

# Air-conditioning vs. presence of pathogenic fungi in hospital operating theatre environment<sup>1</sup>

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**ABSTRACT.** Infections related to modern surgical procedures present a difficult problem for contemporary medicine. Infections acquired during surgery represent a risk factor related to therapeutical interventions. Eradication of microorganisms from hospital operating theatre environment may contribute to reduction of infections as the laminar flow air-conditioning considerably reduces the number of microorganisms in the hospital environment. The objective of the study was to evaluate the occurrence of fungi in air-conditioned operating theatre rooms. The study was carried out in one of the hospitals in Kraków during December 2009. Indoor air samples and imprints from the walls were collected from five operating theatre rooms. A total of fifty indoor air samples were collected with a MAS-100 device, and twenty five imprints from the walls were collected using a Count Tact method. Fungal growth was observed in 48 air samples; the average numbers of fungi were within the range of 5–100 c.f.u. in one cubic metre of the air. Fungi were detected only in four samples of the wall imprints; the number of fungi was 0.01 c.f.u. per one square centimetre of the surface. The mould genus *Aspergillus* was most frequently isolated, and the species *A. fumigatus* and *A. versicolor* were the dominating ones. To ensure microbiological cleanness of hospital operating theatre, the air-conditioning system should be properly maintained. Domination of the *Aspergillus* fungi in indoor air as well as increase in the number of moulds in the samples taken in evenings ( $p < 0.05$ ) may suggest that the room decontamination procedures were neglected.

**Key words:** operating theatre, moulds, air conditioning, nosocomial infections

## Introduction

The increase in the number of microorganisms in hospital rooms can be prevented by use of proper ventilation and air-conditioning systems. The conditions in hospital operating rooms must comply with the requirements defined in the instructions of the Ministry of Health and Social Welfare. According to these instructions, the operating theatres as well as post-operative and nearby rooms should be air-conditioned. These requirements derived from the increasing number of nosocomial infections acquired during surgery. The prevalence of such infections may be reduced by creation of a microorganism free environment, and shortening the surgical procedure time. Knochen et al. [1] compared the environment in operating rooms which floor was disinfected after each surgery to

those where the floor was cleaned only when dirt was visible; none of those procedures had any effect related to the number of infections in the patients after surgery. Air-conditioning with laminar air flow is a sufficient measure that limits the number of microorganisms in the indoor air.

In modern air-conditioning system, the air flows laminarly from the ceiling. In operating theatres, it should provide a sufficient quantity of fresh air and a proper hourly air exchange rate. Endoorthopedical surgical procedures are thought to be at the higher risk related to the presence of microorganisms in the indoor air. Therefore, such procedures should be performed in the 1st class cleanness operating rooms; according to Polish guidelines, the number of microorganisms in such rooms should not exceed 70 c.f.u. per 1 m<sup>3</sup> of indoor air. The analysis carried out by Kramer et al. [2] demonstrated that the

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requirements concerning classes of microbiological cleanness in the operating rooms should be based on the assessment of risk related to surgery but not on epidemiological studies. One may disagree with such opinion as it is easier to assess infection risk than to perform epidemiological study. The latter is usually performed after patient's exposure to bioaerosol but under such circumstances it can hardly be determined what microorganisms were present in the air and whether they were causative agent of the infection. On the other hand, not every microorganism is pathogenic, so its isolation from the air not always provide evidence of its direct relation to the infection of exposed patient. The objective of the study was to evaluate the occurrence of pathogenic fungi in the environment of an air-conditioned hospital operating theatre.

## Materials and methods

The study was carried out in one of the hospitals in Kraków, Poland, in December 2009. The study comprised evaluation of contamination with fungi of the indoor air and the walls as well as measurements of temperature and humidity in five air conditioned, laminar air flow rooms of an operating theatre. The air-conditioning system in these rooms was equipped with absolute filters. The measurements were carried out for five days (Table 1), and the samples were collected twice daily, at approximately 7 AM (morning) and 7 PM (evening) (Table 1). A total of fifty indoor air samples were collected using a MAS 100 device (Merck), and twenty five imprints from the walls were collected using a Count-Tact method. Two hundreds litres of indoor air were sampled at a time using the impact

Table 1. Number of colonies (N) and the species (S) of pathogenic fungi isolated from the indoor air of the hospital operating theatre rooms: M, morning; E, evening.

