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Ten-year air sample analysis of *Aspergillus* prevalence in a university hospital

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Summary Airborne fungal samples were collected on a monthly basis for 10 years, from 1995 to 2005, at a tertiary university hospital. Paired samples were cultured at 25 and 37 °C. Data were interpreted according to the air filtration systems serving each location. Samples cultured at 37 °C from the patient care areas had a mean recovery of 18% of the mean recovery from outdoor air (22 versus 122 cfu/m^3). Recovery of *Aspergillus* spp. at 37 °C in the high-efficiency particulate air (HEPA)-filtered locations was positive for *Aspergillus* spp. approximately one-third of the time; the rest of the patient care areas were positive half of the time and the outdoor samples were positive 95% of the time. We found 48 sporadic bursts at 37 °C which produced counts >3 SD above the mean. Hospital-acquired infection was related to high recovery of *Aspergillus fumigatus* on at least one occasion. We have found it impossible, without implementing impractical measures, to provide an environment completely devoid of *Aspergillus* spp. We conclude that routine air sampling is not an effective means of predicting hospital-acquired infections. However, a transient spike, or burst, may be useful in identifying an in-house source of contamination and may be used to consider additional interventional treatments for patients at risk. Emphasis should be placed on maintaining high-efficiency filtration of the outside air and on ensuring that other environmental control methods are used to prevent dissemination of environmental opportunistic fungal spores.

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Introduction

Certain patient populations have a greater risk of developing invasive aspergillosis (IA).[1–3] Due to the high mortality associated with IA, the standard of care for at-risk patient populations is to provide chemoprophylaxis and an enhanced environment.[4–7] An enhanced environment includes: positive pressure rooms, point-of-use high-efficiency particulate air (HEPA) filters, protection against infiltration and filter bypass, high air exchange rates and elimination of in-hospital sources of opportunistic fungi.[7,8]

Studies have been conducted to determine the prevalence of *Aspergillus* spp. in the hospital environment.[9–13] Although a quantifiable level of contamination leading to an increased risk of infection has not been determined, increases in airborne *Aspergillus* spp. have been correlated with increased incidence of IA.[14,15] Studies have indicted contaminated surfaces and water supplies as potential sources for infection by *Aspergillus* spp.[16,17] Hospital construction has often been shown to cause an increase in the total airborne fungal counts.[7,18–21] Bursts (sporadic deviations from the norm) in the quantity of airborne *Aspergillus* spp. occur in a transient manner and the levels may vary within a given day.[22] These transient spikes, or bursts, probably occur when an accumulation of spores is disturbed by some external source. These events have been described and quantified rarely.

This study was conducted to evaluate the long-term effectiveness of measures taken to protect the air quality in a university hospital, and to analyse the usefulness of the burst phenomenon in determining a meaningful way to interpret data obtained through air sampling.

Methods

Environmental sampling was performed prospectively during a 10 year period at a University hospital. This hospital was designed and constructed to control airborne fungi using increased filtration, pressurisation and high air exchange rates.[23] Sampling was conducted in order of expected cleanliness; the HEPA-filtered patient care areas (PCAs) were sampled first, and the outdoors last. Expected cleanliness was determined according to the respective building's ventilation system (Table I). There are four stacked PCAs, labelled A, B, C and D, on separate floors. The PCA ventilation system is designed such that a fan provides 90–95% filtration (ASHRAE 52-92 Dust Spot Method) to the same PCA on each floor (i.e. fan S-12 supplies the PCA 'D' on each floor). The paediatric Bone Marrow Transplant unit has a dedicated fan, but the adult BMT unit is a HEPA-filtered extension of the fan that supplies the PCA 'B'. The four PCA zones were analysed separately.

Table I Ventilation in each location sampled

Location	Filtering efficiency of fan (%)	ΔP[a]	Air changes per hour
BMT suite (32 rooms)	99.97	0.03	12
Patient care units	90–95	[b]	3
Intensive care unit	90–95	[b]	6
Indoor reference	65	NA	NA
Lobby	90–95	NA	NA

BMT, bone marrow transplant; NA, not applicable.
[a] Pressure differential of patient's room, with the door closed.
[b] Open door, no ΔP maintained.

