Click HERE to go back to MoldAcrossAmerica

ASPERGILLUS SURVEILLANCE PROJECT AT A LARGE TERTIARY CARE HOSPITAL

By Luke Curtis[1], Salvatore Cali[1], Lorraine Conroy[1], Kirk Baker[1], Chia-Hao Ou[1], Ronald Hershow[2], Felice Norlock-Cruz[1], and Peter Scheff[1].

University of Illinois at Chicago. [1]Department of Environmental and Occupational Health Science and [2] Department of Epidemiology, Chicago, Illinois 60612, USA.

Corresponding Author

Luke Curtis MS, CIH

University of Illinois at Chicago

1328 Greenwood

Wilmette, Illinois 60091 USA

(847) 256-3562

lukec@uic.edu

Keywords: Aspergillus, aspergillosis, hospital infections, ventilation, asp f1.

Running Title: HOSPITAL ASPERGILLUS SURVEILLANCE

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 analyzed 74 duct dust samples for *Aspergillus fumigatus* mycelial asp f1 protein. Mean total fungi (in colony forming units per cubic meter of air or cfu/m^3) was 257.8 outdoors, 53.2 in all indoor samples and 83.5 in patient rooms in the bone marrow transplant patient rooms. Mean total *Aspergillus* (cfu/m^3) was 6.8 outdoors, 12.1 in all indoor samples and 7.3 in the bone marrow transplant patient rooms. The five most prevalent *Aspergillus* species collected inside the hospital (mean cfu/m^3) were *A. niger* 7.57, *A. candidus* 1.72, *A. flavus* 0.97, *A. fumigatus* 0.88 and *A. glaucus* 0.45. In rooms undergoing duct cleaning, mean *Aspergillus fumigatus* concentrations reached 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. *Aspergillus niger* levels reached 680 cfu/m^3 in one of the organ transplant rooms following a small water leak from a ceiling pipe. Total *Aspergillus* concentrations rose to 77 cfu/m^3 in a bone marrow patient room following improper sealing and water infiltration of the units dedicated HEPA system. Total *Aspergillus* levels of 160 cfu/m^3 were recorded in a renovation area during wood cutting.

The significantly higher indoor concentrations of *Aspergillus* seen inside the hospital versus outdoors and these various moisture/HEPA filter/ renovation incidents argue against major infiltration of *Aspergillus* spores from outside and suggest that numerous small to moderate sources of *Aspergillus* exist in the hospital. Approaches to controlling *Aspergillus* infections in hospitals must be multi-factorial and involve moisture/water control, HEPA filters and sealed positive pressure rooms, environmental monitoring and medical surveillance and epidemiology of immunocompromised patients.

Introduction

The incidence of life threatening invasive *Aspergillus* infections has been increasing with increasing numbers of immunocompromised bone/organ transplant patients and patients with leukemia, lymphoma and other malignancies. Incidence rates of invasive *Aspergillus* infection are about 17-26% in lung transplant patients, 5-24% in acute leukemia 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 *A. flavus*, *A. terreus*, *A. niger* and other species also frequently cause invasive aspergillosis [3,4].

Even with intensive medical treatment and strong anti-fungal medications *Aspergillus* infections are often fatal. Denning [5] studied 1,223 invasive *Aspergillus* cases and reported crude mortality rates of 86%, 66% and 99% for pulmonary, sinus and cerebral aspergillosis. Aspergillosis has become the leading infectious cause of death for bone marrow/ organ transplant patients. A large 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 stage although use of immunological testing such as the PCR and ELISA may be somewhat useful [7,8].

Given the poor treatment outcomes in invasive aspergillosis, environmental control to prevent infection is critical. Aspergillosis has long been 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[4-16], dusty air conditions [17] , hospital plants [18], clothing [19] and other sources [20-22].

Several studies which have employed HEPA filters in the rooms of bone marrow transplant/ 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 kilometer 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

Andersen Sampling and Culture

A total of 842 bacterial and fungal samples were collected using an Andersen (Atlanta, Georgia) one-stage bioaerosol sampler (N-6) between September 9, 1998 and September 16, 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 were collected every six days as follows: 1) two pairs of samples from patient rooms of two of the four units studied (cardiac ICU, AIDS ward, organ transplant, bone marrow transplant), 2) two pairs of samples in the corridors of two the units collected than 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 9th floor rooftop, 6) 2 blank samples loaded onto air samplers, unloaded and cultured to test for sterility. During the following sampling period- samples were taken from the ward 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* previously seen in the study.

