ASPERGILLUS AIR POLLUTION IN OPERATING THEATRES

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ABSTRACT
Aim of this paper is to present the results of a survey, performed in a teaching hospital of Rome, in order to evaluate the fungal air pollution of operating theatres (OTs) in which also high risk patients underwent an operation. The study was performed in 14 OTs. In each OT, 9 air samples were collected, using an active sampler SAS-PBI. Rodac plates with Sabouraud cloramphenicol and nutrient agar were used; the CFU were counted after incubation for 48h at 37°.

Eight (57%) OTs showed at least one sample positive for Aspergillus. 12 (9,5%) of the 126 samples collected were positive. 33,4% of positive samples were collected near the air conditioning system outlet (*A. fumigatus*, *A. flavus*, *A. niger*) and a further 33,4% near the surgical table (*A. niger, A fumigatus, A. nidulans, A. flavus*). The remaining strains were isolated away from such critical places. All Aspergilli isolated were thermo-resistant.

INDEX TERMS
Aspergillus, air pollution, operating theatres, monitoring, pathogenicity

INTRODUCTION
In the last two decades several studies have shown an increase of hospital infections due to opportunistic fungi (Fraser et al, 1979, Pertowski et al, 1995, Groll et al, 1996). The most frequently involved species are *Candida* (Pfaller, 1988) and *Aspergillus* (Bodey and Vartivarian, 1989). The rising incidence of these infections is mainly due to the widespread use of aggressive cancer chemotherapy regimes, the increasing number of transplant programs and the occurrence of AIDS epidemic (Bodey, 1988, Drohuet E, 1989, Manuel and Kibbler, 1998).

Despite the development of new therapeutic approaches there infections are still responsible for a high mortality that exceeds 50% of cases (Hospenthal, 1998); therefore it is important to introduce effective control measures to avoid further risks for patients.
Aspergillus spp are common environmental saprophytes, often found in soil, water, fomites, ventilation systems, building materials, potted plants and other sources like foods. *Aspergillus* species account for up to 40% of the fungal flora in hospital and home environment (Manuel and Kobbler, 1998).

There exist about 180 *Aspergillus* species and about 30 of them are potentially pathogens for man and animals; in particular: *A. fumigatus, A. flavus and A. niger* (Chabasse et al, 1995).

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Invasive Aspergillosis often occurs in immunocompromised patients; major adjuvant factors are: the underlying disease, the neutropenia (neutrophils <500/ml), the administration of high-doses and extended treatments with immunosuppressive drugs. The transmission of infections occurs when inhaled spores reach pulmonar alveoli and invade the lungs, thanks to their tissues adaptability and the riduced immune defenses of the host.
Another way of transmission, less frequent than the first one, is the direct contamination of deep tissues after burns, crushes, or during a surgical procedure (Bodey and Vartivarian, 1989). Wound contamination can take place from aerodispersed spores or from spores adherent to surfaces or fabrics (bandage, medical dresses). Several Authors demonstrated the association between *Aspergillus* spp contamination of the OTs and the occurrence of deep infections like endocarditis (Petheram and Seal, 1976, Metha, 1990) and cerebellar infections (Morin et al, 1989).

The interruption of the skin barrier due to surgery makes a deep contamination possible. Switch from contamination to infection depends on several factors, such as the fungal capability to grow up at 37° and to adhere to the tissues of the host. It has been shown that in vitro the spores of potentially pathogen species, particularly *A fumigatus*, bind to C3 of the complement, to the fibrinogen and to laminin. In vivo (e.g. in a surgical wound), the deep tissues contamination comes after the deposition of spores on surgical wound: the basal membranes‘ s exposure, the laminin accessibility, the inflammatory reaction, etc, promote the adherence to the deep tissues, and finally induce moltiplication (Annaix et al, 1992, Bouchara et al, 1988, Bouchara et al, 1994).

The infection will take place if the immune defences (mostly phagocitosis) are inadequate like for immunocompromised patients, where the severe neutropenia and the necrotic lesions of mucosae due to antiblastic drugs make the occurrence of Invasive Pulmonary Aspergillosis (IPA) easy. Some tissues are particularly susceptible to *Aspergillus* spores, also in immune-competent people: neural tissue (Darras-Joly, 1996, Morin et al, 1989), endocardium and endotelium of large vessels (Petheram and Seal, 1976, Metha, 1990, Gilbert, 1996), cornea (Heidemann, 1995), etc; but the switch from contamination to infection is still obscure.
Aim of this paper is to present the results of a survey, performed in a large hospital of Rome, in order to evaluate the fungal air pollution of operating theatres in which also high risk patients underwent an operation.

