Analysis of fungal air pollution using different samplers

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ABSTRACT

**Purpose:** The aim of this study was the analysis of fungal air pollution in different rooms using different the air samplers.

**Material and methods:** Air for mycological studies was collected from various hospital rooms. To monitor fungal air pollution were used samplers: SAS SUPER 100 (pbi-international), MAS 100 (Merck) and AIR IDEAL (BioMerieux).

**Results:** We found differences in CFU/L values in depending on hospital room and the air sampler type. The CFU/L values of air samples taken by the AIR IDEAL ranged from 350 to 850 and (724.2 ± 159.9), and the CFU/L values of air samples taken by the sampler SAS SUPER 100 ranged from 160 to 800 (455.3 ± 250.73). The CFU/L values of air samples taken by the MAS 100 sampler varied from 50 to 1340 (302.5±56.6) From the air samples of both samplers was incubated 6 genera/species of fungi. *Candida albicans* fungi species and genus *Penicillium species* were most frequently isolated from SAS Super 100 sampler, and *Penicillium species* from the AIR IDEAL sampler. From the air samples of MAS 100 was isolated 11 types/species of fungi and in air samples of SAS Super 100 was isolated 7 types/species.

**Conclusions:** Significant differences of CFU/L values in the tested rooms were found in depending on the used sampler. Mycological analysis of the obtained cultures from air samples suggests that there is not same isolation of fungi using the different samplers.

**Key words:** fungi, air, AIR IDEAL, SAS SUPER 100

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INTRODUCTION

According to the World Health Organization (World Health Organization - WHO), more than three billion people suffer from diseases caused by air pollution indoors [1].

It is believed that an average 87% of the time people is spending in the enclosed buildings, and about 6% of the time means of transport. Therefore, the potential sources of pollutants and their concentration in the air have become an important internal factor in personal exposure for most people. Housing and air spaces, properly operated and maintained hygienically, not much different from the clean air outside [2].

Air, as a factor contributing to the development of various diseases and the cause of development of certain infectious diseases, took into account the Hippocrates (460-377 BC), writing about it in his book "Corpus Hippocraticum: " When air pollution is infected hostile to the human race, man becomes ill " [3].

Many authors emphasize that "buildings can live in several species of bacteria (mainly Gram negative), more than 400 species of fungi (mainly Aspergillus, Cladosporium, Penicillium, Fusarium), several species of fungi causing decay processes of wood and wood-based materials, and many species of algae[2,4-10].

In 1982, it was introduced the concept of the sick building syndrome "(SBS) under which the team understands the various problems created by the long-term presence in the building, construction and equipment which may have harmful effects on human health [4-10]. In 1987 the WHO has established a list of symptoms and findings, which may result in "sick buildings". Further research in the following years led to the renaming of SBS to BRI term "building - related illness" - "condition causally related to being in the building" [4-10].

The results of studies [11] conducted in the Netherlands, Finland, Canada and the United States and showed that the increase in the concentration of dust diameter of 10 mm in the air reduces the life expectancy of men by 0.8 to 1.37 years.

It is important and it seems necessary to monitor the indoor air quality and raise awareness of existing threats. In Poland, there are unfortunately no date set standards for microbiological purity of the air inside homes and public premises.

Moulds readily enter indoor environments by circulating through doorways, windows, ventilation systems, and air conditioning systems. Spores in the air also deposit on people and animals, bags, and pets common carriers of mold into indoor environments. The most common indoor moulds are Cladosporium, Penicillium, Aspergillus, and Alternaria [12,13].

Since Feinberg's studies (1955), it has been well established that fungal spores play a major role in allergic diseases such as asthma, hay fever and hypersensitivity pneumonitis, and that they may cause serious systemic infections in some areas. In most cases, there is no exposure to true or opportunistically pathogenic fungi, but there are species that can act as allergens or cause other non-allergic symptoms [12,14].