Samples were incubated at 25 ^{o}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 ^{o}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 (100-1000x) and classified to genera with the help of standard references for fungal identification [30,31]. Species of *Aspergillus*, *Penicillium* and several other fungal genera were classified to species with the help of standard references for *Aspergillus*, *Penicillium* and other species [31,32]. *Aspergillus* with teleomorph forms (such as *Eurotium Aspergillus glaucus*) were included in the total *Aspergillus* level.

Ventilation Characterization

The ventilation system was characterized through several interviews with hospital physical plant staff 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 and/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 vacuum bags used by hospital maintenance staff to clean the duct covers in patient rooms and other areas. This bag samples were collected opportunistically and the sampling protocol was not directly tied 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 milligrams of dust was taken out of each bag and analyzed using the Sandwich ELISA (Enzyme Linked Immunosorbent Assay) method of Arruda [33]. This method measures the asp f1 protein which is found in the mycelia of Aspergillus fumigatus but not in the spores. Various concentrations of asp f1 protein standard and blanks were analyzed to get a standard curve to calculate asp f1 concentrations. Eight blank samples were analyzed and the detection limit was set as 2 standard deviations higher than the blank samples.

Statistics

Differences between mean indoor and outdoor levels of fungi were determined by using a 2 tailed t-test with unequal variances. Differences in fungi levels inside/ outside renovation areas and during activity/ no activity were determined by a 2 way ANOVA program. All statistical analyses were done using SPSS 6.1 program (1994 SPCC, Incorporated- Chicago, Illinois, 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 Intensive Care 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 Intensive Care, 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. Our 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 of the units are designed to supply positive pressure in the patient rooms relative to the corridors, and our 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 average temperatures dropped from averages in the mid-50 ^{o}F range to the mid-30 ^{o}F range immediately before the elevated *Aspergillus* concentration incident. The April incident occurred shortly after average daily temperatures jumped from the mid-30 ^{o}F range in mid-March to the mid-60 ^{o}F range in early April. The less extreme incidents in July occurred during very hot and humid temperatures.

Air changes per hour (ACH) were calculated by the maximum of intake or exhaust in each area compared to the total volume. The ACH values

are listed in Table I below.

Table I- Measured Air Changes Per Hour (ACH) in 4 Hospital Area Sampled

Area	Number of days sampled	Mean Air Changes per Hour (ACH) +/-SD	Range of Air Changes per Hour (ACH)
Cardiac Intensive Care Unit	8	3.8 +/- 4.0	0.5 to 13.2
Organ Transplant Unit	12	8.1 +/- 7.9	2.3 to 30.7
HIV Unit	11	2.9 +/- 2.7	0.6 to 8.2
Bone Marrow Transplant Unit	7	26.6 +/- 13.2	16.2 to 53.9

Table III below summarizes total Fungi and total *Aspergillus* collected from the various sampling sites. Table III below summarizes the concentrations of various *Aspergillus* species collected at various parts of the hospital. Table IV summarizes mean indoor and outdoor concentrations of the 10 most common non-Aspergillus fungi.

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	% of samples positive for *Aspergillus* (%)	*Aspergillus* as % 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 (21-724)	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- 2 tailed 2 test with unequal variances			0.017	0.006		

Table III- Species Composition of *Aspergillus* Collected at Various Hospital Sites- All Values in mean cfu/m^3

UNIT/ ACTIVITY	AREA	Total number of samples	Total *Aspergillus*	*A. candidus*	*A. flavus*	*A. fumigatus*	*Eurotium A. glaucus*	*A. niger*	*A. ochraceus*	*A. parasiticus*	*A. terreus*	*A. ustus*	*A. 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	0.09
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 Patient Rooms	62	8.4	1.75	0.45	0.26	0.33	5.41	0.0	0.04	0.0	0.0	0.16
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	76	6.4	1.91	2.07	0.52	0.26	1.29	0.0	0.0	0.0	0.0	0.33

Table III- Species Composition of *Aspergillus* Collected at Various Hospital Sites- All Values in mean cfu/m^3

