Journal of Hospital Infection (2004) **57**, 149–155

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Environmental investigations and molecular typing of *Aspergillus flavus* during an outbreak of postoperative infections

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Received 18 September 2003; accepted 3 February 2004

Available online 12 May 2004

KEYWORDS
Aspergillus flavus;
Surgical-site infection;
Environmental survey;
RADP typing

Summary After an outbreak of sternal surgical-site infections (SSSI) with *Aspergillus flavus* following cardiac surgery, a mycological survey of air and surfaces (41 and 149 samples, respectively) was performed throughout the surgical ward (SW) and in other areas of the hospital. Results showed massive contamination by *A. flavus*: more than 100 cfu per contact plate were frequently observed in some areas of the SW. The distribution of the *A. flavus* spores in the building, and especially in the SW, enabled the location of a possible source within the non-medical part of the SW, but the true source could not be identified. Four other surveys were made to follow up the decontamination process; the contamination level did not fall rapidly, needing repetitive cleaning operations. Strains from patients and from the hospital environment selected all over the SW were typed by random amplification of polymorphic DNA (RAPD), using two different primers (ERIC-1, BG-2). All these strains showed the same genotype, proving the clonal single-source of the environmental contamination and the intra-operative acquisition of *A. flavus* in the SSSI outbreak.

Introduction

Aspergillus species, commonly found in soil, decaying organic matter, dust and air, are ubiquitous filamentous fungi that can cause severe opportunistic human diseases. In immunocompromised hosts, the inhalation of conidia may provoke pulmonary, invasive and disseminated infections associated with high mortalities. The most common aetiologic agent of aspergillosis is *Aspergillus fumigatus*, but *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and some other thermotolerant species of the genus are also involved in these diseases.[1] *Aspergillus* spp. infections in immunocompetent patients are less frequent; cases of postoperative infections by *A. fumigatus*, *A. flavus* or *A. terreus*[2–6] have nevertheless been described, particularly after cardiac surgery.

Outbreaks of aspergillosis are most often hospital

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doi:10.1016/j.jhin.2004.02.007

acquired.[7,8] The contamination of hospital environments by fungal spores was frequently implicated as the cause of aspergillosis in haematological wards or in intensive care units.[9–12] Reported by Gage *et al.*[13] and Peterham and Seal[14] postoperative *Aspergillus* spp. infections were related to environmental contamination by *A. fumigatus* in a surgical ward (SW). More recently, the link between an aortic prosthesis infection with *A. flavus* and the contamination of the operating room (OR) was clearly demonstrated by molecular typing methods.[15]

The present study reports the extensive environmental sampling performed after an outbreak of *A. flavus* sternal surgical-site infection (SSSI) after cardiac surgery,[16] in order to localize the origin of the contamination. Strains isolated from the environment were typed and compared with the patients' strains by molecular methods to ascertain the hospital acquisition of the SSSI cases.

Materials and methods

Setting

St Jan General Hospital is a 774-bed secondary-care hospital located in Bruges, Belgium. The SW is located on the fourth floor of the building and consists of three wings. Fourteen ORs, equipped with HEPA (high-efficiency particulate air) filtration, are distributed in the two lateral wings (ORs 1–6 in wing A, ORs 7–14 in wing C), as well as recovery, rooms sterile supplies and storage rooms. In each of these wings, a peripheral corridor allows the evacuation of waste and used material directly from the ORs. Wing B is composed of offices, meeting rooms, lounges, utilities and a few additional sterile supplies and storage rooms; a lift enables provision of materials from the central supply located in the basement. There is no air-conditioning system in wing B.

Outbreak and its management

In April 1998, *A. flavus* was recovered from SSSIs in two patients after cardiac surgery. A retrospective finding study,[16] based on three criteria (clinical or radiological signs of deep infection or fistulae, isolation of *A. flavus* from deep operative wound samples and the patient having undergone cardiac surgery in the affected unit), demonstrated nine *A. flavus* SSSI cases from 18 January to 17 April (among others, a patient with strain P1), whereas no case had been noticed in the previous year.