The quality of air and the number of pathogens depend on the condition and cleanliness of the building, appropriate humidity and temperature and good ventilation, access to light, oxygen and water [15-18]. Some indoor moulds have the potential to produce extremely potent toxins called mycotoxins. Mycotoxins are lipid-soluble and are readily absorbed by the intestinal lining, airways, and skin.

Until recently, there was only one published report in the United States linking airborne exposure to mycotoxins with health problems in humans [20]. This report described upper respiratory tract irritation and rash in a family living in a Chicago home with a heavy growth of Stachybotrys atra). The investigators documented that this mold was producing trichothecene mycotoxins. The symptoms disappeared when the amount of mold was substantially reduced.

Microscopic observations and culture results showed that filters from five of the hospitals were colonized with fungi, including species of Acremonium, Alternaria, Aspergillus, Cladosporium, Epicoccum, Penicillium, and Rhinocladiella, and a Beauveria-like fungus. Several of these commonly airborne species, e.g. Epicoccum purpurascens and Rhinocladiella had not been previously reported to colonize air filters [17,21, 22].

The aim of this study was to analyze the results of air pollution in different rooms' fungi using different the air samplers.

MATERIAL AND METHODS

The study was conducted after approval by the Bioethics Committee of the Medical University of Bialystok, resolution no.: RI-002/97/2008.

The material for mycological examination was taken from the air spaces of rehabilitation, before entering them and the entrance to the building of the hospital. During measurement, the test indoors, doors and windows were closed. To monitor air pollution in our study, fungi were used the air samplers SAS SUPER 100 (pbi international), sampler MAS 100 (Merek) and AIR IDEAL (BioMerieux). The air sampler SAS SUPER 100 (pbi international) is the newest
generation of instruments that meet the requirements of world standards of measurement.

To measure fungi, we used Sabouraud plates with a diameter of 90 mm, and the collection of air samples is fully standardized, with the ability to program devices.

The sampler was placed at a height of 1.5 m from the floor. At the time of measurement in the test room doors and windows were closed after removing tiles from the camera they were incubated in the incubator. After the incubation, the number of colonies was determined and calculated number of CFU - colony forming unit - a unit forming colony per 1000L (1 m$^3$) of air. The calculations were carried out in accordance with the recommendations of camera manufacturers. Identification of the yeast-like fungi was carried out using ground CandiSelect, and fungi based on direct examination of the material DMSO, appearance, culture and characteristics of microcultures were used.

The Wilcoxon’s test was used for statistical analysis. A value of $p$ <0.05 was considered statistically significant. The data were analyzed with the statistical package Statistica v. 7.1 PL.

**RESULTS**

For monitoring of airborne fungi operating rooms Hospital Kavala was used the SAS SUPER 100 air sampler. In the air of operating rooms was isolated a significant number of the fungal colonies 0 to 110. In the morning, the highest number of fungi was isolated at the hall of entrance into the operating department and afternoon in the central sterilization. The following fungal pathogens isolated from air were *Candida albicans*, *non-Candida albicans*, *Mucor species*, *Penicillium species* and *Aspergillus species*. Mean number of fungi colonies isolated from air was 180 ± 140.3, mean temperature 25.3 ± 0.7 and humidity 57.5 ± 2.1. (data are not shown)