A total of 1523 fungal samples were collected between 5 January 1995 and 28 December 2005. A slit impactor sampler was used at each location to collect the samples. The volume sampled in the filtered indoor air was 1400 L, and 700 L were sampled outdoors.[24]

Samples were collected monthly, and all locations were sampled within 1 h. The samples collected in areas of patient care were collected ~1 m off the floor in a corridor adjacent to a nurses' station. The samples were incubated on Inhibitory Mould Agar for seven days at 25 and 37 °C prior to enumeration and identification. The number of colonies recovered per plate was adjusted to colony-forming units per cubic metre of air (cfu/m^3). Filamentous fungi were incubated at 37 °C and identified by classification to genus level on the basis of the macroscopic and microscopic characteristics of the colony, according to standard conventions.[25,26] The genus *Aspergillus* was further identified to species level, and *Aspergillus* with teleomorph forms, such as *Eurotium*/*Aspergillus glaucus* were included in the total *Aspergillus* counts.[27]

Results

The mean total airborne fungal concentration was analysed in relation to the fan system filtering PCA locations. In general, isolate recovery was greater when the sample was incubated at room temperature (25 °C) when compared to incubation at body temperature (37 °C). Of the 1141 samples obtained from areas of patient care, an average of 34 cfu/m^3 was recovered at 25 °C, compared with 22 cfu/m^3

at 37 °C, respectively. The indoor reference and the lobby were sampled 253 times, and averaged 81 cfu/m^3 at 25 °C and 21 cfu/m^3 at 37 °C, respectively. Although the lobby had the same 90—95% filtration rate as the areas of patient care, it had higher mean recovery for two identifiable reasons. First, one set of the double doors to the outside was often held open so there was direct exposure to the outside air. Second, the inherently higher traffic levels cause more air turbulence and may introduce airborne mould spores, both from outdoors and from in-house reservoirs. The outdoor air near the hospital was sampled 129 times and had a mean recovery of 848 cfu/m^3 at 25 °C and 122 cfu/m^3 at 37 °C. Samples from areas of patient care showed a 96% reduction from the outdoor mean recovery when cultured at 25 °C, and an average mean reduction of 82% when cultured at 37 °C. The recovery values for each location sampled are displayed in Table II.

As shown in Table II, the mean and median values, at both temperatures, are quite different. The mean value, in every instance, is greater than the median, which indicates that the data are skewed toward the higher values. The greatest difference between the maximum recovery and the mean recovery from a hospital ward occurred on a floor filtered (at a 90—95% reduction) by fan S-11; this difference was 10 SD away from the total average recovered fungi at 37 °C. Around 3% of the 1523 samples incubated at 37 °C could be identified as a potential spike, or burst, because they were >3 SD away from the mean (Figure 1). These deviations may represent the greatest risk for a short-term, high-dose exposure.

The mean total airborne fungi recovered at both 25 and 37 °C provide a 'clean, cleaner, cleanest' rank order interpretation of the expected air quality. The lowest mean recovery, and percentage of samples positive for *Aspergillus*, occurred on the HEPA-filtered areas, with the mean recovery increasing in the other areas of patient care, the other indoor areas (i.e. the lobby) and outdoors. The mean recoveries from the PCAs were always lower than the outdoor reference counts. The results are especially obvious for the samples cultured at 25 °C: the outdoor reference samples contained >40 times more cfu/m^3 than the areas of patient care with HEPA-filtered fans, and ~25 times more cfu/m^3 than the other patient care units (PCUs). The results obtained at 37 °C provide qualitative evidence of the fans' effectiveness in filtering out potential pathogens.

The level of contamination by *Aspergillus* spp. is displayed in Table III. Sampling of the environment in the BMT units, which is filtered through HEPA-filtered fans, recovered *Aspergillus* spp. about one-third of the time, but only averaged 1 cfu/m^3. Together, the PCAs which are pressurised and have higher air exchange rates had the lowest mean recovery of *Aspergillus* spp. These two fan systems had a narrow distribution of data, because the median and the mean had similar values. The outdoor reference point contained *Aspergillus* spp. more than 90% of the time, and showed the greatest mean recovery (cfu/m^3) of *Aspergillus* spp. The outdoor sample had the largest difference between the median and the mean, and had the greatest range. The samples from patient care areas which did not have HEPA-filtered air and the other reference samples were almost indistinguishable in terms of mean *Aspergillus* spp. recovery (cfu/m^3), but all the PCAs had a significantly smaller percentage of samples which were positive for *Aspergillus fumigatus* when compared with outdoors.

