Journal of Hospital Infection (2005) **59**, 188–196

Available online at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevierhealth.com/journals/jhin

Aspergillus surveillance project at a large tertiary-care hospital

L. Curtis[a,*], S. Cali[a], L. Conroy[a], K. Baker[a], C.-H. Ou[a], R. Hershow[b], F. Norlock-Cruz[a], P. Scheff[a]

[a]*Department of Environmental and Occupational Health Science, University of Illinois at Chicago, Chicago, IL 60612, USA*
[b]*Department of Epidemiology, University of Illinois at Chicago, Chicago, IL 60612, USA*

Received 27 August 2003; accepted 13 May 2004

Available online 12 January 2005

KEYWORDS
Aspergillus; Aspergillosis; Hospital infections; Ventilation; asp f1

Summary A one-year surveillance project was conducted at a large tertiary hospital, which had extensive indoor renovation and extensive demolition/building at several nearby sites. This study collected viable fungi samples in the hospital every six days and analysed 74 duct dust samples for *Aspergillus fumigatus* mycelial asp f1 protein. Mean total fungi were 257.8 cfu/m^3 outdoors, 53.2 cfu/m^3 in all indoor samples and 83.5 cfu/m^3 in the bone marrow transplant patient rooms. Mean total aspergillus was 6.8 cfu/m^3 outdoors, 12.1 cfu/m^3 in all indoor samples and 7.3 cfu/m^3 in the bone marrow transplant patient rooms. The five most prevalent *Aspergillus* species collected inside the hospital (mean cfu/m^3) were *Aspergillus niger* 7.57 cfu/m^3, *Aspergillus candidus* 1.72 cfu/m^3, *Aspergillus flavus* 0.97 cfu/m^3, *A. fumigatus* 0.88 cfu/m^3 and *Aspergillus glaucus* 0.45 cfu/m^3. In rooms undergoing duct cleaning, mean *A. fumigatus* concentrations were 11.0 cfu/m^3. Forty-eight of 74 (65%) duct dust samples had measurable levels of asp f1 protein, with a mean level of 0.41 ppm and maximum level of 1.94 ppm. Three major incidents involved increased hospital aspergillus concentrations. *A. niger* levels reached 680 cfu/m^3 in an organ transplant room after a water leak from a ceiling pipe. Total aspergillus concentrations rose to 77 cfu/m^3 in a bone marrow transplant patient room after improper sealing and water infiltration of the unit's dedicated high-efficiency particulate air filter system. Total aspergillus levels of 160 cfu/m^3 were recorded in a renovation area during wood cutting. The higher concentrations of aspergillus seen inside the hospital compared with outdoors and the various moisture/HEPA filter/renovation incidents suggest that numerous small to moderate sources of aspergillus exist in the hospital.

*Corresponding author. Address: University of Illinois at Chicago, 1328 Greenwood, Wilmette, IL 60091, USA. Tel.: +1-847-256-3562.
E-mail address: lukec@uic.edu

0195-6701/$ - see front matter
doi:10.1016/j.jhin.2004.05.017

Introduction

The incidence of life-threatening invasive aspergillus infections has been increasing with increasing numbers of immunocompromised bone or organ transplant patients and patients with leukaemia, lymphoma and other malignancies. Incidence rates of invasive *Aspergillus* spp. infection are about 17–26% in lung transplant patients, 5–24% in acute leukaemia patients, 5–15% in allogenic bone marrow transplant patients, 2–13% in heart transplant patients, and 1–3% in lymphoma patients.[1,2] *Aspergillus fumigatus* is the most common species found in invasive aspergillus infections, although *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and other species also frequently cause invasive aspergillosis.[3,4]

Even with intensive medical treatment and antifungal medications, aspergillus infections are often fatal. Denning[5] studied 1223 invasive *Aspergillus* cases and reported crude mortalities of 86%, 66% and 99% for pulmonary, sinus and cerebral aspergillosis, respectively. Aspergillosis has become a leading infectious cause of death for bone marrow and organ transplant patients. A study of 2496 bone patients in a US tertiary cancer hospital reported 143 deaths due to invasive aspergillosis.[6] Invasive aspergillus infections are hard to diagnose at an early state, although use of immunological testing methods, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) may be useful.[7,8]

Given the poor treatment outcomes in invasive aspergillosis, environmental control to prevent infection is critical. Aspergillosis is known to be an airborne infection,[9] and construction work and increased dust loads have been associated with higher rates of airborne aspergillus and nosocomial aspergillosis.[10–14] Recent information suggests that hospital water supplies may also be an important vector for spread for aspergillosis.[15] Several detailed studies have found aspergillus spores in many parts of hospitals, including shower heads,[16] dusty air conditioners,[17] hospital plants,[18] clothing[19] and other sources.[20–22]