For monitoring of airborne fungi gynecological departments were used apparatus SAS SUPER 100. The assessments of the indoor air contamination of fungi at the different contamination at the gynecological departments in Białystok (Poland) and Kavala (Greece). In Białystok from the air samples of 16 rooms of the gynecological department were isolated the different number of colonies ranged from 0-560 CFU/1000 L of air. No fungi from the air samples of preparation room, septic room, operating room and room of family births was isolated. The highest number of fungi colonies was isolated from the patient rooms. From the air samples were isolated the following pathogens: *Candida albicans*, *non-Candida albicans*, *Penicillium citricum*, *Cladosporium herbarum*, *Aspergillus niger*. The highest temperature was detected in nurses’s rooms, preparation room no 2 (20.5 and 20$^9$ C) and patient room no. 1 (20$^9$C). The highest humidity were detected in lavatory (85.2%) and preparation room no.1 (79.5%). In Kavala from the air samples of 8 rooms the gynecological department isolated the different number of colonies ranged from 10-200 CFU/1000 L of air. The highest number of fungi colonies were isolated from a dirty room. From the air samples were isolated the following pathogens: *Candida albicans*, *non-Candida albicans*, *Penicillium citricum*. The highest temperature was detected in the dirty room (27$^9$ C), pre-birth room (26.9$^9$ C) and nurses’s room (26.7$^9$ C). The highest humidity were detected in corridor, at the entrance to the Department (61.4%) and the dirty room (59.2%).

For monitoring of airborne fungi in the Pediatric Rehabilitation rooms was used the SAS SUPER 100 sampler. The highest concentrations of fungi were isolated in the surgery room (p=0.01) and gymnasium (p=0.001). The lowest concentrations of fungi were found in the paraffin room, and cloakroom for patients near the gymnasium. Significantly, (p<0.05) higher number of (*Candida albicans*) was isolated from the matters compared to (*Penicillium, Aspergillus, non-Candida albicans*). The highest temperature was detected near the gymnasium with devices but the lowest in the first corridor. The highest humidity was found in the second corridor and the lowest in the gymnasium. Significant correlation between humidity and number of isolated fungi in the tested rooms was found (beta=0.3147; p=0.021). (Data are not shown)

For monitoring of airborne fungi in the oncology care rooms was also used the SAS SUPER 100 sampler. The air from the Oncological Department in Lublin showed the higher number of fungi colonies compared with Białystok. The air of the Oncological Department in Lublin was characterized with a larger variety of mycological flora. Almost twice higher number of the fungi colonies in the air outside the hospital building in Lublin in comparison with Białystok was found.

For monitoring of airborne fungi in the departments of chronic care rooms was used the MAS 100 sampler. The highest number of fungi was isolated from not self-reliant patients' rooms at ground floor (mean 235 ± 24.8 j.k.t./m$^3$ of air), at the corridor on the ground floor of not self-reliant patients' rooms (535 j.k.t/m$^3$), at bathroom (320 j.k.t/m$^3$) and dirty room (120 j.k.t/m$^3$). The highest temperature was noted at not self-reliant patients' rooms on the ground floor (24.2 ± 0,1), and humidity at patient's rooms on the first floor (42.1 ± 1.98). Outside of building was isolated 200 j.k.t of fungi/m$^3$. From air of the patient rooms most often was isolated *Penicillium sp.*, *Cladosporium herbarum* and *Myelia sterilia*. At corridors was isolated *Myelia sterilia* and
Penicillium sp. from the walls of patient rooms Stachybotrys sp., Mycelia sterilia, Penicillium sp., and Mucor; and outside of building Penicillium sp., and Cladosporium herbarum. (data are not shown) Responders in self-assessment rarely declared incidence in their rooms: stains on the walls and ceiling, moulds, water stains mustiness. Only in rooms with the highest number of fungi it was reported the following complaints: skin cracks of hands, ardor, hands blushing, itching of skin hands, nose dryness, cough, limbs tingle, edema of eyelids, eyes itching and necessity of rest during the bath or wearing. Complaints were reported during the exposition to dust and cold.

To compare the results of fungal air pollution in the long-term care departments using the SAS SUPER 100 and AIR IDEAL samplers. The CFU/L values ranged from 350 to 850 (724.2 ± 159.9) taken by the sampler AIR IDEAL differ significantly (p=0.0016) compared with the sampler SAS SUPER 100 - 160 to 800 (455.3 ± 250.73). (Tab.1) From the air samples of both samplers was incubated 6 genera/species of fungi. Candida albicans fungi species and genus Penicillium species were most frequently isolated from SAS Super 100 sampler, and Penicillium species from the AIR IDEAL sampler. (Fig.1)

Table 1. The CFU values in depend on the tested room and used the air sampler.