Table II Colony-forming units per cubic meter sampled at each fan-specific location sampled

Location (Fan #)	Samples (*N*)	Total fungal counts at 25 °C				Total fungal counts at 37 °C			
		Mean	Median	SD	Range	Mean	Median	SD	Range
Adult BMT (S-11)	122	18	11	32	0—320	3.2	1.4	4.2	0—25
Paediatric BMT (S-9)	127	22	14	27	0—158	16	2.8	98	0—784
PCA									
'A' (S-8)	122	47	41	35	4.2—232	9.1	7	8.5	0—36
'B' (S-11)	123	46	27	103	2.8—1120	16	4.2	91	0—1008
'C' (S-14)	275	45	25	97	1.4—1120	9	5.6	15	0—151
'D' (S-12)	279	28	19	31	0—280	6.4	4.2	9	0—79
Intensive care unit (S-13)	93	26	19	21	2.8—95	8	4.2	20	0—186
Hospital lobby	126	97	66	91	7—582	21	11	42	1.4—428
Indoor reference	127	65	36	111	0—677	21	7.1	74	0—714
Outdoors	129	848	406	1076	17—5830	122	50	325	0—2540

PCA, patient care area.

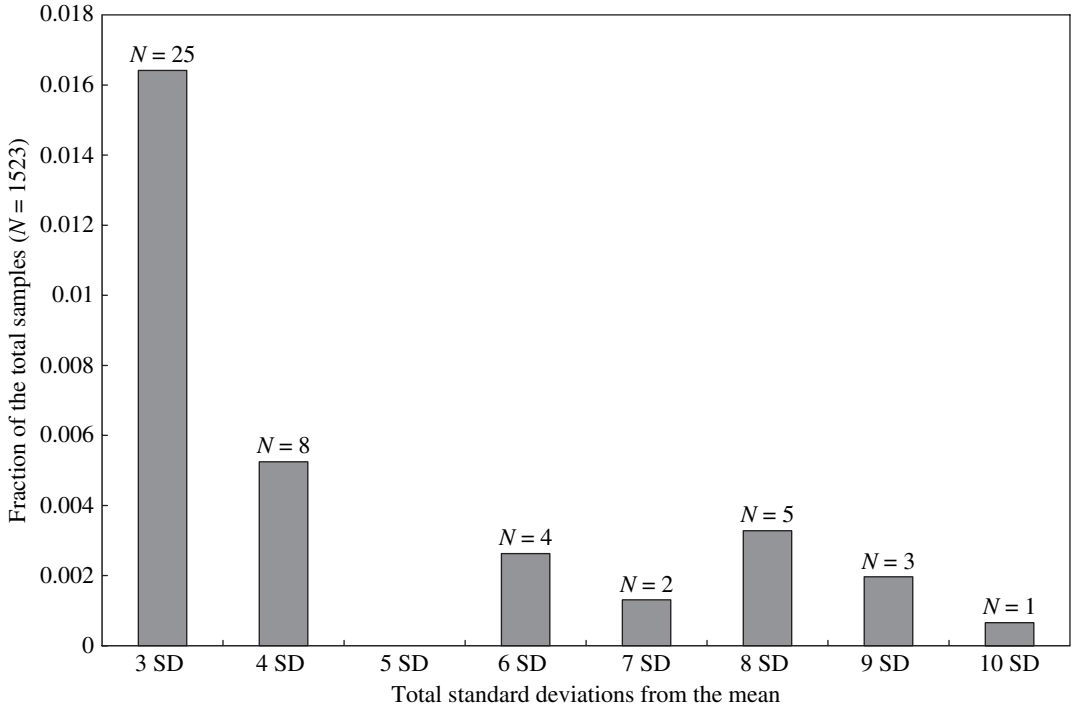

Figure 1 Total plates ($N = 48$) with recovery $\geq$3 SD from the mean at 37 °C.