Several studies that have employed high-efficiency particulate air (HEPA) filters in the rooms of bone marrow transplant and haematological malignancy patients have noted significant declines in both airborne levels of aspergillus and in aspergillosis infection rates.[11,23–27] Some of these studies also employed additional controls for aspergillus such as intranasal amphotericin B,[24] or use of sealed rooms, replacement of perforated ceiling tiles and dust-accumulating blinds, and use of anti-fungal copper-8-quionolate paint.[23]

This year-long aspergillus study was conducted at the request of a major tertiary-care hospital, which had about 200 bone marrow and organ transplant patients per year. The hospital was concerned about possible aspergillus infections due to renovation work inside the hospital and several large demolition and building projects being undertaken within 1 km of the hospital. The study involved air sampling for aspergillus and other viable fungi, dust sampling for asp f1 protein, and ventilation studies.

Materials and methods

Air sampling and culture

A total of 842 bacterial and fungal samples were collected using an Andersen (Atlanta, GA, USA) one-stage bioaerosol sampler (N-6) between 9 September 1998 and 16 September 1999. For each air sample, 0.050 m^3 to 0.400 m^3 of air was collected, depending upon the expected fungal concentrations. The Andersen samplers were loaded with autoclaved malt extract agar medium for fungal isolation.[28]

During each sampling visit, a total of 16 air samples were collected in eight pairs. Sampling pairs ware collected every six days as follows: (1) two pairs of samples from patient rooms of two of the four units studied [cardiac intensive care unit (ICU), acquired immunodeficiency syndrome (AIDS) ward, organ transplant, bone marrow transplant], (2) two pairs of samples in the corridors of two of the units collected that day, (3) one pair of samples inside rooms where either construction/renovation or duct cleaning was conducted, (4) one pair of samples just outside rooms where construction/renovation or duct cleaning was conducted, (5) one pair of outdoor samples from ninth floor rooftop, and (6) two blank samples loaded on to air samplers, unloaded and cultured to test for sterility. During the following sampling period, samples were taken from the ward that was not sampled earlier, so that every unit was sampled every 12 days. A total of 64 days of sampling was performed. Of these, three sets were unusable due to problems with media or air collection equipment. One extra sampling period collected only six samples to confirm high levels of aspergillus seen in the study.

Samples were incubated at 25 °C for three days, after which time the number of viable fungal colonies were recorded. The samples were then

incubated for a further two to four days at 25 °C to allow maximum growth of isolates. Where confluent growth occurred by day five to seven, the three-day count was used. For samples with 20 or more viable spores per plate, a count correction factor was used to overcome the problem of undercounting as a result of more than one spore being present in each hole.[29]

Fungal colonies were examined using a light microscope (100–1000 ×) and classified to genera with the help of standard references for fungal identification.[30,31] *Aspergillus* spp., *Penicillium* spp. and several other fungal genera were classified to species with the help of standard references for *Aspergillus* spp., *Penicillium* spp. and other species.[31,32] Aspergillus with telemorph forms (such as *Eurotium Aspergillus glacus*) were included in the total aspergillus level.

Ventilation characterization

The ventilation system was characterized through several interviews with physical plant staff at the hospital and by performing intermittent ventilation measurements in a total of 30 patient rooms over 22 dates interspersed throughout the sampling period. The ventilation measurements were made with an Alnor Balometer or a TSI Velocicalc thermo-anemometer air velocity measurement device at supply and exhaust grills.

Dust sampling

Seventy-four dust samples were collected from portable HEPA filter vacuum bags used by hospital maintenance staff to clean the duct covers in patient rooms and other areas. These bag samples were collected opportunistically and the sampling protocol was not directly linked to the airborne sampling. The maintenance staff marked the duct cleaning bags by location and date, and the bags were transported to our laboratory. Approximately 100 mg of dust was taken out of each bag and analysed using the sandwich ELISA method of Arruda.[33] This method measures the asp f1 protein, which is found in the mycelia of *A. fumigatus* but not in the spores. Various concentrations of asp f1 protein standard and blanks were analysed to get a standard curve to calculate asp f1 concentrations. Eight blank samples were analysed and the detection limit was set as two standard deviations higher than the blank samples.