A. flavus was also isolated from other patients who underwent cardiac surgery in the same period, but the SSSI cases did not match the three defined criteria (patient with strain P2) or was from bronchoaspiration in a patient with atelectasis (patient with strain P3).

At first, *A. flavus* was isolated from cardiac surgery ORs (OR 4 and 6 in wing A) on environmental culture performed by the infection control practitioner from the hospital. Each OR, their related entrance and exit anterooms, and the recovery rooms were thoroughly cleaned between 15 April and 7 May, and *A. flavus* was still detected.

After a request from the hospital authorities, a extensive environmental survey was performed on 5 May by a specialized team from the Scientific Institute of Public Health; only ORs 2, 12 and 13 had not yet been cleaned. The results of this sampling led the hospital authorities to close each wing successively and have them cleaned and disinfected by a specialized company: wing A on 11–15 May, wing B on 8–10 and 16–17 May, wing C on 18–20 and 25–26 May. New environmental surveys were performed on 18 and 28 May, 15 June and 18 August, in order to assess the results of the decontamination.

Environmental samplings

During the five surveys, a total of 440 surfaces were sampled with Rodac contact plates, filled with malt extract agar plus chloramphenicol, and 129 samples of air (200 L in 2 min) were taken using a Merck air sampler (MAS 100) using the same medium. Colonies were counted after 48 and 72 h of incubation at 37 °C, and all were identified to species level, based on macroscopic and microscopic morphology. In case of confluence of colonies, the colonies were nevertheless sufficiently distinguishable to evaluate their number on 1 cm^2.

When no colony was observed in air samples, the quantitative result recorded was the limit of detection of fungal spores taking into account the total volume sampled: 0 cfu from one sample of 0.2 m^3 was recorded as <5 cfu/m^3, 0 cfu from 10 samples (total volume sampled of 2 m^3) was recorded as <0.5 cfu/m^3. Qualitative results consists of the incidence of *A. flavus* in the total fungal flora from air and surfaces.

Typing

Three patients' strains (two from SSSIs and one from bronchoaspiration from a patient who also underwent cardiac surgery), and 15 from air and surfaces in the SW were selected for typing (Table I); each strain was subcultured in order to obtain monosporales. Strain

E1 was collected in OR 4 on the day of the operation of P1 in that OR. The discrimination power of the method was validated with 30 unrelated isolates from different human or environmental origins. All the strains typed are referenced and available in BCCM/IHEM Culture Collection, Brussels, Belgium (www.belspo.be/ihem).

DNA was prepared according to a previously described protocol by Symoens *et al.*[17] Primers used, ERIC-1 (5′ CAC TTA GGG GTC CTC GAA TGT A 3′) and BG-2 (5′ TAC ATT CGA GGA CCC CTA AGT G 3′), and random amplification of polymorphic DNA (RAPD) conditions were performed according to Leenders *et al.*[18]

Results

Environmental investigation

The results of 5 May environmental sampling in the SW and in some other areas of the hospital are summarized in Table II.

Table I Strains from patients and from the hospital environment typed in relation with the outbreak

Ref.	Date isolation d/m/y	Strain origin/patient data
P1	23/04/98	Sternal wound, CAGB in OR 4 on 15/4, SSSI
P2	30/03/98	Sternal wound, MVR in OR 4 on 18/3, SSSI
P3	26/04/98	Bronchoaspiration, CAGB on 30/1, SSSI and atelactasis
E1	15/04/98	Wing A, OR 4, air
E2	21/04/98	Wing A, OR 3, extraction air vent
E3	5/05/98	Wing A, anteroom of OR 4, furniture
E4	5/05/98	Wing A, anteroom of OR 6, furniture
E5	5/05/98	C wing, anteroom of OR 10, furniture
E6	5/05/98	C wing, OR 13, furniture
E7	5/05/98	Wing A, peripheral corridor, furniture
E8	5/05/98	Wing A, recovery, equipment
E9	5/05/98	Wing A, storage room, furniture
E10	5/05/98	Wing B, administrative office, furniture
E11	5/05/98	Wing B, Dr's office, air
E12	5/05/98	Wing B, restroom furniture
E13	5/05/98	Wing B, storage room, air
E14	5/05/98	Wing B, central corridor, floor
E15	5/05/98	Wing C, storage room, equipment

CABG, coronary artery bypass graft; MVR, mitral valve replacement; SSSI, sternal surgical-site infection; OR, operating room.