<table>
<thead>
<tr>
<th>CFU/Air sampler type</th>
<th>CFU/ m³</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>SAS</td>
<td>455.3 ± 250.73</td>
<td>724.2 ± 159.9</td>
</tr>
<tr>
<td>AIR Ideal</td>
<td>390</td>
<td>320</td>
</tr>
<tr>
<td>Outside building</td>
<td>140 ± 56.7</td>
<td>70 ± 84.9</td>
</tr>
<tr>
<td>23 the long-term care departments rooms</td>
<td>180</td>
<td>10</td>
</tr>
</tbody>
</table>

Air was sampled from the rehabilitation rooms, corridors and at the entrance to hospital building. The study of fungal air contamination was performed using SAS SUPER 100 (pbi-international) and MAS 100 (Merck). The CFU/L values taken by the MAS 100 sampler varied from 50 to 1340 and mean of CFU value of all measures was 302.5±56.6. The CFU/L values taken by the SAS SUPER 100 sampler varied from 0 to 200 and mean CFU/L value of all measures was 104.8±84.9. From air taken by the MAS 100 sampler was isolated 11 types/species of fungi and from air samples of the SAS Super 100 was isolated 7 types/species. The same fungi strains were isolated only in one sample from physiotherapy room, kinesiotherapy I, and corridor. From air samples taken by the MAS 100 sampler was isolated 4 types/species of fungi but in contrast none fungi were isolated from air sampled by the SAS SUPER 100.
DISCUSSION

In the literature [1,23-27] meets descriptions use different methods to assess microbial air pollution. In Poland, the methods for assessing microbial air pollution are not standardized. This follows eg. from the use of different measurement methods, different samplers and unequal size plates (diameter 55 mm to 90 mm) [1, 23-27].

Traditional methods for the enumeration of airborne fungi are slow, tedious, and rather imprecise [28]. In this study, the possibility of using flow cytometry (FCM) for the assessment of exposure to the fungus aerosol was evaluated. Epifluorescence microscopy direct counting was adopted as the standard for comparison. Setting up of the method was achieved with pure suspensions of Aspergillus fumigatus and Penicillium brevicompactum conidia at different concentrations, and then analyses were extended to field samples collected by an impinger device. Detection and quantification of airborne fungi by FCM were obtained combining light scatter and propidium iodide red fluorescence parameters. Since inorganic debris is unstainable with propidium iodide, the biotic component could be recognized, whereas the preanalysis of pure conidia suspensions of some species allowed us to select the area corresponding to the expected fungal population. A close agreement between FCM and epifluorescence microscopy counts was found. Moreover, data processing showed that FCM can be considered more precise and reliable for any of the tested concentrations [28].

Metha et al. [29] monitored at five locations (three in an office/laboratory building and two in a private residence) in a series of experiments designed to compare the efficiency of four air samplers: the Andersen two-stage, Burkard's portable, RCS Plus, and SAS Super 90 samplers. A total of 280 samples was collected. The four samplers were operated simultaneously, each sampling 100 L of air with the collection on trypticase soy agar. The data were corrected by applying positive hole conversion factors for the Burkard portable, Andersen two-stage, and SAS Super 90 air samplers, and were expressed as log10 values prior to statistical analysis by analysis of variance. The Burkard portable air sampler retrieved the highest number of airborne cultivable bacteria at four of the five sampling sites, followed by the SAS Super 90 and the Andersen two-stage impactor. The number of bacteria retrieved by the RCS Plus was significantly less than those retrieved by the other samplers. Among the predominant bacterial genera retrieved by all the samplers were Staphylococcus, Bacillus, Corynebacterium, Micrococcus, and Streptococcus [29].