Statistics

Differences between mean indoor and outdoor levels of fungi were determined using a two tailed *t*-test with unequal variances. Differences in fungi levels inside and outside renovation areas and during activity or no activity were determined by a two-way analysis of variance (ANOVA) program. All statistical analyses were done using SPSS version 6.1 (1994 SPCC, Incorporated-Chicago, IL, USA).

Results

Ventilation of units

All four patient units had different ventilation configurations. All of them are humidified when necessary by steam sprayed into the systems downstream from the supply fans, and dehumidified when necessary by the respective cooling coils in each system.

The cardiac ICU is supplied by ceiling supply and exhaust vents. These are fed with 100% fresh air conditioned for heat by heating coils above the ceiling, and cooled by a chiller located near the air handling unit for that area.

The AIDS treatment area is ventilated by window induction units supplied with air from a dedicated fan. The air is expelled to the rooms by a jet that causes air in the room to be induced into the unit face and back out through the top of the unit. The air is conditioned by hot and cold coils located in the induction unit. This air is mostly re-circulated, with some being exhausted to bathroom exhausts or through the doorway by positive pressure.

The organ transplant unit is ventilated in a manner similar to that of cardiac ICU, except the air is not 100% fresh. The air is re-circulated with general hospital air.

The bone marrow transplant unit is ventilated by a dedicated, HEPA filtered system that is designed to keep the rooms and unit very strongly positively pressurized relative to other areas. This area is also heated by coils in the ceiling. An evaluation of the HEPA filters, performed in June 1999, indicated that the filters were properly installed and operating according to the rated efficiency for such filters.

All the units are designed to supply positive pressure in the patient rooms relative to the corridors, and ventilation measurements indicated that this was generally true. With four different ventilation systems throughout the hospital, it is difficult to find a systemic source of growth and dissemination, unless it is related to humidification. In December 1998, outside daily temperatures dropped from averages in the mid-50 °F range to the mid-30 °F range immediately before the

elevated aspergillus concentration incident. The April incident occurred shortly after average daily temperatures jumped from the mid-30 °F range in mid-March to the mid-60 °F range in early April. The less extreme incidents in July occurred during very hot and humid temperatures.

The number of air changes per hour (ACH) were calculated by the maximum intake or exhaust in each area compared with the total volume. The ACH values are listed in Table I.

Table II shows that mean total viable fungi concentrations are much higher outdoors (257.8 cfu/m^3) than indoors (means ranged from 34.6 to 83.5 fungal cfu/m^3 in all the patient areas sampled). Paired *t*-test with unequal variances indicated that mean total fungal levels were significantly lower in the hospital rooms compared with outside. *P*-values for these comparisons were 0.001 for the cardiac ICU, 0.018 for the AIDS unit, 0.001 for the organ transplant unit, 0.001 for the bone marrow transplant units and 0.001 for the renovation rooms. Total fungi were only marginally lower in renovated rooms than outdoors ($P = 0.067$).

However, mean aspergillus concentrations were much lower outdoors (6.8 cfu/m^3) than in the air of the indoor patient areas (7.3–30.6 cfu/m^3). Paired *t*-test with unequal variances indicated that mean total aspergillus levels were significantly higher in the organ transplant rooms ($P = 0.028$) and in the cardiac ICU ($P = 0.05$) compared with outdoors. Mean aspergillus concentrations were not significantly different from outdoors in the AIDS unit, bone marrow transplant unit, duct cleaned rooms or renovated rooms. Aspergillus comprise only 2.6% of total outdoor viable fungi but comprise 11.1% to 36.6% of the total fungi in the indoor hospital sites. The much higher aspergillus concentration indoors suggests that there are multiple sources of aspergillus inside the hospital. The bone marrow units had the lowest average concentrations of aspergillus. The organ transplant unit and the cardiac ICU had considerably higher mean aspergillus concentrations inside the rooms than in the corridors outside the rooms.

The 120 blank plates yielded 12 contaminants with an average of 0.10 colonies per plate of contamination. Contaminants reported included *Penicillium brevicompactum* (six colonies), *Penicillium chrysogenum* (four colonies), *Mucor racemosus* (two colonies), and *A. niger* (one colony).

Figure 1 illustrates two major incidents when high concentrations of airborne aspergillus were collected in December 1998 and April 1999, and some relatively smaller incidents in June and July 1999. These incidents were characterized by high concentrations in samples collected in all four treatment areas, especially inside patient rooms. The presence of aspergillus in these units persisted for several sampling periods, suggesting that the indoor aspergillus spore levels were elevated for 12 to 30 days. Several sources of aspergillus were probably present inside the hospital.