METHODS
The study was performed in 14 OTs, located in 5 Surgical Wards (SW) of a large hospital of Rome (Italy), all supplied with air conditioning systems (turbolent flow), HEPA filters and positive pressure. In each OT, 9 air samples were collected in clean OTs (t_0), during (t_1) and after (t_2) surgical activities, in 3 different places:

- next to the surgical table at 150 cm height (point 1);
- at the same height, but far from the surgical table (point 2);
- next to the air outlet of the conditioning system (point 3).

Samples were collected using an active sampler SAS-PBI and the duration of each sampling was 1 minute, corresponding to a volume of 180 litres of air.

At t_0, in each OT, 10 samples were collected from at risk surfaces, on or next to surgical table (surgical table, operating lamps, surgical aspirators, technical units for general anaesthesia, mobile technical units, etc) or in other surfaces far from it, using Rodac plates (24 cm^2). Rodac plates with Sabouraud cloramphenicol and nutrient agar were used; the CFU were counted after incubation for 48h at 37°. The isolated *Aspergilli* were further seeded and

incubated at 45° in order to test their thermic resistance. Species identification was defined with a new seeding in Czapek, supplemented with 0,5‰ baking powder.

RESULTS

Table 1 summarizes the main study results. 8 (57%) OTs showed at least one air sample positive for *Aspergillus* species. 12 (9.5%) of the 126 samples collected were positive. 33.4% of positive samples were collected near the air conditioning system outlet (*A. fumigatus*, *A. flavus*, *A. niger*) and a further 33.4% near the surgical table (*A. niger*, *A fumigatus*, *A. nidulans*, *A. flavus*). The remaining strains were isolated away from such critical places.
In 4 OTs 22.2% of samples were positive for *Aspergillus* species. The positive samples collected in SW1-OT4 showed different *Aspergillus* species: *A. niger* and *A. fumigatus*. 4 isolations (33.4%) were *A. fumigatus* and 2 of them (50%) came from cardiosurgery OTs.
Among 140 surface samples collected, 9 (6.4%) were positive for aspergilli (table 2) and, among them, 3 (33.4%) refer to surface on or next to the surgical table. All positive samples came from 3 OTs (21.4%).

All *Aspergillus* species isolated were thermo-resistant. Only in 4 OTs (25%) the last HEPA filters change took place less than 3 months before the study; furthermore, in several OTs, the air positive pressure was frustrated because of the health workers'use to open frequently the doors during surgical activities. In 6 OTs (43%) there were, in average, 5.8 health workers present during the OTs activities.

Table 1. *Aspergillus* species collected in the OTs air

SW	OT	# Positive air samples (%)	Positive sampling points	Isolations	Incubation (37°/45°)
SW 1	OT 1	2 *(22.2)*	far from surgical table	*A. niger*	+/+
			next surgical table	*A. niger*	+/+
	OT 2	0 *(0.0)*	-	-	-/-
	OT 3	0 *(0.0)*	-	-	-/-
	OT 4	2 *(22.2)*	far from surgical table (t_0)	*A. niger*	+/+
			far from surgical table (t_1)	*A. fumigatus*	+/+
SW 2	OT 1	1 *(11.1)*	oulet conditioning system	*A. flavus*	+/+
	OT 2	0 *(0.0)*	-	-	-/-
	OT 3	0 *(0.0)*	-	-	-/-
	OT 4	1 *(11.1)*	outlet conditioning system	*A. niger*	+/+
SW 3	OT 1	0 *(0.0)*	-	-	-/-
	OT 2	2 *(2.2)*	far from surgical table	*A. flavus*	+/+
			next surgical table	*A. flavus*	+/+
	OT 3	0 *(0.0)*	-	-	-/-
SW 4	OT 1	1 *(11.1)*	outlet conditioning system	*A. fumigatus*	+/+
	OT 2	1 *(11.1)*	next surgical table	*A. nidulans*	+/+
SW 5	OT 1	2 *(22.2)*	next surgical table	*A. fumigatus*	+/+
			outlet conditioning system	*A. fumigatus*	+/+

DISCUSSION

The study highlights some interesting issues. First, in 57% of the OTs monitored there was an air pollution due to *Aspergillus* and in 33.4% of them the pollution came from the air conditioning systems. Second, all *Aspergillus* species isolated were thermo-resistant, which could be a factor of pathogenicity (Chabasse, 1995), and in other 33.4% of cases the pollution

was observed next to the surgical table. Third, the management of OTs shows relevant hygienic faults: lack of HEPA filter renovation in 75% of cases; lack of OTs activities isolation and access protection from visitors. Fourth, it has to be stressed that, although the surface samples were collected early in the morning, before the start of surgical activities, in 6.4% of cases surfaces resulted positive for *Aspergillus* and 33.4% of positive samples was collected next to the surgical table on high risk surfaces. Although the study regards only 14 OTs, all belonging to the same hospital, and it shows the results obtained in 1 day-surveillance for each OT, the similarity of the results and the faults observed, among the OTs examined, seem to support their validity. In order to evaluate the reliability of these results it could be useful to repeat the survey more times in each OTs and to extend the survey to other OTs.

