Intensive care unit environment contamination with fungi

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Abstract

Purpose: The purpose of the study was evaluation of the fungal presence in the environment of an intensive care unit.

Material and methods: The environment testing was carried out at a chest clinic intensive care unit in Cracow, in December 2004. The materials to mycological examinations were sampled simultaneously from indoor air and room walls in 15 rooms: air samples twice daily while samples from the walls once daily, for five days. The findings were processed statistically. The t-test (Student) and F-test (Snedecor) were used. The border value of significance was 0.05.

Results: No fungi were found in 6 air samples out of 150 taken in 15 rooms. The mean number of fungi in the particular rooms in the whole sampling period varied from 172 to12 c.f.u.×m^{-3}. Out of 75 samples from the walls, fungi were present only in 19 of them. The mean numbers varied from 0 to 0.37 c.f.u.×cm^{-2}. The moulds *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp. as well as yeast-like fungi *Rhodotorula rubra*, *Candida* sp. were most frequently isolated from the indoor air and the walls.

Conclusion: Significant difference between the numbers of fungi sampled in the morning vs in the evening occurred on the first, third and fourth days of sampling ($p<0.001$). Yeast-like fungi *Rhodotorula rubra* and moulds *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp. were isolated from indoor air in all of the rooms tested.

Key words: fungi, indoor air, intensive care unit.

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Introduction

Nosocomial infections are a serious medical problem, particularly in intensive care units. Even though the percentage of patients there does not exceed 10% of all of the hospitalisations, nosocomial infections comprise 25 per cent of the total number of such infections [1]. A high correlation is reported between the prevalence of infection and the duration of hospitalisation. There is evidence that 80% of nosocomial infections are transmitted through the medical staff's hands [2-4].

A dramatic increase in the prevalence of fungal infections was observed in the recent years. Opportunistic fungal infections lead to considerable increase in mortality rate at invasive treatment wards. Particularly, systemic mycoses are considered a cause of death in 88% of the cases [5]. The aetiological profile of fungal infections is changing: fungal species, formerly considered as harmless, increase their virulence. This results from the resistance of fungal strains to numerous antifungals, prolonged antimicrobial treatment, but also from poor general condition of the patients, caused by the underlying disease [6-8].

An epidemiological study performed by Kao et al. [9] gives evidence that candidaemia occurred in eight patients out of 100 000 people population yearly. In 19% of the patients, candidaemia developed before or on the day of hospital admittance. *Candida species* other than *C. albicans* were detected in 47% of the cases: *C. parapsilosis* 21%, *C. glabrata* 12%, *C. tropicalis* 10%, and *C. krusei* 4%.

Unlike usually endogenous invasive candidiasis, invasive aspergillosis is exogenous. Abundant sporulation, tiny size of the spores ubiquitous in the environment and their ability to survive in a wide range of temperatures enable them to reach pulmonary alveoli [2,10-13]. Nosocomial *Aspergillus* infections may be serious problem at hospital wards with patients suffering from neutropenia if construction works are done near of them [14]. In such circumstances, there is a high density of mould spores in the indoor air, particularly *Aspergillus*.

The objective of the study was evaluation of the fungal presence in the environment of an intensive care unit.

Material and methods

The environment testing was carried out at a chest clinic intensive care unit in Cracow, Poland, in December 2004. The materials to mycological examinations were sampled simultaneously from indoor air and room walls in 15 rooms: four bays, a treatment room, three bathrooms, a nurse's station, a doctor's room, a rest room, a corridor, a dirty annex, a ward kitchen and a washing room. The air samples were taken twice daily and the samples from the walls once daily, for five days.

To evaluate the presence of fungi in the indoor air, 150 air samples were colleted using aspiration method by means of a MAS 100 (Merck) device in 15 rooms of the ward.