A peak in the aspergillus concentration occurred in one of the organ transplant rooms; levels of *A. niger* were 680 cfu/m^3 on 14 December 1998 and 20 cfu/m^3 on 18 December 1998. A slight plumbing leak was found above the room, which was repaired several days later. Aspergillus levels then fell to zero or near zero in the organ transplant unit for several months. Another room in the organ transplant unit had total aspergillus levels of 465 cfu/m^3 on 1 April 1999 (426 *A. niger*, 28 *A. fumigatus* and 11 *A. flavus*). Some hospital personnel noted some moisture condensation in this room in April.

A high aspergillus concentration (160 cfu/m^3 *A. candidus*, 155 cfu/m^3 *A. niger*) was recorded on 12 June 1999 inside a room being renovated and undergoing wood-cutting operations. A lot of sawdust was present on the floor, much of it damp.

Another peak in the aspergillus concentration occurred in the patient rooms of the bone marrow transplant unit. Aspergillus concentrations in the transplant unit rooms were 77 cfu/m^3 on 1 April 1999 (63 *A. niger*, 11 *A. flavus*, three *A. fumigatus*) and 73 cfu/m^3 on 13 April 1999 (52 *A. niger* and 21 *A. flavus*). At about this time, a roof leak above the bone marrow transplant unit, which caused visible ceiling tile damage outside the unit's entrance

Table I Measured air changes per hour in the four hospital areas sampled

Area	Number of days sampled	Mean air changes per hour ± SD	Range of air changes per hour
Cardiac intensive care unit	8	3.8 ± 4.0	0.5–13.2
Organ transplant unit	12	8.1 ± 7.9	2.3–30.7
AIDS unit	11	2.9 ± 2.7	0.6–8.2
Bone marrow transplant unit	7	26.6 ± 13.2	16.2–53.9

Table II Summary of total fungi and total aspergillus collected in various locations in the hospital

Unit activity	Area	Total number of samples	Mean viable fungi (range) cfu/m^3	Mean aspergillus (range) cfu/m^3	Samples positive for aspergillus (%)	Aspergillus as percentage of total organisms
Cardiac intensive care unit	Inside patient rooms	58	54.9 (0–360)	16.8 (0–167)	43	30.7
	Outside patient rooms	57	34.6 (0–180)	8.4 (0–45)	55	24.2
AIDS unit	Inside patient rooms	58	41.7 (0–198)	7.6 (0–42)	41	18.1
	Outside patient rooms	58	37.1 (0–276)	10.0 (0–140)	38	27.0
Organ transplant unit	Inside patient rooms	71	83.5 (2–680)	30.6 (0–680)	44	36.6
	Outside patient rooms	62	44.8 (2–248)	12.7 (0–100)	34	28.4
Bone marrow transplant unit	Inside patient rooms	62	41.0 (0–260)	7.3 (0–68)	31	17.9
	Outside patient rooms	62	40.7 (0–220)	8.4 (0–96)	19	20.5
Duct cleaning	Inside patient rooms	23	192.8 (21–274)	22.6 (0–87)	35	11.7
	Outside patient rooms	21	70.4 (8–176)	17.5 (0–100)	38	24.8
Renovation	In containment–inactive	76	38.1 (8–226)	6.4 (0–85)	28	16.7
	Out containment–inactive	68	37.5 (5–310)	4.2 (0–95)	32	11.1
	In containment–active	22	100.6 (0–323)	13.2 (0–88)	55	13.2
	Out containment–active	22	57.4 (4–130)	14.7 (0–30)	41	25.6
All indoor samples		720	53.2 (0–724)	12.1 (0–680)	40	22.7
Rooftop samples (outdoors)		122	257.8 (10–1340)	6.8 (0–40)	43	2.6
P-value difference between means of indoor and outdoor samples- two tailed *t*-test with unequal variances			0.017	0.006		

doors, was seen. The leak was apparently caused by the installation of the dedicated air handling system for the unit. The roof membrane was cut for the installation, and the edges were not properly scaled. The water leaked in through the edges of the air handling unit, flowed across a concrete roof layer, and leaked into the hospital through breaches in the concrete layer. This leak was repaired in April. For the next seven sampling sessions, total aspergillus concentrations averaged only 2 cfu/m^3.

There were numerous reports of a chronic window leak throughout the entire sampling period. Apparently the seals around the window assemblies had failed over a long period of time. Precipitation running down the outside walls of the building was drawn into the window areas where the seals failed by a capillary effect. This, together with normal window condensation, could have permitted constant growth for *Aspergillus* spp. and other fungal organisms. Also, changes in indoor humidity and duct wind speed may have played a crucial role in disseminating the spores.