In 2003, our routine sampling uncovered a burst of *Aspergillus fumigatus* in an oncology PCU. There was a case of hospital-acquired IA due to *A. fumigatus* in a patient on this PCU which was verified one month after the recovered burst. In every other month of that year, no *A. fumigatus* was recovered from that PCU and there were no subsequent nosocomial cases (Figure 2). This November burst was 10 SD away from the mean recovery of *A. fumigatus* observed in all the hospital PCAs, and

Table III Recovery of *Aspergillus* spp. from different test locations, in rank order by mean count obtained at each test location

Location	Mean (cfu/m^3)	Median (cfu/m^3)	Range (cfu/m^3)	Percentage of samples positive for *Aspergillus* spp.	Samples positive for *A. fumigatus* (% of total *Aspergillus* recovered)	Samples positive for *A. niger* (% of total *Aspergillus* recovered)	Samples positive for *A. flavus* (% of total *Aspergillus* recovered)
S-11 (HEPA BMT)	0.9	0	0—19	36	3.9	63	9.6
S-9 (HEPA paediatric BMT)	1.2	0	0—18	27	12	78	4.6
S-8 (A wards)	2.3	1.4	0—24	54	12	30	3.7
Hospital lobby	6.7	1.4	0—428	66	23	24	4.6
S-11 (B wards)	10	1.4	0—1008	51	7.2	26	2.4
Mayo reference site	15	1.4	0—714	62	25	24	3.2
Outdoors	45	14	0—2217	94	36	16	3

HEPA, high-efficiency particulate air filtration.
All samples were cultured at 37 °C.
Other *Aspergillus* spp. were recovered but not included, so columns 6—8 do not add up to 100%.

did not coincide with a high level of *A. fumigatus* in the outdoor control. The potential source of this burst was not located; however, this PCU had extensive water damage in 1994.[28]

Discussion

The recovery of *Aspergillus* spp. was lower in the BMT units than was shown a similar study which sampled inside the patients' rooms, but slightly higher than a study which had a HEPA-filtered unit with laminar air flow.[10,29]

Pathogenic fungi, especially of the genus *Aspergillus*, are frequently recovered in PCAs and may represent a higher percentage of the total airborne fungi than an outdoor sample.[10,19] We have noted that bursts of thermotolerant fungi have a high probability of containing some *Aspergillus* spp. Rather than using the total airborne fungi levels, it may be more useful to analyse recovery of *Aspergillus* spp. separately. By simply analysing our data in terms of the mean total airborne fungi, we would have missed three incidents of bursts >3 SD away from the hospital PCA mean recovery of *Aspergillus* spp. At our institution, a burst in June 1997 was 100% *A. flavus*, a species known to produce a great risk of infection. However, there were no associated cases of infection with *A. flavus*. In a one-year surveillance project by Curtis *et al.*, four bursts of *Aspergillus* spp. occurred in patients' rooms and were identified as in-house sources. Since no standard deviations were reported, it is unclear how far removed from the mean these results were. It is also unknown whether these bursts led to a hospital-acquired infection.[10]

In hospitals a burst can be an important exposure because the uncontrolled release of airborne fungi may pose a significant risk to a susceptible host. In our climate (Minnesota, USA) the efficacy of the filtration system should be verified through a demonstration of particle reduction to rule out the ventilation systems as a source of airborne fungal exposure. This reduction validation provides baseline data to confirm that the source of a burst is from an independent source, such as indoor uncontrolled construction. We recorded fungal levels which were >3 SD away from the mean in hospital recovery and which indicated potential in-house sources of mould spores.

Spore exposure can be the result of either failure of ventilation controls or a local disruption of a source of fungal spore accumulation. Recent literature has placed great emphasis on the