Two hundred litres of indoor air were sampled in each of the rooms at a time. For this purpose, the sampling device was positioned in the middle of the room, 0.5 m above the floor. The windows and the doors of the room were closed during the sampling period. The air was aspirated to a Petri dish with Sabouraud Glucose Selective Agar medium, with gentamicin an chloramphenicol (manufactured by Oxoid Company). The antibiotics were added to prevent bacterial growth on the medium. Petri dishec with the material aspirate were then incubated at 27°C. The cultures were inspected, the fungal colonies were counted, and their morphology was evaluated after three days of incubation. The period of incubation depended on the fungal genus detected but id did not exceed fourteen days. After the incubation period, the real number of the fungal colonies was obtained using a statistical calculation table for the MAS 100, and then the number of fungi in one cubic metre was calculated using a formula:

$$X = \frac{a \times 1000}{V}$$

where a – the number of fungal colonies grown from the indoor air sample; V – the volume of the air sample aspirated (litres); and X – the number of fungi in one cubic metre of the air expressed in terms of the number of colony forming units in one cubic metre (c.f.u./m^{-3}).

The presence of fungi was also evaluated on the walls of the rooms, in which the air was tested. A total of 75 samples was taken using a Count-Tact technique (bioMerieux). The imprints on the plates with Sabouraud glucose medium with chloramphenicol were taken using a bioMèrieux applicator from a dry wall surface 1.5 m above the floor. In the rooms inhabited by mothers with children, the samples were taken above the mother's bed. The plates with the material samples were incubated at 37°C for three days and then moved to a thermostat with 27°C. The number of colonies on the plate was then counted and the number of fungi on one square centimetre of the wall surface was calculated using a formula:

$$X = \frac{a}{\pi r^2}$$

where a – the number of fungi on the imprint plate; r – the diameter of the plate in cm; and X – the number of colony forming units per one square centimetre of the wall (c.f.u./cm^2).

The fungal colonies grown in the cultures were counted according to the accepted standards and identified using procedures accepted in mycology. Moulds were evaluated macroscopically an microscopically on the basis of their appearance in the culture as well as their morphological features in direct preparations stained with lactophenol and methylene blue (Merck). When the evaluation of a preparation was doubtful, a slide microculture was made for further identification. The yeast-like fungi were Gram-stained and cultured on starvation media. Photographs were taken of the macroscopical appearance of the fungi.

The findings were processed statistically. The t-test (Student) and F-test (Snedecor) were used. The border value of significance was 0.05.

Results

Out of 150 air samples taken in the rooms, only 6 did not contain fungi. During the morning sampling, no fungi were isolated in the bay № 4 on the first day of testing, in the bays № 1 and № 2 on the second day, and in the treatment room on the fourth day. During the evening sampling, no fungi were detected in the indoor air in the bay № 4 and in the nurses' room on the first day of sampling.

The highest number of fungi (720 c.f.u.×m^{-3}) was detected in bay № 2 on the third day of testing. The remaining numbers of fungi sampled in the bays did not exceed 70 c.f.u.×m^{-3}, i.e. did not exceed Polish standards for the bays.

In other rooms such as corridors, bathrooms, rest room and kitchen, higher number of fungi were sampled in evening. The results are shown in *Fig. 1*.

The average number of fungi in one cubic metre of air within the whole testing period varied between 172 c.f.u.×m^{-3} in the bay № 2 to 12 c.f.u.×m^{-3} in the bay № 4. The comparative analysis using the F test revealed significant differences ($p<0.001$) between the numbers of fungi sampled in the morning vs. in the evening on the first, third and fourth days of sampling which is shown in *Fig. 2*.

Out of 75 samples taken from the walls, fungi were present in 19 of them: their mean number varied between 0 and 0.37 c.f.u.×cm^{-2}. No fungi were found on the walls in the treatment room, bays № 2 and № 3, nurse's station and bathroom № 2. (*Fig. 3*).