Forty-eight of the 74 duct dust samples (65%) had levels of asp f1 protein exceeding the detection limit. The mean, median, standard deviation (SD) and range of dust asp f1 levels were 0.41, 0.20, 0.56 and −0.10 to 1.94 ppm. None of the nine dust samples with asp f1 levels exceeding 1 ppm came from samples for the four wards sampled. These nine samples came from a variety of areas on the second to sixth floors and included a surgical suite on the third floor. These significant levels of asp f1 protein seen in ducts are consistent with the significant mean airborne levels (11.0 cfu/m^3) of *A. fumigatus* collected in rooms undergoing duct cleaning (Table III). This suggests that there may be some growth of *A. fumigatus* mycelia in the hospital air ducts.

Discussion

The mean total aspergillus concentration for all 720 indoor hospital samples was 12.1 cfu/m^3, with the seven most common *Aspergillus* species being *A. niger* 7.54 cfu/m^3, *A. candidus* 1.27 cfu/m^3, *A. flavus* 0.97 cfu/m^3, *A. fumigatus* 0.88 cfu/m^3, *Eurotium A. glaucus* 0.45 cfu/m^3, *A. versicolor* 0.18 cfu/m^3 and *A. parasiticus* 0.14 cfu/m^3. The mean airborne aspergillus concentrations in this study were considerably lower than the mean total aspergillus concentration (101.1 cfu/m^3) seen in an Austrian hospital.[21] The Austrian study was conducted with 164 air samples from January to

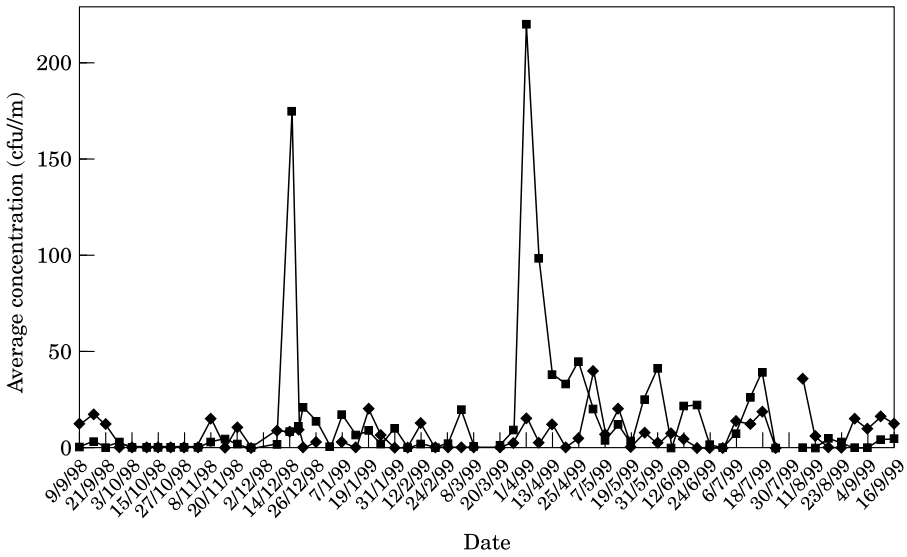

Figure 1 Average total Aspergillus concentrations indoors/outdoors during the sampling period; ■ indoor Aspergillus concentrations; ◆ outdoor Aspergillus concentrations.

July.[21] The seven most common *Aspergillus* species collected in the Austrian study were *Eurotium A. glaucus* 53.6 cfu/m^3, *A. versicolor* 18.0 cfu/m^3, *A. fumigatus* 12.8 cfu/m^3, *Emericella A. nidulans* 3.5 cfu/m^3, terreus 2.8 cfu/m^3, *A. sydowii* 2.4 cfu/m^3 and *A. niger* 1.4 cfu/m^3 (Rainer). Thus this study had much higher levels of *A. niger* and much lower levels of total aspergillus, *Eurotium A. glaucus*, *A. veriscolor*, *A. fumigatus*, *A. sydowii and A. terreus* compared with the study of Rainer *et al*.[21]

Total aspergillus concentrations averaged 7.2 cfu/m^3 in the bone marrow ward rooms (which have a dedicated HEPA system) in this study. This is comparable with a mean total aspergillus concentration of 6.77 cfu/m^3 seen in the bone marrow transplant ward in another hospital during construction periods,[23] but is much higher than that reported in another bone marrow unit with HEPA air filter units and a laminar air flow (0.009 cfu/m^3).[22] In this study, the four most common *Aspergillus* spp. collected in bone marrow transplant patient rooms were *A. niger* 5.47 cfu/m^3, *A. flavus* 0.77 cfu/m^3, *A. candidus* 0.72 cfu/m^3 and *A. fumigatus* 0.18 cfu/m^3.