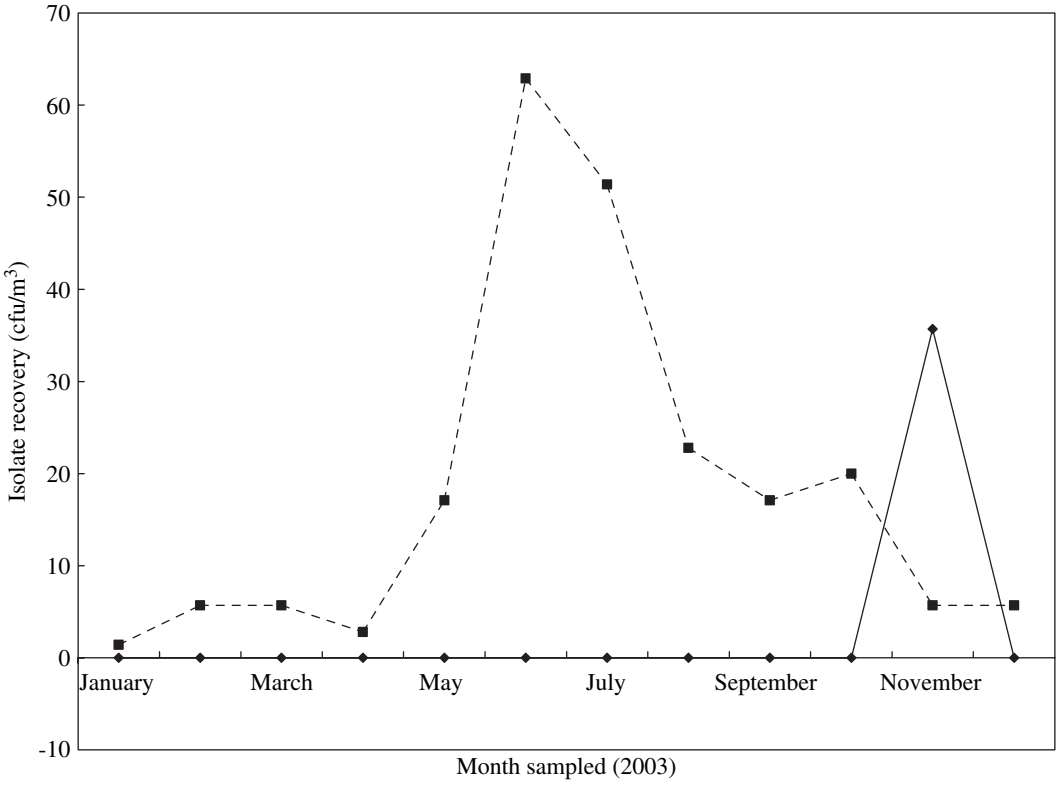

Figure 2 Monthly samples of outdoors and patient care areas (PCAs) with burst of Aspergillus fumigatus on an oncology patients care unit in November, 2003. ◆, PCAs; ■, outdoors.

analysis of air sampling in predicting risks for nosocomial infections.[9,10,28] However, sampling airborne fungi with the intent of identifying a potential exposure risk, such as during construction, is not likely to yield a timely infection control response. The data obtained from culture plates are not available for approximately one week, and come too late to identify the source of the transient burst of fungi. The value of the data obtained from routine air sampling should be used for validation rather than prediction. During our 10 year survey, it became clear that at 37 °C *Aspergillus* spp. are routinely found in areas of patient care; between 27 and 54% of the samples taken from respective PCAs were positive for *Aspergillus* spp. Could a burst of a particularly virulent species of mould, such as *A. fumigatus*, be used as justification to initiate anti-fungal therapy in at-risk patients? The specific environmental contamination marker could be targeted using molecular diagnostics and used in place of the usual signs and cultures to switch from standard prophylactic treatment.

The frequent recovery of airborne *Aspergillus* spp. from samples taken in a hospital environment is widely misunderstood in current literature. For example, a recent publication stated that virtually every case of nosocomial aspergillosis could be attributed to an airborne source, and concluded that patients at risk for IA 'should not be exposed to *Aspergilli*.'[29] This misinterpretation regarding exposure risks reflects an idealism that is not possible because air and environmental sterility is not a cost-effective option. The preventive measures to control the hospital environment cannot always eliminate in-house sources of *Aspergillus* spp.

The burst phenomenon is an important theory and can be described as short- or long-term exposure potential.[16,22] No amount of air sampling will yield a preventive response to such a phenomenon, and, although air sampling can be reassuring of high quality air, it will not prevent infection. The emphasis should be on maintaining the environmental controls and minimising the in-house release and containment of mould spores. Arnow *et al*. demonstrated that a lack of maintenance causes in-hospital production of mould spores.[21] We have repeatedly found mould sources in this institution. Infectious aerosol control is a challenge especially in climates where mould spores are common, such as agricultural regions to the west of Minnesota. Hospitals should maintain air quality control with dilution ventilation, high-efficiency filtration, pressure management, and operational procedures during both demolition and construction. Air sampling should be used as one method to assure a safe environment of care and not as an early response plan.

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