UNIT/ ACTIVITY	AREA	Total number of samples	Total *Aspergillus*	*A. candidus*	*A. flavus*	*A. fumigatus*	*Eurotium A. glaucus*	*A. niger*	*A. ochraceus*	*A. parasiticus*	*A. terreus*	*A. ustus*	*A. versicolor*
	- Inactive												
	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 - Active	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		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- 2 tailed 2 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

Table IV Indoor and Outdoor Concentrations of the 10 most common genus or species non Aspergillus fungi collected

Species	Mean Indoor cfu/m^3	Mean Outdoor cfu/m^3	Ratio Indoor to Outdoor mean Concentrations	P value difference- 2 tailed t-test with unequal variances
Alternaria	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

Figure 1 below notes average total Aspergillus concentrations indoors/outdoors during the sampling period.

FIGURE 1- MEAN ASPERGILLUS LEVELS (CFU/M^3) OVER TIME

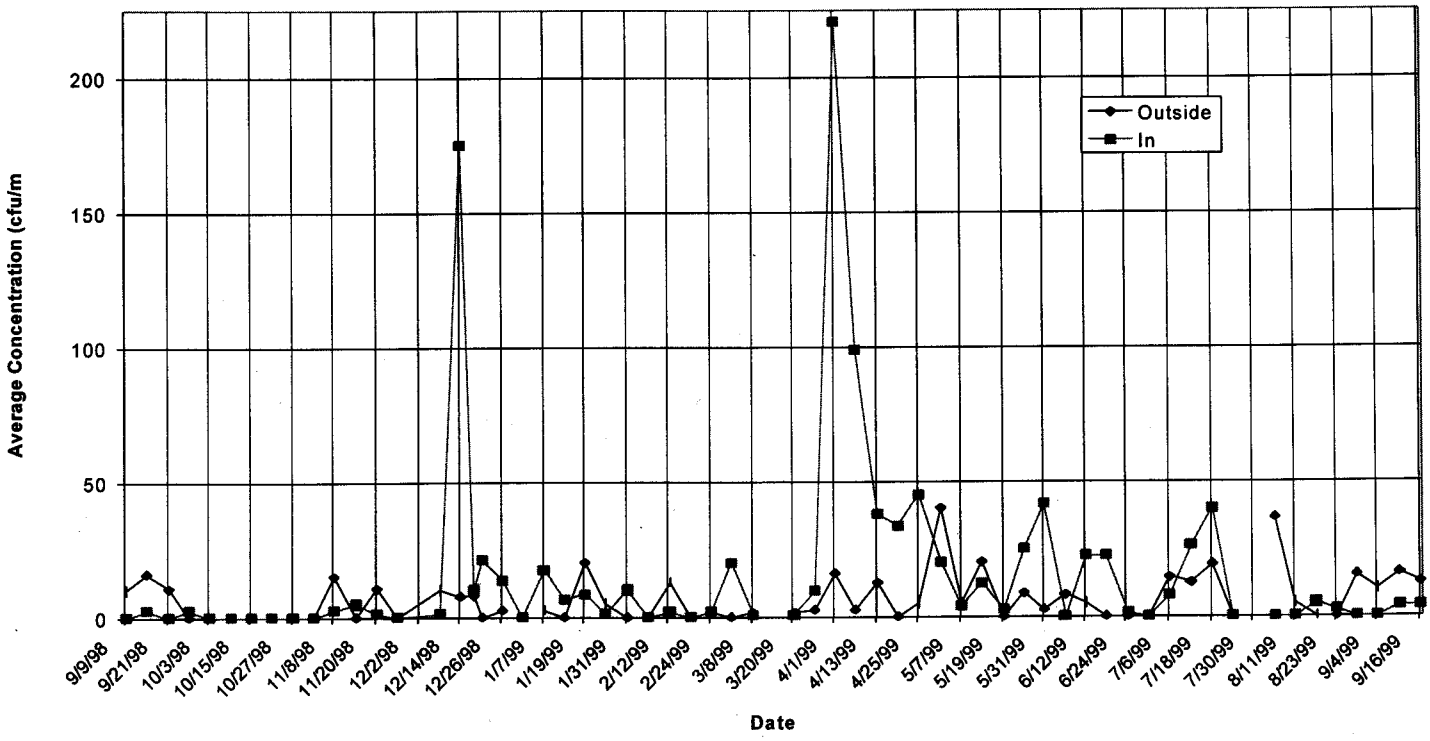
Average Concentration (cfu/m
200
150
100
50
0
Outside
In
9/9/98
9/21/98
10/3/98
10/15/98
10/27/98
11/8/98
11/20/98
12/2/98
12/14/98
12/26/98
1/7/99
1/19/99
1/31/99
2/12/99
2/24/99
3/8/99
3/20/99
4/1/99
4/13/99
4/25/99
5/7/99
5/19/99
5/31/99
6/12/99
6/24/99
7/6/99
7/18/99
7/30/99
8/11/99
8/23/99
9/4/99
9/16/99
Date

