
August 2007	5-198 46.3±58.6	25-101 61±28.05	20-101 49.6±22.0	0	0-80 36.8±28.5	0-5 0.5±1.5	105-383 193.7±84.5
November 2007	0-35 8.6±10	0-40 14.5 ±11.9	0-5 0.9±2.0	0-5 0.5±1.5	0-35 18.6±17.1	0-20 1.8±6	5-80 45±21.7
April 2008	0-5 0.5±1.50	0-25 6.5±8.1	0-5 1±2.1	0	20-70 38±13.8	0-5 0.5±1.5	25-70 46.5±13.7
June 2008	0-35 7.7±10.0	0-100 21.8±29	0-45 19.5±15	0-5 0.5±1.5	0-55 35.4±19	0-50 7.7±14.6	0-205 85.5±55.0
August 2008	0-5 1.9±2.5	5-45 24.4±14.7	35-115 67.5±26.6	0	20-70 52.5±16.4	0-10 3.1±3.7	110-205 149.4±30.9
October 2008	0-205 33.5±63.6	0-65 21±18.4	5-110 28.5±31.5	0-5 1±2.1	0-160 53±43.3	0-80 11.5±24.2	25-440 149±130.8
June 2009	0-10 1.8±3.37	0-80 28.63±25.3	5-95 35±24.1	0	10-65 32.3±16.3	0-10 3.1±4.0	25-205 100.9±53

*filamentous fungi excluding *Aspergillus, Penicillium, Cladosporium, Alternaria,* and *Mucoraceae* representatives
**strains belonged to the taxa: *Rhodotorula, Cryptococcus laurentii, Candida parapsilosis,* and *Candida* spp.

the number of fungal colonies and the volume of the air sample, according to the manufacturer's instructions. The fungi were identified by morphology (moulds) and biochemical tests (yeasts).

Student's t test and the STATISTICA software system (version 8.0.; StatSoft, 2008) were used for statistical analysis.

Results

The results of the air examinations in the HAEMU and BMTU are presented in Tables 1-3. The fungal contamination of the air in the BMTU rooms equipped with the HEPA filter system ranged from 0 to 75 CFU/m^3 and was significantly lower than in the HAEMU ($p<0.0005$). Potentially pathogenic species of *Aspergillus* were found in the air of the BMTU in 2006 and 2007 and *Mucor* species were noted in November 2007. In December 2007 the HEPA filters of the BMTU air-conditioning system were exchanged and then no *Aspergillus* or *Mucor* were isolated from the air samples. *Penicillium* and *Cladosporium* genera, known to be non-pathogenic, were dominant among the fungal biota in the BMTU.

In the HAEMU the concentrations of fungi were higher in the summer months than in the spring and autumn. The highest concentration of fungi were observed in August 2007. In September 2007 the unit was disinfected and the MFI equipment was installed. The air examination performed in November 2007 revealed that the fungal contamination of the air decreased significantly ($p<0.005$). In the next months, except for April 2008, the concentration of fungi increased, but did not achieve the level of August 2007. The analysis of fungal species in the HAEMU showed that *Cladosporium* and *Penicilium* dominated in all the seasons, whereas the frequency of *Aspergillus* representatives varied in particular years. Elevated concentrations of *Aspergillus* in the air and higher numbers of patient rooms with airborne *Aspergillus* were observed in 2006 and 2007 (about 100%) (Tables 2 and 3). *A. fumigatus* was the most frequently isolated *Aspergillus* species. Other pathogenic filamentous fungi, such as *Fusarium* and *Mucoraceae* spp., were observed only in a few cases. Yeasts (mainly *Rhodotorula* and *Candida parapsilosis*) were isolated rarely, whereas *C. albicans* was not found in any tested air sample.

Like in the HAEMU patient rooms, the concentration of fungal aerosol in the treatment rooms ranged from 15 to 195 CFU/m^3, with the maximum in August of 2006 and 2008. The distribution of the fungal species in the air of the treatment rooms was also similar to the remaining part of the HAEMU. *Cladosporium* and *Penicillium* were predominant and the occurrence of *Aspergillus* was highest in November 2006 (40 and 20 CFU/m^3), whereas in the rest of the examinations it ranged from 0-10 CFU/m^3.

Discussion

Airborne fungi are regarded as the major cause of nosocomial aspergilosis. However, epidemiological examination with the use of molecular methods usually does not support this if environmental and clinical isolates represent different genotypes [7, 8]. In the present study we tested

Table 3. Number of rooms contaminated with *Aspergillus* species.