Several earlier studies have established that construction/renovation/remodelling and other activities producing copious dust can increase levels of airborne aspergillus.[10,12,13] Total fungal concentrations averaged 100.6 cfu/m^3 in the containment area during active renovation work, but averaged only 37.5 to 57.4 cfu/m^3 in the containment area during inactive periods or just outside the containment area during active or inactive periods. A two-way ANOVA of the areas with active/inactive renovation and inside/outside renovation areas as independent variables found that activity was related to significantly higher aspergillus levels ($P = 0.049$) but not significantly higher total fungi levels ($P = 0.739$). Total fungi ($P = 0.719$) and total aspergillus levels were not significantly different inside or outside the renovation rooms. On 12 July 1999 in a renovation area where wood was being cut with a power saw, 160 cfu/m^3 *Aspergillus* spp. was collected (including 155 and 5 cfu/m^3, respectively, of *A. niger* and *A. candidus*). On 20 March 1999 in a renovation area that was dusty and where painting was undertaken earlier in the day, total aspergillus levels reached 112 cfu/m^3, including 102 cfu/m^3 *A. flavus*, 5 cfu/m^3 *A. fumigatus* and 5 cfu/m^3 *A. versicolor*.

Tables II and III note that the highest indoor concentrations of mean total indoor fungi (192.8 cfu/m^3) and *A. fumigatus* (11.0 cfu/m^3) were collected in the rooms that were undergoing duct cleaning. Anderson *et al*.[34] reported *A. fumigatus* levels of 65 cfu/m^3 near a vaccum that was running in a paediatric cancer unit. This suggests that the ducts are a significant source of *A. fumigatus* spores and fungal spores in general. Workers should wear respiratory protection during duct cleaning and immunocompromised patients should not be in the area during cleaning. Additional studies of residence times of the *Aspergillus* spp. spores after cleaning might yield some useful information (Table IV).

This study found viable aspergillus propagules in all parts of the hospital at concentrations somewhat greater than those found outside. In addition, levels of total fungi were significantly higher outside the hospital than indoors. Therefore,

Table III Species composition of aspergillus collected at various hospital sites

Unit activity	Area	Total number of samples	Total aspergillus	*Aspergillus candidus*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Eurotium Aspergillus glaucus*	*Aspergillus niger*	*Aspergillus ochraceus*	*Aspergillus parasiticus*	*Aspergillus terreus*	*Aspergillus ustus*	*Aspergillus versicolor*
Cardiac intensive care unit	Inside patient rooms	58	16.8	0.85	2.96	0.52	0.0	10.72	0.0	1.75	0.0	0.0	0.0
	Outside patient rooms	57	8.4	0.88	1.50	0.63	0.61	4.77	0.0	0.0	0.0	0.0	0.0
AIDS unit	Inside patient rooms	58	7.6	1.73	0.68	1.18	0.0	3.64	0.0	0.0	0.0	0.0	0.36
	Outside patient rooms	58	10.0	0.77	0.09	0.40	0.0	8.35	0.31	0.0	0.0	0.0	0.9
Organ transplant unit	Inside patient rooms	71	30.5	1.25	0.73	1.25	1.50	25.0	0.0	0.0	0.0	0.0	0.86
	Outside patient rooms	62	12.7	3.53	0.34	0.30	0.90	7.54	0.0	0.0	0.0	0.0	0.07
Bone marrow transplant unit	Inside patient rooms	62	7.3	0.72	0.77	0.17	0.08	5.47	0.08	0.0	0.0	0.0	0.0
	Outside patients rooms	62	8.4	1.75	0.45	0.26	0.33	5.41	0.0	0.04	0.0	0.0	0.0
Duct cleaning	Inside patient rooms	23	22.6	1.13	3.48	11.00	0.0	5.88	1.08	0.0	0.0	0.0	0.0
	Outside patient rooms	21	17.5	6.67	0.0	1.24	0.77	8.82	0.0	0.0	0.0	0.0	0.0
Renovation	Inside containment—inactive	76	6.4	1.91	2.07	0.52	0.26	1.29	0.0	0.0	0.0	0.0	0.33
	Outside containment—inactive	68	4.2	0.52	0.15	0.22	0.07	3.11	0.04	0.0	0.0	0.0	0.07
	Inside containment-	22	13.2	7.03	0.0	0.35	0.46	3.67	0.46	0.0	0.0	1.23	0.0
	Outside containment—inactive	22	14.7	1.60	0.0	0.0	2.15	10.95	0.0	0.0	0.0	0.0	0.0
All indoor samples		720	12.1	1.72	0.97	0.88	0.45	7.59	0.08	0.14	0.01	0.05	0.18
Rooftop samples (outdoors)		122	6.8	1.23	0.80	0.68	0.22	2.71	0.46	0.0	0.34	0.0	0.35
P-value difference between means of indoor and outdoor samples- two tailed *t*-test with unequal variances			0.006	0.40	0.71	0.66	0.19	0.010	0.042	0.18	0.050	0.29	0.17