Sampling site	Sampling time		7 December	8 December	9 December	10 December	11 December
Room no. 1	M	N	30	55	25	10	5
		S	–	<i>A. fumigatus</i> <i>A. terreus</i>	<i>A. terreus</i>	<i>A. versicolor</i>	<i>A. versicolor</i>
	E	N	5	70	15	20	10
		S	–	<i>A. fumigatus</i>	<i>A. versicolor</i>	<i>A. versicolor</i> <i>A. fumigatus</i>	<i>A. versicolor</i>
Room no. 2	M	N	5	35	15	5	5
		S	<i>A. versicolor</i>	<i>A. versicolor</i>	<i>A. versicolor</i>	<i>A. versicolor</i>	–
	E	N	25	25	0	10	5
		S	<i>A. versicolor</i> <i>A. fumigatus</i>	<i>A. ochraceus</i> <i>A. nidulans</i>	–	<i>A. fumigatus</i> <i>A. versicolor</i>	–
Room no. 3	M	N	25	45	35	5	0
		S	<i>A. fumigatus</i>	<i>A. versicolor</i>	<i>A. fumigatus</i> <i>A. versicolor</i>	<i>A. versicolor</i>	–
	E	N	35	90	35	30	10
		S	<i>A. terreus</i>	–	<i>A. fumigatus</i> <i>A. versicolor</i>	<i>A. fumigatus</i>	–
Room no. 4	M	N	50	25	30	10	5
		S	–	–	–	<i>A. versicolor</i>	<i>A. versicolor</i>
	E	N	55	55	20	15	5
		S	–	<i>A. versicolor</i>	–	<i>A. fumigatus</i>	–
Room no. 5	M	N	40	55	60	20	25
		S	<i>A. fumigatus</i>	<i>A. versicolor</i>	<i>A. fumigatus</i>	<i>A. versicolor</i>	<i>A. fumigatus</i>
	E	N	80	100	50	30	15
		S	<i>A. versicolor</i> <i>A. fumigatus</i>	<i>A. versicolor</i> <i>A. fumigatus</i>	<i>A. terreus</i> <i>A. versicolor</i>	<i>A. fumigatus</i>	–

method, directly on the Petri dish with Sabouraud medium, supplemented with chloramphenicol (bioMérieux). The air samples were taken at the level of approximately 1.5 metre above the floor in the middle of the room in the morning before the surgical procedures, and in the evening just after surgical procedures. The samples were incubated at 27°C, the colonies were counted after three days, and their morphology was evaluated. First, the actual number of colonies was adjusted using a table for the MAS-100 device and then expressed by the number of colony forming units (c.f.u.) in one cubic metre of the air (c.f.u./m<sup>3</sup>). The cleanness of the walls was tested using a Count-Tact method with disposable plates with Sabouraud Glucose medium with chloramphenicol (bioMérieux) using an applicator (bioMérieux). The samples were collected in the evening from the same rooms where the indoor air was tested. The samples were initially incubated at 37°C for three days, and then transferred to an incubator set up for 27°C. The colonies grown on the plates were counted and their number per one square centimetre was calculated for each of the rooms, and presented as c.f.u./cm<sup>2</sup>. The fungi were identified using routine procedures for mycological diagnostics. The mould colonies were evaluated macroscopically and microscopically on the basis of their appearance and morphological features in preparations stained with lactophenol and methyl blue (Merck). The data were analyzed statistically using the Fischer test; it was concluded that  $p < 0.05$  was the borderline of significance.

## Results

Fungal growth was observed in 48 out of 50 indoor air samples, with their average numbers ranging from 5 to 100 c.f.u./m<sup>3</sup>. Only in three air samples collected in the evening the numbers of fungi exceeded standards accepted for first class cleanness in operating rooms: 80 and 100 c.f.u./m<sup>3</sup> in the room #5, and 90 c.f.u./m<sup>3</sup> in the room #3 (Table 1). Based on the Fisher test, the comparison of the numbers of fungi sampled in the morning vs. evening, revealed that the number of fungi sampled in the evening was significantly higher. Out of the 25 wall imprints, fungal growth was detected only in four samples. The mean number of fungi was 0.01 c.f.u./cm<sup>2</sup>. The following fungal genera were isolated from the operating theatre environment; *Aspergillus* sp. (35% of the total of fungi isolated),

*Penicillium* (24%), and *Cladosporium* (22%). Other moulds detected in the samples comprised 19% of all of the fungi. Single colonies of pathogenic fungi, such as *Verticillium* sp. and *Stachybotrys* sp. were isolated. The most pathogenic fungi isolated from the hospital environment belonged to the species: *A. fumigatus*, *A. versicolor*, *A. terreus*, *A. ochraceus* and *A. nidulans* (Table 1).