In the SW itself, the contamination level was higher in wing A and much higher in wing B than in wing C, on surfaces as well as in the air. Moreover, the highest local contamination observed on one surface was 52 cfu/plate in wing C, while plates with more than 100 cfu were observed in wing A (8% of the plates) and in wing B (65%). In wing B, these heavily contaminated surfaces were mainly distributed in the administrative office, the restroom, and the corresponding part of the central corridor (see Figure 1). On 5 May, 9510 cfu of *A. flavus* were isolated from surfaces in the whole SW while only 11 cfu of this species were cultured from the air in the same area. The highest local contamination of the air was 6 cfu in the sample of 0.2 m^3 from the restroom. *A. flavus* was the predominant species in the total flora from each area of the SW.

In the peripheral corridors, the fungal contamination reflected the contamination in the wings themselves with a proportion of *A. flavus* high on side A, low on side C. No fungal spores were detected on surfaces or from the air in the lift opening on to the most contaminated area of wing B, but *A. flavus* was abundantly present in the air from the lift shaft; it was also detected in areas close to this lift on other floors but at lower levels than in the SW (53% of the total fungal flora); it was not observed at the entrance of the SW. *A. fumigatus*, *A. niger*, *Aspergillus ustus*, *Paecilomyces variotii* and mucorales were the other thermotolerant fungi isolated in the hospital environment.

Table III lists the follow-up of the fungal contamination in the three wings of the SW from 5 May to 18 August. On 18 May, sampling occurred mainly in wing A; only one surface sample and one air sample were taken in wing B; wing C being closed for cleaning. On 15 June sampling concerned exclusively wing B. Contact plates with 100 cfu or more were still observed in wing A on 18 and 28 May (5% and 2% of the plates) and in wing B on 18 May (only one plate), 28 May and 15 June (8% both plates). On 18 May, 1000 cfu were observed on the unique surface sampled despite the previous cleaning. *A. flavus* was still the predominant species in all parts of the SW in May and June, but fell to 21% of the fungal flora in the SW in August behind *A. ustus* (43%) and *P. variotii* (24%).

Typing results

Thirty unrelated strains of *A. flavus* isolated from various origins, geographical areas, and/or pathologies, typed with primer ERIC-1, showed very

Table II Environmental contamination in the surgical ward and in some other areas of the hospital on 5 May

Areas	Contamination of surfaces			Airborne contamination			*A. flavus* in the total flora (%)
	Number of samples	Mean contamination (cfu/plate)	Min–Max	Number of samples	Mean contamination (cfu/m^3)	Min–Max	
Surgical ward itself							
Wing A	50	34	0–600	13	1.7	<5–10	98
Wing B	26	295	2–830	7	7.9	<5–30	99
Wing C	55	4	0–52	13	0.4	<5–5	70
Peripheral corridors							
Wing A	2	130	122–139	2	<2.5	<5–<5	99
Wing C	3	0.7	0–1	2	115	85–145	2
Elevator	2	0	0–0	1	<5	–	0
Elevator shaft[a]	–	–	–	1	115	–	100
Other floors[b]	10	2	0–11	1	<5	–	53
Fourth floor at the entrance of the SW	1	2	–	1	70	–	0

[a] Sampler placed between the doors of the lift and the lift itself.
[b] Other floors: areas close to the elevator on the basement, and on the first, third and fifth floors.

diversified RADP patterns (Figure 2a). The three strains from patients and all the strains selected from all over the SW ($N = 15$) had accurately the same genotype (Figure 2b). These results were confirmed with primer BG-2 (data not shown).