All values in mean cfu/m^3.

Table IV Indoor and outdoor concentrations of the 10 most common genus or species of non-aspergillus fungi collected

Species	Mean indoor (cfu/m^3)	Mean outdoor (cfu/m^3)	Ratio indoor to outdoor mean concentrations	*P*-value difference two tailed *t*-test with unequal variances
Altemaria	0.70	8.04	0.09	0.000
Cladosporium herbareum	9.87	149.9	0.07	0.000
Fusarium species	1.01	10.5	0.09	0.000
Mucor racemosus	4.80	1.75	2.77	0.027
Penicillium brevicompactum	5.20	4.96	1.05	0.897
Penicillium chrysogenum	5.10	9.45	0.54	0.099
Penicillium citrinum	1.74	3.90	0.45	0.186
Rhizopus oryzae	4.08	5.18	0.80	0.517
Trichoderma species	7.96	3.79	2.10	0.010
Yeasts—several genera	2.47	3.05	0.80	0.415

infiltration of outside air cannot be the primary mechanism for producing the airborne aspergillus levels in the hospital. Significantly higher levels of aspergillus, especially *A. fumigatus*, were seen during duct cleaning operations. There were probably numerous small to moderate aspergillus sources in the hospital, including dust from duct cleaning and renovation and sites of water damage and moisture condensation. Approaches to controlling aspergillus infections in hospitals must be multi-factorial and involve moisture/water control, HEPA filters, sealed positive-pressure rooms, and environmental monitoring of aspergillus. In the future, the use of ELISA,[33] DNA hybridization or PCR[35] methods may be a very useful addition to the standard microbiological methods to monitor aspergillus in hospitals and other environments.

Acknowledgements

The authors acknowledge the full cooperation, extensive help and financial support that a major hospital in the US Midwest gave us to conduct this study. This study was also supported in part by the University of Illinois Occupational Safety and Health Educational and Research Center, National Institute of Occupational Safety and Health Grant T42/CCT510424-4.