The most abundant fungi in the rooms tested were moulds: *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp. Those fungal genera were found in each of the rooms tested during the entire testing period. The dominating species of yeast-like fungi, present in all of the rooms was *Rhodotorula rubra*, while *Candida* sp. was also frequent. Fungi belonging to this genus were present in the indoor air in four rooms; three of them were inhabited by the patients (*Tab. 1*).

Discussion

According to Polish guidelines there are three classes of hospital wards cleanness. The intensive care units are classi-

Figure 1. **Numbers of fungi c.f.u.×m^{-3} isolated from the indoor air in the morning and evening in the rooms during entire assay period**

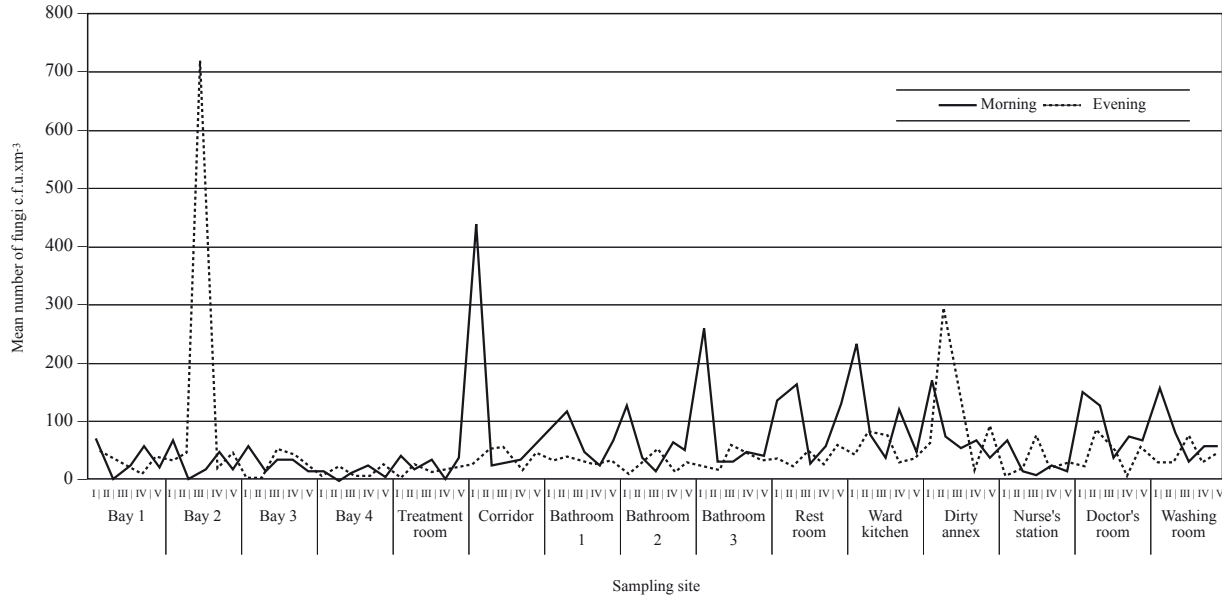

Figure 2. **Medians of the mean numbers of fungi isolated from the indoor air of the rooms tested**