Discussion

After several cases of SSSI, the protocol of an extensive environmental survey was developed to allow the rapid localization of the contamination source and its eradication. The sampling of 5 May was thus planned in the different ORs and related rooms, in the non-medical parts of the SW, in other places on the same floor, and on other floors next to the lift opening in the SW; surfaces were sampled, as well as the air.

Air sampling data were not helpful in evaluating the contamination because of the low percentage of positive samples and the narrow range of the results (0 to 6 cfu per sample of 0.2 m^3). Conversely, as previously described,[15,19,20] results of surface sampling were more useful with up to

Table III Evolution of the fungal contamination in the three wings of the surgical ward

Areas	Dates	Contamination of surfaces			Airborne contamination			*A. flavus* in the total flora (%)
		Number of samples	Mean contamination (cfu/plate)	Min–Max	Number of samples	Mean contamination (cfu/m^3)	Min–Max	
Wing A								
	5 May	50	34	0–600	13	1.7	<5–10	98
	18 May	39	18	0–500	8	2.5	<5–5	98
	28 May	57	8	0–215	13	0.8	<5–5	94
	18 August	33	0.3	0–3	14	<0.4	<5–<5	30
Wing B								
	5 May	26	295	2–830	7	7.9	<5–30	99
	18 May	1	1000	–	1	5.0	–	100
	28 May	13	29	0–201	6	3.3	<5–10	94
	15 June	39	23	0–254	9	18.3	<5–40	82
	18 August	20	3	0–14	9	0.6	<5–5	17
Wing C								
	5 May	55	4	0–52	13	0.4	<5–<5	70
	28 May	51	3	0–62	13	0.8	<5–5	72
	18 August	38	0.4	0–10	15	<0.3	<5–<5	29