Yao et al. [30] investigated the overall performances of the SMA MicroPortable, BioCulture, Microflow, Microbiological Air Sampler (MAS-100), Millipore Air Tester, SAS Super 180, and RCS High Flow portable microbial samplers when collecting bacteria and fungi both indoors and outdoors. The performance of these samplers was compared with that of the BioStage impactor. Results showed that the sampling environment can have a statistically significant effect on sampler performance, most likely due to the differences in airborne microorganism composition and/or their size distribution. Data analysis using analysis of variance showed that the relative performance of all samplers (except the RCS High Flow and MAS-100) was statistically different (lower) compared with the BioStage. The MAS-100 also had statistically higher performance compared with other portable samplers except the RCS High Flow. The Millipore Air Tester and the SMA had the lowest performances. The relative performance of the impactors was described using a multiple linear regression model (R(2) = 0.83); the effects of the samplers' cutoff sizes and jet-to-plate distances as predictor variables were statistically significant [30].

Sautour et al. [31] conducted an 18-month survey of indoor fungal contamination in one haematology unit during a period of construction work. Air was sampled with a portable Air System Impactor and surfaces with contact Sabouraud plates. During this survey, the mean concentration of viable fungi in the air was 4.2 CFU/m(3) and that for surfaces was 1.7 CFU/plate. At the beginning of construction work, there were increases in airborne fungal spores (from 3.0 to 9.8 CFU/m3) in the unit, but concentrations did not exceed 10 CFU/m(3) during the 18-month period. The most frequently recovered airborne fungi were Penicillium spp., (27-38%), Aspergillus spp., (25%) and Bjerkandera adusta, Basidiomycete identified with molecular tools (7-12%). Blastomycetes accounted for more than 50% of the fungal flora on surfaces. Investigating the impact of a new air-treatment system (mobile Plasmair units), there were significant reductions in fungal contamination for the Plasmair -treated rooms, and in these rooms, we observed the same level of fungal load, whether construction work was in progress or not [31].

Drevova [32] examined the occurrence of fungi in the air of units where patients with hematology disorders, naturally predisposed to fungal infections, are hospitalized. We performed the search at four sampling sites with the different grade of reverse isolation. (unit for allogeneic transplantation, unit for autologous transplantation, intensive care unit and standard three-bed room. Air
samples were collected by a special instrument for aerosopic sampling (Biotest RCS Plus) during two consecutive periods in 2002, in summer (June - July) and in fall (November - December), and compared with each other. Forty-four samples of air were collected, which yielded 147 fungal isolates, representing 41 genera. The growth of fungi was recorded in 34 samples of air (77.3%), 10 samples were free of fungi. Yeasts were not taken into consideration in this study. The isolates belonged mostly to the genera Cladosporium (33.9%), Penicillium (23.9%), Aspergillus (12.8%), Acremonium (5.6%) and Alternaria (5.6%), the other genera represented 18.2% altogether [32].

Kemp [33] analyzed in the ventilation, and air conditioning (HVAC) systems of two large office buildings in different climate zones. Fungal samples were taken in each of the walk-in chambers of the HVAC systems using a six-stage Andersen Sampler with malt extracted agar. Results showed that fungal species changed with different locations in the HVAC systems. The outdoor air intake produced the greatest filtration effect for both the counts and species of outdoor air fungi. The colony forming unit (CFU) counts and species diversity was further reduced in the air directly after the filters. The cooling coils also had a substantial filtration effect. However, in room air the CFU counts were double and the mixture of fungal species was different from the air leaving the HVAC system at the supply air outlet in most locations. Diffusion of outdoor air fungi to the indoors did not explain the changes in the mixture of airborne fungi from the outdoor air to the indoor air, and some of the fungi present in the indoor air did not appear to be transported indoors by the HVAC systems [33].