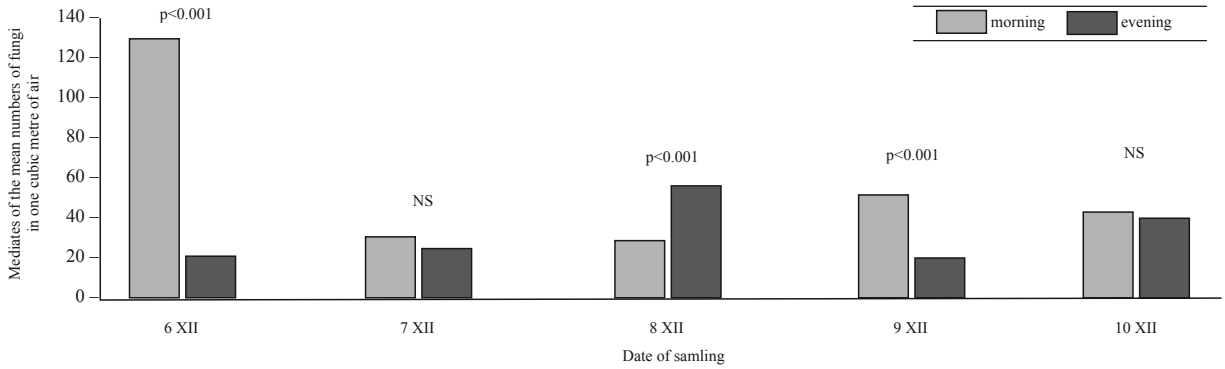

Figure 3. **Numbers of fungi isolated from the walls during the entire testing period**

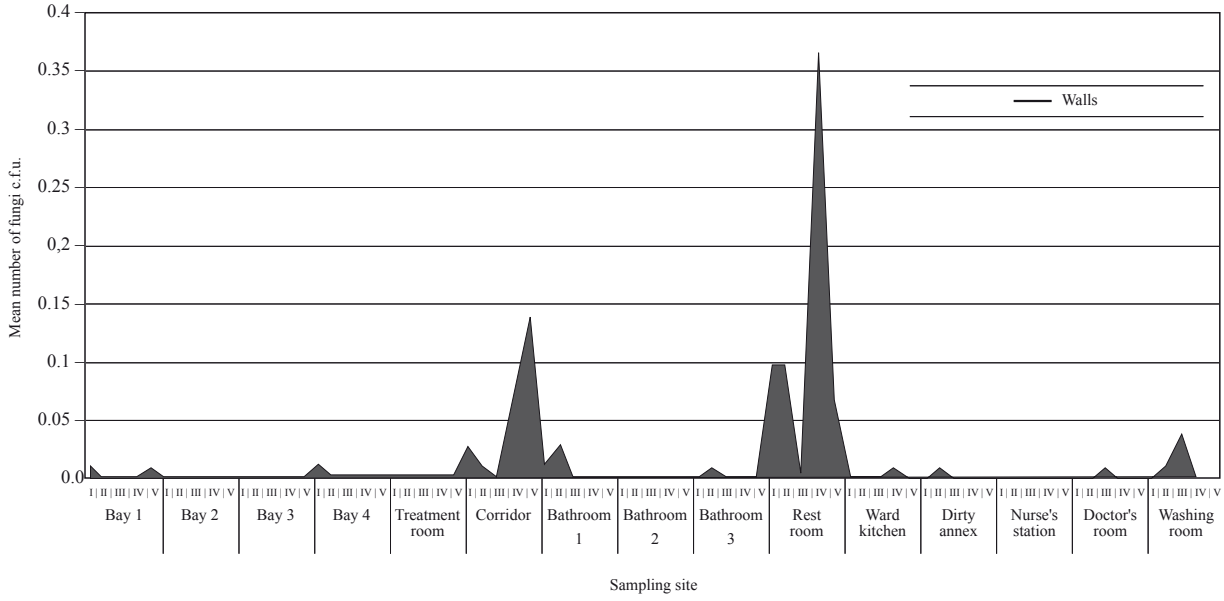

***Table 1*. Genera and species of the fungi isolated from the indoor air of the rooms during the entire testing period**

Genera and species of the fungi isolated from the indoor air	Room number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Candida sp.	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+
Rhodotorula rubra	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cladosporium sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Botritis sp.	-	-	-	-	+	+	-	+	-	-	+	-	-	+	+
Stachybotrys sp.	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+
Acremonium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Alternaria sp.	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-
Rhizopus sp.	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Mucor sp.	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Other moulds	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+