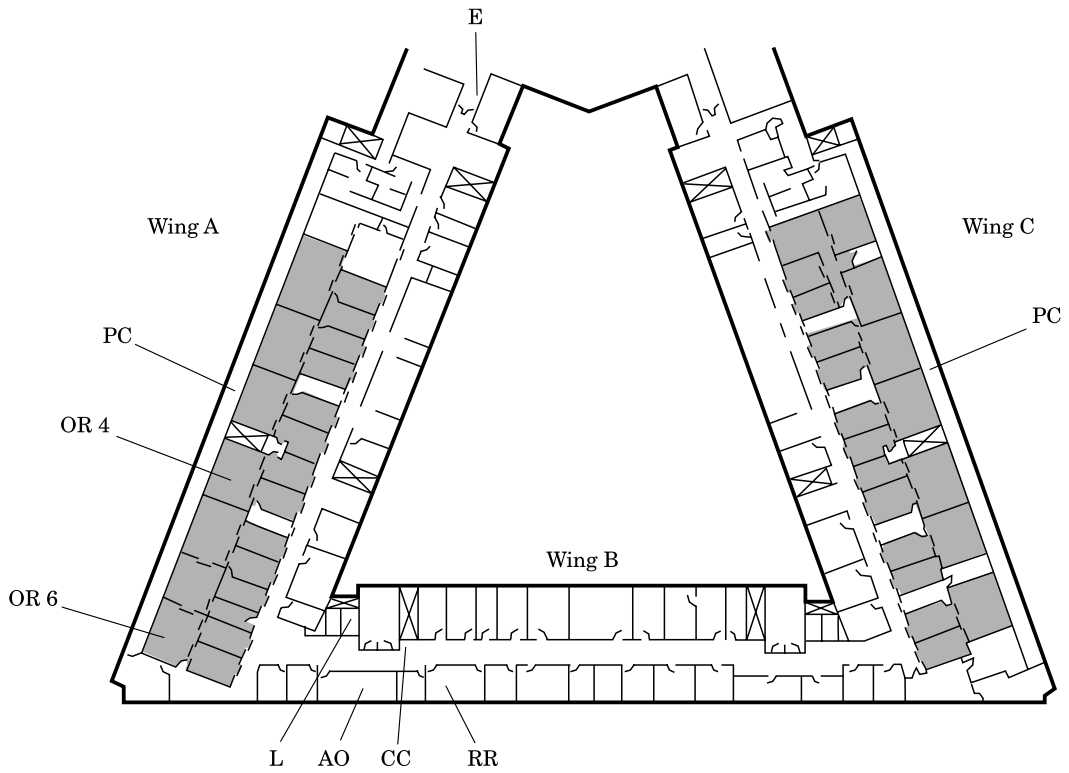

Figure 1 Plan of the surgical ward. Operating rooms (OR) and their entrance/exit anterooms are shaded. L, lift; AO, administrative office; RR, restroom; CC, central corridor; PC, peripheral corridors; E, entry.

1000 cfu per sample; they revealed a gradient of contamination reaching its highest values in wing B, in the restroom and the adjacent areas. In accordance with Alberti *et al.*,[9] this observation showed the importance of sampling in non-protected areas, as well as in protected ones. As the lift was actually located in the most heavily contaminated zone, the possibility that the fungal spores came from another floor via the lift[21] was examined. *A. flavus* was not isolated in the lift but was abundantly recovered from the air of the lift shaft; moreover, this species was cultured from other floors next to the lift, but never with such a high concentration as in the SW. These results obviously demonstrated that the source of the fungal spores was actually in the SW itself and that they were spread to the other floors by the motion of the lift.

Because of these results, the restroom and the administrative office were immediately closed, apparently being the places where the fungus could have likely developed. A water leakage was noticed when dismantling the banquette benches of the restroom which could implicate the damaged wood or the damp stuffing material as potential growing substrates for *A. flavus*. In the same way, Arnow *et al.*[10] showed that *A. fumigatus* and *A. flavus* could proliferate on common hospital materials when moistened. However, in the present case, direct examination of the damaged furniture, in order to reveal possible fungal *Aspergillus* spp. heads, could not be performed because the furniture was discarded too rapidly.

A growing source of spores inside the SW itself and the long dispersal period—the first operation taken into account by the retrospective study dates back to January[22]—explained the heavy contamination. Moreover, the spores generated in wing B had time to accumulate in an infinity of reservoirs in the whole SW even up to the ORs equipped with HEPA filtration. During the cleaning process, some of the surface spores may have dispersed into the air, escaped disinfection, and re-settled. This explains the need for successive cleaning operations to obtain the definitive decrease of the contamination. The highest contamination on a surface was observed on 18 May in wing B corridor; this was likely due to extensive air movements caused by moving all the furniture from wing C to wing A, through wing B, in the previous days, in order to clean wing C.

Closing the SW, dismantling of equipment and ceilings, cleaning and disinfection of each wing successively by a specialized company, changes in the behaviour of the healthcare workers towards hygiene were certainly important factors which