Table 2 shows that mean total viable fungi concentrations are much higher outdoors (257.8 cfu/m^3) than indoors (means range from 34.6 to 83.5 fungal cfu/m^3 in all of the patient areas sampled.) Paired t-test with unequal variances indicated that mean total fungal levels were significantly lower in the hospital rooms versus outside. P values for these comparisions were 0.001 for the cardiac care unit, 0.018 AIDS unit, 0.000 for organ transplant unit, 0.000 for bone marrow transplant units and 0.000 in the renovation rooms. Total fungi was only marginally lower in renovated rooms than outdoors (p=0.067).

However, mean *Aspergillus* concentrations are much lower outdoors (6.8 cfu/m^3) than in 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 intensive care unit (p=0.05) as compared to 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* cfu/m^3 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 mean *Aspergillus* concentrations considerably larger inside the rooms than in the corridors outside the rooms.

The 120 blank plates yielded 12 contaminants for average of 0.10 colonies per plate of contamination. Contaminants reported included *Penicillium brevicompactum* six colonies, *Penicillium chrysogenum* four, *Mucor racemosus* two, and *Aspergillus niger* one.

Figure 1 illustrates 2 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 of 1999. These incidents were characterized by high concentrations in samples collected in all four treatment areas, especially inside patient rooms. 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 high *Aspergillus* event occurred in one of the organ transplant rooms, levels of *Aspergillus* niger were 680 cfu/m^3 on 12-14-1998 and 20 cfu/m^3 on 12-18-1998. 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 April 1, 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* level of 160 cfu/m^3 (5 cfu/m^3 *A. candidus*, 155 cfu/m^3 *A. niger*) was recorded on 6-12-1999 inside a room being renovated and undergoing wood cutting operations. Much sawdust was present on the floor, much of it damp.

Another high *Aspergillus* event occurred in the patient rooms of the bone marrow transplant unit. *Aspergillus* concentrations in the transplant unit rooms were 77 cfu/m^3 on 4-1-1999 (63 *A. niger*, 11 *A. flavus*, 3 *A. fumigatus*) and 73 cfu/m^3 on 4-13-1999 (52 *A. niger* and 21 *A. flavus*). About this time, a roof leak above the Bone Marrow Transplant Unit that caused visible ceiling tile damage outside the unit entrance 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 sealed. 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 problem 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 provided a constant growth area for *Aspergillus* 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 *Aspergillus* asp f1 protein exceeding detection limit. The mean, median, 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 9 samples came from a variety of areas on the second through sixth floors and included a surgical suite on the third floor. These significant levels of asp f1 protein seen in ducts is consistent with the significant mean airborne levels (11.0 cfu/m^3) of *Aspergillus fumigatus* collected in rooms undergoing duct cleaning (see table III above). This suggests there may possibly be some growth of *Aspergillus fumigatus* mycelia in the hospital air ducts.