Table 2. *Aspergillus* species collected on the OTs surfaces

SW	OT	# Positive air samples (%)	Positive sampling surfaces	Isolations	Incubation (37°/45°)
SW 1	OT 1	1 *(11.1)*	far from surgical table	*A. niger*	+/+
	OT 2	0 *(0.0)*	-	-	-/-
	OT 3	0 *(0.0)*	-	-	-/-
	OT 4	3 *(33.3)*	on surgical table	*A. nidulans*	+/+
			far from surgical table	*A. niger*	+/+
			far from surgical table	*A. fumigatus*	+/+
SW 2	OT 1	0 *(0.0)*	-	-	-/-
	OT 2	0 *(0.0)*	-	-	-/-
	OT 3	3 *(33.3)*	far from surgical table	*A. fumigatus*	+/+
			far from surgical table	*A. niger*	+/+
			on mobile technical unit	*A. niger*	+/+
	OT 4	1 *(11.1)*	far from surgical table	*A. unguis*	+/+
SW 3	OT 1	0 *(0.0)*	-	-	-/-
	OT 2	0 *(0.0)*	-	-	-/-
	OT 3	1 *(1.1)*	on operating lamp	*A. niger*	+/+
SW 4	OT 1	0 *(0.0)*	-	-	-/-
	OT 2	0 *(0.0)*	-	-	-/-
SW 5	OT 1	0 *(0.0)*	-	-	-/-

CONCLUSIONS AND IMPLICATIONS

The need for the biological monitoring of hospital wards and of OTs is a *vexhata questio* (Hospenthal et al, 1998, Paugam te al, 1999). The environmental monitoring of Aspergilli is usefull mainly to evaluate the effectiveness of air filtration systems and the hygienic management of the OTs (Lajonchere and Feuilhade De Chauvin, 1994).

As stated before, deep aspergillosis is not only represented by IPA in immunocompromised patients, but also by other pathological situations such as endocarditis, endophthalmitis, post-surgical cerebral abscesses, etc. Such pathologies are less common and less life-threatening for patients than IPA, but they represent critical entities, uneasy to diagnose.

Positive pressure air conditioning systems with HEPA filters, with or without laminar flow, are employed to keep some hospital environments free from microbiological air pollution, potentially pathogenic Aspergilli included; such a protection is real only when such plants are

correctly managed and monitored. Environmental safety depends also on adequate disinfection of surfaces and on a rational circulation of the personnel within the OTs.

Only a correct biological monitoring of the protected OTs can guarantee that such rules are respected. It must be made clear that neither standardized instructions for air or surface sampling exist, nor instructions are available to define the biological risk from opportunistic Aspergilli detected in protected environments (Deruin, 1996). Some authors underline that very seldom *Aspergillus* spores can be detected in rooms where air is supplied through absolute filters, while it is even more difficult to trace them when also a laminar flow is operating (Bocquet and Brucker, 1993).

The presence of *Aspergillus* spores in the OTs examined in this study, all equipped with absolute filters, must be considered abnormal, and even more abnormal if one reflects that such spores were present in samples from clean OTs, before the starting of daily activity. The mycological monitoring, therefore, is advisable in order to verify the effectiveness of air conditioning in OTs such as the ones under our control, especially if in such OTs surgical interventions are performed on tissues at special risk.

The objective “zero Aspergilli” has been recently suggested by a “Consensus conference” which took place in Paris in March 2000 on mycological surveillance of hospital environments protected by positive pressure or laminar flow air conditioning with HEPA filters. The Conference report suggests to prefer surfaces samples to air samples, because spores tend to deposit on surfaces of protected environments, where they can develop easily due to their limited nutritional requests. Air sampling should therefore be considered only as a support for surface sampling (Conférence de consensus, 2000).

In conclusion, when a serious infectious risk is ahead, even infrequent, but from which a definite pathologic event can evolve, it is necessary to implement all the cost-effective preventive procedures available: this is the case for infectious risk from *Aspergilli* during a surgical treatment. A periodical monitoring of OTs contamination by *Aspergilli* using surface samples represent an appropriate and unexpensive method of environmental surveillance capable to detect, just on time, a specific infectious risk situation in those OTs where complex interventions take place. In particular, such OTs must be equipped according to the suggestions of the “Consensus conference on at risk hospital wards”, where the “zero Aspergillus” goal is seeked.

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