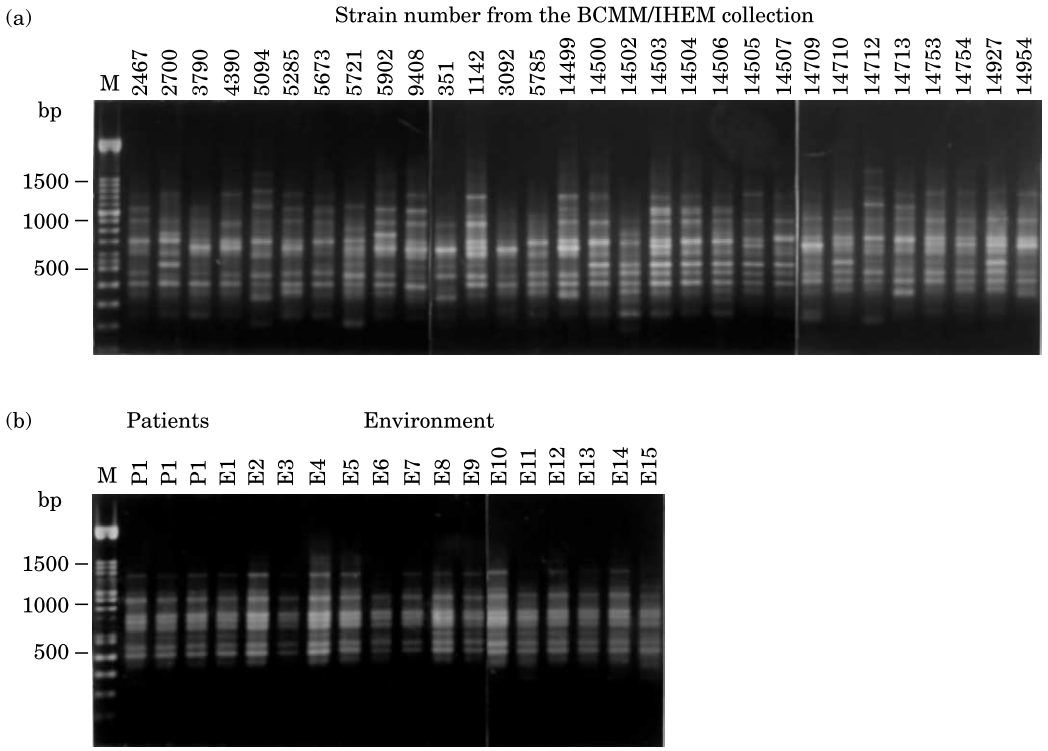

Figure 2 (a) Typing of 30 epidemiologically unrelated strains. (b) Typing of the strains from patients (P1 to P3) and from the hospital environment (E1 to E15) in the context of the outbreak.

resolved the problem. Nevertheless, complete decontamination was obtained after some weeks after the closing of the rooms where the fungal spores were likely to have originated (the restroom and administrative office); this was sufficient to eradicate the source of *A. flavus* spores.

In August the contamination was more 'usual' according to our experience in non-epidemic context in other SW (contamination <1 cfu per plate on surfaces and <1 cfu/m^3 in the air) and no new case of SSSI occurred.

The occurrence of an exceptional contamination of the SW by *A. flavus* spores and clustered cases of SSSI due to this species could be sufficient to presume an intra-operative acquisition. Nevertheless, proof of nosocomial acquisition was supplied by molecular typing establishing the relationship between environmental and patient isolates. In this case the RAPD technique was chosen because it is easy to perform and to interpret. The validation of the RAPD conditions was done with 30 unrelated strains of *A. flavus* from the BCCM/IHEM culture collection. The nosocomial acquisition of the post-operative infections and the clonality of the environmental contamination could be clearly proved: the three patients' isolates (two from SSSIs and one from bronchoaspiration) shared exactly the same RAPD pattern with the two primers as all the environmental strains from all over the SW.

A case of *A. flavus* nosocomial infection after cardiac surgery has been reported previously by Diaz-Guerra *et al.*:[15] the source of *A. flavus* could not be elucidated, but two strains from a cooler–heater unit in the OR had a RAPD pattern identical to the patient's strain.

These data highlight the importance of molecular typing to prove nosocomial acquisition of *A. flavus* infections. Molecular typing could also prove the hospital-acquisition of *A. terreus* invasive pulmonary aspergillosis after surgery, as well as the clonality in colonized cystic fibrosis patients and in environment samples.[17,23] These two species have a lower occurrence in the environment than *A. fumigatus*, for which the ineffectiveness of molecular typing to assess the environmental acquisition of an infection has already been proved. Indeed, for this species a highly varied environmental genotypic diversity was observed[24,25] and patients can be simultaneously or successively infected by several genotypes.[26,27]

This outbreak of SSSI due to an exceptional contamination of the SW by *A. flavus* was stopped after extensive environmental sampling, the

elimination of the source of the spores, the decontamination of the premises and the improvement of the hygiene precautions in the SW.

Acknowledgements

The authors are grateful to Christel Binard for English proofreading.

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