## Discussion

A three-step filtration with absolute filter integrated with the ventilator is a standard for operating rooms air-conditioning. The air passing through the absolute filter is in fact free of microorganisms. Constant air flow through the filter is necessary to maintain proper air quality. When the contamination of the filter increases, the resistance to air flow also increases which may result in reduction of the air exchange. Such a situation causes increase in the number of microorganisms in the rooms and loss of the required class of air cleanness. The contaminated filter is a reservoir for fungal spores [3]. The rooms under study were air-conditioned with absolute filters, however, mould spores were present in the environment. It is assumed that the presence of the microorganisms could be either a result of filter contamination or microorganisms were spread by the medical staff. A loose surgical apron worn by a surgical staff person may emit to the environment up to 5000 c.f.u. per one minute [3].

According to the manufacturer's instruction, the exchange of filters is not a sufficient measure ensuring microbiological cleanness of the air in operating rooms. According to Kaiser [4], periodical ventilation of the air-conditioning system as well as drying the ventilation pipes and elements of equipment is necessary. He also states that air-conditioning system should not be completely shut off. On the contrary, Dettenkofer et al. [5] suggest that shutting off the ventilation systems in the operating rooms when they are not in use, probably does not increase microbiological contamination of the indoor air when the system is turned back on. From the epidemiological point of view, isolation of fungi of documented pathogenicity from the environment is a problem. The most frequently isolated fungus in the present study was *A. fumigatus*, and then *A. terreus* and *A. versicolor*. The spores of those species are smallest within this genus; *A. terreus* 1.5–2.5 µm, *A. fumigatus*

2.5–3  $\mu\text{m}$ , and *A. versicolor* 2–3.5  $\mu\text{m}$ . The spores are large enough to be eliminated by HEPA (High Efficiency Particulate Air) filters used in air-conditioning systems; the filters are able to eliminate spores over 0.3  $\mu\text{m}$ . When the filter is properly maintained, the spores should remain on its surface. However, such spores were most abundant in the environment under study. The presence of those pathogenic fungi increases the risk of infection as *A. fumigatus* isolated from hospital environment is highly cytotoxic and may cause opportunistic infection [6].

## Conclusions

To ensure microbiological cleanness of the hospital operating theatre air, the air-conditioning system should be properly maintained.

Domination of the *Aspergillus* fungi in indoor air as well as statistically significant increase in the number of moulds in the samples collected during the evenings ( $p < 0.05$ ) suggest that decontamination procedures in the operating theatre rooms have been neglected.

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## Stosowanie klimatyzacji a występowanie grzybów chorobotwórczych w środowisku sal bloku operacyjnego

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Zakażenia związane ze stosowaniem nowoczesnych metod leczenia stanowią nie rozwiązany problem współczesnej medycyny. Infekcje nabyte w trakcie zabiegów operacyjnych to kolejne zagrożenie związane z podejmowaniem działań leczniczych. Eliminacja drobnoustrojów ze środowiska bloku operacyjnego może przyczynić się do zmniejszenia liczby zakażeń, a stosowanie klimatyzacji z laminarnym przepływem powietrza znacząco obniża liczbę drobnoustrojów w środowisku pacjenta.

Celem pracy była ocena występowania grzybów w pomieszczeniach sal bloku operacyjnego wyposażonego w klimatyzację.

Badania przeprowadzono w jednym ze szpitali w Krakowie w grudniu 2009 roku, gdzie pobierano próbki powietrza oraz odciski ze ścian pięciu sal bloku operacyjnego. Za pomocą aparatu zderzeniowego MAS 100 uzyskano 50 próbek powietrza, a metodą odciskową Count Tact pobrano 25 materiałów.

Łącznie w 48 próbkach powietrza obserwowano wzrost grzybów, gdzie średnie liczby grzybów mieściły się od 5 do 100 c.f.u. w 1 m<sup>3</sup> powietrza. Z odcisków pobranych ze ścian tylko w 4 przypadkach wyhodowano grzyby, a uzyskane liczby wynosiły 0,01 c.f.u. w 1 cm<sup>2</sup> powierzchni. Najczęściej spośród izolowanych grzybów stwierdzano grzyby pleśniowe z rodzaju *Aspergillus*, a spośród nich gatunki *A. fumigatus* i *A. versicolor*.

Zapewnienie czystości mikrobiologicznej sal bloku operacyjnego związane jest z koniecznością prawidłowej eksploatacji urządzeń klimatyzacyjnych. Dominacja w powietrzu grzybów z rodzaju *Aspergillus*, a także zwiększanie się liczby grzybów pleśniowych w próbkach pobranych wieczorem ( $p < 0,05$ ) może świadczyć o braku przestrzegania procedur związanych z dekontaminacją pomieszczeń.

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