1, 2, 3, 4 – bays, 5 – treatment room, 6 – corridor, 7-9 – bathrooms, 10 – rest room, 11 – ward kitchen, 12 – dirty annex, 13 – nurse's station, 14 – doctors' room, 15 – equipment washing room

fied as class 2 which means presence of microorganisms not exceeding 300 colony forming units in one cubic metre (300 c.f.u.×m^{-3}) of indoor air [15]. The number of c.f.u.×m^{-3} allowed in operating theatres is 0 while in treatment rooms – 50. In other hospital rooms – 200 c.f.u.×m^{-3} [16]. In this study, the average number of fungi varied from 12 to 172 c.f.u.×m^{-3}, i.e. did not exceed the class 2 of cleanness.

In the present study, lower numbers of fungi were observed in the evening vs the morning sampling. Detailed comparative statistical analysis with the F-test for two samples showed ($p<0.001$) that the above phenomenon occurred on the first, third and fourth days of the measurements. The findings are consistent with those obtained by the investigators in Cracow and Białystok [14,17-19]. The decrease of the number of fungi in the evening might have been caused by ventilation of the rooms and/or lower number of people present in the rooms by day.

Even though *Candida albicans* is still the most frequent fungal pathogen at intensive care units, an increase of infections caused by other than *C. albicans Candida* species is observed. Particularly dangerous and hard to treat are the strains *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis*. Those fungal species are often resistant to azole antifungals [1,2,4,7].

As a rule, candidiasis is an endogenous infection, however, exogenous infections are also possible. Numerous studies give evidence that *Candida* fungi are detected in the indoor air at, e.g., surgical, haematological and obstetric wards; they are a potential source of infections, especially in risk group patients [5,9,11-13,20-22]. In the present study, *Candida* fungi were isolated from indoor air and from the walls in four rooms out of fifteen tested at the intensive care unit. Three of the rooms were bays.

The role of infections caused by the moulds *Mucor* and *Rhizopus* in severely ill patients at intensive care units is increasing. The fungi may invade patients with inhaled air as well as through the equipment used in diagnostics and care of the patients.

In a study carried out at an intensive care unit in Spain, a gastrointestinal tract zygomycosis was caused by *Rhizopus microsporus*. The infection was transferred through spatulas used by the medical staff. Zygomycosis was a complication of the underlying disease and contributed to an increase in mortality [22]. In our study, *Mucor* and *Rhizopus* were isolated only from indoor air in corridor and washing room, and from the walls in a rest room.

Moulds belonging to the *Botrytis* genus play a role in hypersensitivity reactions and may cause allergic alveolitis, the so-called vineyard worker's lung. The presence of those fungi in the indoor air may lead to allergy [3].

In our study, moulds belonging to the *Botrytis* genus were found in several rooms: in the treatment room, doctors' room, bathroom №1, corridor and kitchen. Probably, the moulds were brought to the ward with contaminated grapes. Similar contamination was detected in other our study at a invasive diagnostics ward in chest clinic, where this fungal species was isolated in six rooms [18].

The mortality in systemic aspergillosis is high as compared with other systemic mycoses. Most often, infection with *Aspergillus* occurs *via* inhalation. The tiny spores readily invade upper and lower airways and may produce lung aspergillosis in risk group patients [11-13,23].

In France, a systemic *Aspergillus fumigatus* infection occurred in a patient eleven days after liver transplantation. At the same time, lung aspergillosis caused by the same fungal species was detected in two patients at an intensive care unit [20]. Large amounts of *Aspergillus* were isolated in all of the rooms at the intensive care unit.

It is not surprising that large amounts of *Aspergillus* were found in the indoor air tested because that fungal genus is ubiquitous. Even though it is harmless for healthy people, it may be dangerous for the patients of risk groups, including those treated in surgical wards and intensive care units. Therefore, it appears, that indoor air monitoring focused on the presence of fungi is an important procedure in wards where risk group patients are treated. Such a procedure should be routine in hospitals, and particularly at intensive care units.

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