References

1. Patterson JE, Peters J, Calhoon JH, *et al*. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2000;**2**:22—28.
2. Kontoyiannis D, Bodey G. Invasive aspergillus in 2002: an update. *Eur J Clin Microbiol Infect Dis* 2002;**21**:161—172.
3. Pagano L, Girmenia C, Miele L, *et al*. Infections caused by filamentous fungi in patients with haematological malignancies. A report of 391 cases by GIMEMA Infection Program. *Haematologica* 2001;**86**:862—870.
4. Rinaldi M. Invasive aspergillosis. *Rev Infect Dis* 1993;**5**: 1061—1067.
5. Denning D. Therapeutic outcome in invasive aspergillosis. *Clin Infect Dis* 1996;**23**:608—615.
6. Wald A, Leisenring W, Von Burik J, Bowden R. Epidemiology of aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 1997;**175**:1459—1466.
7. Jones ME, Fox AJ, Barnes AJ, *et al*. PCR—ELISA for the early diagnosis of invasive pulmonary aspergillosis in neutropenic patients. *J Clin Pathol* 1998;**51**:652—656.
8. Kappe R, Schutz-Berge A, Sonntag HG. Evaluation of eight antibody tests and one antigen test for diagnosis of invasive aspergillosis. *Mycoses* 1996;**39**:13—23.
9. Hajjeh R, Warnock D. Counterpoint: invasive aspergillosis, and the environment-rethinking our approach to prevention. *Clin Infect Dis* 2001;**33**:1452—1459.
10. Arnow P, Sadigh M, Costas C, Weil D, Chudy R. Endemic and epidemic aspergillosis associated with in-hospital replication of aspergillus organisms. *J Infect Dis* 1991;**164**:998—1002.
11. Cornet M, Levy V, Fluery L, *et al*. Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against aspergillus airborne contamination during hospital renovation. *Infect Control Hosp Epidemiol* 1999;**20**: 508—513.
12. Goodley JM, Clayton YM, Ray RJ. Environmental sampling for aspergilli during building construction on a hospital site. *J Hosp Infect* 1994;**26**:27—35.
13. Opal S, Asp AA, Cannady PB, *et al*. Efficacy of infection control measures during an outbreak of disseminated aspergillosis associated with hospital construction. *J Infect Dis* 1986;**153**:634—637.
14. Panackal A, Dahlman A, Keil K, *et al*. Outbreak of invasive aspergillosis among renal transplant patients. *Transplantation* 2003;**75**:1050—1053.
15. Annassie E, Costa S. Nosocomial aspergillosis is waterborne. *Clin Infect Dis* 2001;**33**:1546—1548.
16. Anaissie E, Stratton S, Rex J. Hospital water as a source of aspergillosis: evidence for possible nosocomial transmission (Abstract 1322). *Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (Toronto, Ontario, Canada)*. Washington, DC: American Society of Microbiology; 2000. p. 375.
17. Lentino J, Rosenkranz M, Michaels JA, Kurup VR, Rose HD, Rytel RW. A retrospective review of airborne disease secondary to road construction and contaminated air conditioners. *Am J Epidemiol* 1982;**116**:425—430.
18. Lass-Florl C, Rath P, Niederwiesser D, *et al*. *Aspergillus terreus* in haematological malignancies: molecular epidemiology suggests association with in-hospital plants. *J Hosp Infect* 2000;**46**:31—35.
19. Potera C. Clothing spreads spores. *Environ Health Perspect* 2001;**109**:A365.
20. Solomon W, Burge H, Boise JR. Airborne *Aspergillus fumigatus* levels outside and within a large clinical center. *J Allergy Clin Immunol* 1978;**62**:56—60.
21. Rainer J, Peintner U, Poder R, *et al*. Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologica* 2000;**149**:87—97.
22. Sheretz R, Belani A, Kramer B, *et al*. Impact of air filtration on nosocomial aspergillus infections: unique risk of bone marrow transplant patients. *Am J Med* 1987;**83**:709—718.
23. Loo V, Bertrand C, Dixon C, *et al*. Control of construction associated nosocomial aspergillus in an antiquated hematology unit. *Infect Control Hosp Epidemiol* 1996;**17**:360—364.
24. Withington S, Chambers S, Beard ME, *et al*. Invasive aspergillosis in severely neutropenic patients over 18 years of age; impact of intranasal amphotericin B and HEPA filtration. *J Hosp Infect* 1998;**38**:11—18.
25. Oren I, Haddad N, Finkelstein R, Rowe J. 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.
26. 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.
27. Hahn T, Cummings K, Michalek AM, Lipman B, Segel B, McCarthy P. Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies. *Infect Control Hosp Epidemiol* 2002;**23**:525—531.
28. Morey P, Otten J, Burge H, *et al*. Guidelines for sampling for

viable aerosols in office environments. *Appl Indust Hyg* 1986; **1**:R19—R23.
29. Andersen AA. New sampler for collecting, sizing and enumeration of viable airborne particles. *J Bacteriol* 1958; **76**:471—484.
30. Barnett HL, Hunter B. *Illustrated genera of imperfect fungi*. Minneapolis, MN: Burgess Publishing; 1972.
31. Samson R, Hoekstra E, Frisvad J, Fitenborg O. *Introduction to foodborne fungi*. The Netherlands: Centraalburea Voor Schimmelcultures; 1996.
32. Klich M, Pitt J. *A laboratory guide to common Aspergillus species and their teleomorphs*. North Ryde, NSW, Australia: Commonwealth Scientific and Industrial Research; 1994.
33. Arruda LK, Platt-Mills TAE, Longbottom JL, El-Dahr JM. *Aspergillus fumigatus*: identification of the 16, 18, and 45 kD antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. *J Allergy Clin Immunol* 1992; **89**:1166—1176.
34. Anderson K, Morris G, Kennedy H, *et al*. Aspergillosis in immunocompromised pediatric patients: associated with building design, hygiene and indoor air. *Thorax* 1996;**51**: 251—256.
35. Rath PM, Ansorg R. Identification of medically important *Aspergillus* species by single strand conformational polymorphism (SSCP) of the PCR-amplified intergenic spacer region. *Mycoses* 2000;**43**:381—386.