Date of sampling	Number of rooms with positive results/total tested rooms in the particular hospital unit			Species isolated
	BMTU	HAEMU	Operating rooms	
November 2006	1/3	10/11	2/2	*A. fumigatus, A. niger A.* sp
August 2007	2/2	11/11	2/2	*A. clavatus A. candidus A. fumigatus A. ochraceus A.* sp
November 2007	3/3	7/11	1/2	*A. fumigatus A. niger A.* sp
April 2008	0/3	1/11	0/2	*A. fumigatus*
June 2008	0/3	8/11	1/2	*A. fumigatus A.* sp
August 2008	Not tested	3/8	1/2	*A. clavatus A. niger A. fumigatus A. flavus*
October 2008	0/3	6/10	0/2	*A. clavatus A. niger A.* sp
June 2009	Not tested	2/11	1/2	*A. fumigatus A.* sp

fungal contamination in rooms with and without HEPA-filtered air. Until 2006, when the air in our department was tested by the sedimentation method, moulds were cultured very seldom (0-0.1 CFU/m^3), even from samples taken in unprotected rooms [9]. The poor efficiency of this method of detecting moulds has already been described [10]. The introduction of the mechanical air sampler showed the real level of fungal air contamination. The pathogenic fungi *Aspergillus* and *Mucor* were isolated even from the BMTU (Table 1). It should be emphasized that there was no known technical problem with the filter system and that mycological examination was the only available method of detecting air contamination. The preventive procedures, including exchange of the HEPA filters, improved air quality in the BMTU, but fungi were still detected in most air samples. To the best of our knowledge, there are no accepted guidelines recommending interpretative criteria of fungal contamination in particular hospital wards. Some authorities suggest that fungal air contamination in HEPA-filtered rooms should not exceed 0.1 CFU/m^3. The detection of more than 1 CFU/m^3 is a strong indication to evaluate the air system and procedures [10]. Thus the air quality in our BMTU, although much better than in the HAEMU, did not fulfil this criterion except in October 2008. The level of fungal contamination in a HEPA-filtered BMTU reported by other authors [11] was also much higher than 1 CFU/m^3 (mean 18-47 CFU/m^3) and 27-54% of samples were positive for *Aspergillus*.

The concentration of fungi in the air of the naturally ventilated rooms of the HAEMU ranged up to 200 CFU/m^3 *Aspergillus* and 380 CFU/m^3 total fungi. To improve the air quality, equipment based on multifunctional ion technology (MFI) was installed in each patient room. MFI uses electrostatic precipitation and ion beams that push pollutants onto a collection surface, which is subsequently automatically cleaned. This method enables effective removal and killing of fungi and other microbes (manufacturer's brochure; www.genano.fi). Its usefulness in cleaning the air of an ophthalmic operating theatre has been reported recently [12]. Our study is the first that describes the impact of such devices on the mycobiota of air in a HAEMU. After installation of the equipment in November 2007, the concentration of airborne fungi significantly diminished compared with the previous months (Table 2). The low concentration of fungi was maintained for the next months, but in August 2008 an increase was observed. That peak in the number of fungi may be attributed to the lack of a professional air-conditioning system in our department and the seasonal increase in fungal aerosol outdoors. We conclude that the MFI equipment diminished the concentration of fungi, although it is clear that it cannot be effective enough in naturally ventilated rooms.

The role of routine air examination in the prevention of hospital-acquired infections has been widely discussed recently. In some studies a significant relationship was found between the incidence of invasive mycoses and fungal air contamination, especially in hospital wards not equipped with HEPA [13] and those which underwent building demolition or renovation [14]. Falvey and Streifel [11] and Rupp et al. [15], who analyzed the correlation between prospectively tested fungal air contamination in a HEPA-protected ward and the incidence of aspergillosis, indicated the uselessness of air screening in the prognosis of nosocomial airborne infection. In our department, despite the high frequency of isolated *Aspergillus* observed in some periods, the number of aspergillosis cases was low (5 cases among 240 tested patients). The low frequency of diagnosed aspergillosis may be a result of applying antifungal therapy "in advance" (strategies of prophylactic, pre-emptive, and empiric therapies), which possibly precludes the development of infection in many patients. The incidences of mycoses were not related to the detected increase in the air concentration of *Aspergillus*. However, we examined the air only a few times, which is why it is impossible to draw a definitive conclusion about such a relationship.

The development of invasive mycoses is often preceded by colonization of the mucosa of the respiratory and/or gastrointestinal tract. To test the frequency of fungal mucosa colonization, we examined, together with air samples, clinical specimens obtained from all currently hospitalized patients. Mould strains were isolated only from a few clinical samples. None of the fungus-positive patients developed invasive mycoses within two months after mould isolation [16].

Although the presented data did not reveal connections between fungal air contamination, patient colonization, and the development of invasive mycoses, they may present an example of the role which hospital air control plays. The detected air contamination enabled identifying problems with the hospital's air facilities and introduced procedures improving air quality, consequently diminishing the risk of nosocomial mycoses.

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