Li and Hou [34] evaluated bioaerosol characteristics in the hospital clean rooms with different class levels. For total particles, an airborne particle counter was used to determine the particle size ranges (0.1, 0.2, 0.3, 0.5, 1 and 5 microm) for air inlets and patient beds. An Andersen 1-STG sampler was used for bacterial and fungal collection. For aerosol characteristics, it was found that some air inlet particle levels were higher than 100000/foot(3) in class 100000 clean rooms. In addition, it was clearly demonstrated that particle concentrations in patient beds were much higher than those for air inlets. Human activity might play a role in these particle concentration differences. Moreover, it was demonstrated that bacterial and fungal concentrations ranged from 1 to 423 and from 0 to 319 CFU/m(3), respectively. For class 100 clean rooms, no particles were ever found. In addition, bacterial concentrations were found to be in the range of 0-32 CFU/m(3) and there were no fungal aerosols. For operating rooms of class 10000, some of the particle levels observed were higher than 100000/foot(3). Furthermore, the average level of bacterial aerosols was 88 with a range of 13-336 CFU/m(3). In addition, fungal levels ranged from 0 to 51 with a mean value of 4 CFU/m(3). It was indicated that bacterial levels were higher than fungal ones, which might be related to human sources. Moreover, there were weak relationships among class level, particle concentration and bioaerosol levels [34].

Engelhart and Exner [35] applied the recommendations of the newly released technical standards TRBA 405 and 430 to the conditions of non-industrial indoor environments and to investigate the comparative performance of the two recommended filter cassette samplers (Sartorius MD8 and GSP) using the direct method. During two sampling phases (August to beginning of December 2000, and December 2000 to February 2001) a total of 360 short-term samples and 30 long-term samples were analyzed in parallel at 20 sampling days. Mean fungal CFUs varied from 2 x 10(1) to 3 x 10(2) CFU/m3, with the relative recovery of the GSP (vs. MD8) method being 1.00 (range, 0.89 -1.14). We found a good correlation (Pearson's r = 0.95) between the MD8 and the GSP method. Both samplers showed good reproducibility, the coefficients of variation being 19.4% for the MD8, and 13.2% for the GSP sampler. Aspergillus and Penicillium (median proportions > 20% of the fungal CFUs, each) were the most prevalent indoor fungal genera during this season, followed by Wallenium (>10%) and Cladosporium. The coefficients of variation for single genera ranged from 27% to 89%. We conclude that the recommendations of the technical standards TRBA 405 and 430 can efficiently be applied to survey fungal exposure under the conditions of non-industrial indoor environments. If short-term samples by using the MD8 are conducted for orientating purposes, at least two to three samples per sampling site are necessary in order to minimize intra-sampler variability (r > 0.95). Due to easy overloading of the small filter surface of the GSP at higher airborne spore concentrations, the more representative long-term sampling should preferably be done by using the indirect method, however, the results of both methods may not be equated. The interpretation of the fungal spectrum should be conducted with caution, particularly at low absolute concentrations [35].

Pettermel et al. [36] analyzed the relationship between meteorological conditions and Alternaria and Cladosporium spore concentrations in the air of Zagreb in August 2002 and August 2003. These months were chosen because they represented climatic extremes. A 7-day VPPS 2000 Hirst volumetric pollen and spore trap was used for spore sampling. Spores marked as 'others' (Ascospores, Basidiospores, Epicoccum, Ustilago, Pithomyces, Helminthosporium, Stemphylium, ...
**CONCLUSIONS**

1. Monitoring of the microbiological environment in the healthcare facilities is particularly important, therefore, it is advisable to research improvement and modifying methods for air assessing.

2. Significant differences of CFU/L values in the tested rooms were found in depending on the used sampler.

3. Mycological analysis of the obtained cultures from air samples suggests that there is not same isolation of fungi using the different apparatus.

4. The performance of further studies on air fungal contamination with using different samplers are needed.

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