Discussion

The mean total *Aspergillus* collected in for all 720 indoor hospital samples was 12.1 cfu/m^3, with the 7 most common *Aspergillus* species (in cfu/m^3) being *A. niger* 7.54, *A. candidus* 1.27, *A. flavus* 0.97, *A. fumigatus* 0.88, *Eurotium A. glaucus* 0.45, 0.18 *A. versicolor* and *A. parasiticus* 0.14. The mean *Aspergillus* airborne concentrations in this study were considerably lower that the mean 101.1 cfu/m^3 total *Aspergillus* seen in an Austrian hospital [21]. The Austrian study was conducted with 164 air samples from January to July [21]. The 7 most common *Aspergillus* species collected in the Austrian study (in cfu/m^3) were *Eurotium A. glaucus* 53.6, *A. versicolor* 18.0, *A. fumigatus* 12.8, *Emericella A. nidulans* 3.5, terreus 2.8, *A. sydowii* 2.4 and *A. niger* 1.4 (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* as compared to the Rainer study [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 to a mean of 6.77 cfu/m^3 total *Aspergillus* seen in bone marrow ward in another hospital during construction periods [23], but is much higher than the mean 0.009 cfu/m^3 total *Aspergillus* levels reported in another bone marrow unit with HEPA air filter units and a laminar air flow [22]. In this study, the 4 most common *Aspergillus* collected in bone marrow rooms (in cfu/m^3) were *A. niger* 5.47, *A. flavus* 0.77, *A. candidus* 0.72 and *A. fumigatus* 0.18.

Several earlier studies have established that construction/ renovation/ remodeling and other dusty activities can increase levels of airborne *Aspergillus* [10, 12-3].

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 are during inactive periods or just outside the containment area during active or inactive periods. A 2 way ANOVA of the areas with active/ inactive renovation and inside/outside renovation areas as independent variables found that activity was related to significantly more Aspergillus levels (p=0.049) but not significantly more total fungi (p=0.739). Total fungi (p=0.719) and total Aspergillus levels were not significantly different inside or outside the renovation rooms. On July 12, 1999 in a renovation area where wood was being cut with a power saw, 160 cfu/m^3 *Aspergillus* was collected (including 155 and 5 cfu/m^3 respectively of *A. niger* and *A. candidus*). On March 20, 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 *A. flavus*, 5 *A. fumigatus* and 5 *A. versicolor.*

Tables II and III note that highest indoor concentrations of mean total indoor fungi (192.8 cfu/m^3) and *Aspergillus fumigatus* (11.0 cfu/m^3) were collected in the rooms which were undergoing duct cleaning. Anderson [34] reported *Aspergillus* fumigatus levels of 65 cfu/m^3 near a vaccum that was running in a pediatric cancer unit. This suggests the ducts are a significant source of *Aspergillus* 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* spores after cleaning might yield some useful information.

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, infiltration of outside air can not be the primary mechanism for producing the airborne *Aspergillus* levels in the hospital. Significantly higher levels of Aspergillus- especially A. fumigatus was seen during duct clearning operations. There probably were 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

We 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 aspergillosis in 2002: An update. *Eur J Clin Micro 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 MG. Invasive aspergillosis. *Rev Infect Dis* 1983; **5**: 1061-1067.

5. Denning D. Therapeutic outcome in Invasive Aspergillosis. *Clinl 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 Path* 1998; **51**: 652-656.

8. Kappe R, Schulz-Berge A, Sonntag HG. Evaluation of eight antibody tests and one antigen test for diagnosis of invasive aspergillosis. *Mycoses* January-February 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**: 1459-52.

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* July 1999; **20**: 508-513.

12. Goodley JM, Clayton YM, Ray RJ. Environmental sampling for *Aspergilli* during building construction on a hospital site. *J Hospl Infect* 1994; **26**: 27-35.

13. Opal S, Asp AA, Cannady PB, Morse PL, Burton LJ, Hammer PG. 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* April 15 2003; **75**:1050-3.

15. Anaissie 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). In: Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (Toronto, Ontario, Canada). Washington, DC, American Society of Microbiology, 375: 2000.

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**: 430-437.

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. Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologica* 2001; **149**: 87-97.

22. Sherertz 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 Hematology* 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, Chatigny M, Feeley J, LaForce FM, Peterson K: Guidelines for sampling for viable aerosols in office environments. *Applied Industrial Hygiene* 1986; **1**: R19-23.

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*. Burgess Publishing. Minneapolis, Minnesota, 1972.

31. Samson R, Hoekstra E, Frisvad J, Filtenborg O. *Introduction to Foodborne Fungi.* Centraalburea Voor Schimmelcultures, PO Box 273, 3740 AG BAARN, The Netherlands 1996.

32. Klich M, Pitt J. *A laboratory guide to common Aspergillus species and their teleomorphs.* Published by Commonwealth Scientific and Industrial Research, PO Box 52, North Ryde, NSW 2113, Australia, 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: associates 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**: 381-6.

Click HERE to go back to MoldAcrossAmerica