Indoor air studies of fungi contamination of social welfare home in Czerewki in north-east part of Poland

Rolka H[1], Krajewska-Kułak E[1], Łukaszuk C[1], Oksiejczuk E[2], Jakoniuk P[3], Leszczyńska K[3], Niczyporuk W[4], Penar-Zadarko B[5]

[1] Department of General Theory, Mycological Laboratory, Medical University of Białystok, Poland
[2] Białystok Technical University, Faculty of Environmental Management in Hajnówka, Poland
[3] Department of Microbiological Diagnostic, Medical University of Białystok, Poland
[4] Department of Dermatology and Venereology, Medical University of Białystok, Poland
[5] Department of Human Biology and Health Education, University of Rzeszów, Poland

Abstract

Purpose: **The contamination of the indoor environment with yeast-like fungi and moulds in social welfare home in Czerewki was evaluated.**

Material and methods: **The concentration of airborne fungi (in front of the building and in the corridors, patient rooms, study rooms, recreation rooms, kitchens, bathrooms, toilets) was determined using SAS-Super 100 (Pbi International). The fungal concentration on walls was assessed using the Count-Tact applicator and the plate Count-Tact irradiated (BioMerieux). Swabs were taken from the skin of the interdigital spaces of feet and hands, nails and the oral cavity of the residents. The fungi from the swabs were cultured on Sabouraud medium. Fungi were identified using standard microbial procedures.**

Results: **Tests of air and walls revealed significant differences in mycological flora in depending on the place isolation (e.g. corridor, rooms, reading room, nurse, room, kitchen, dining room, bathroom) and season (summer, autumn, winter, spring). A significant increase in the fungi isolated from the air and walls in the social welfare home was found, depending on the season.**

Conclusion: **An increase in the fungi isolated from residents was found in relation to the season.**

Key words: social welfare homes, Fungi, air studies.

ADDRESS FOR CORRESPONDENCE:
Prof. dr hab. med. Elżbieta Krajewska-Kułak
Department of General Theory, Mycological Laboratory
Medical University of Białystok
ul. M. Skłodowskiej-Curie 7a, 15-096 Białystok, Poland,
e-mail: elzbieta.krajewska@wp.pl

Received 21.01.2005 Accepted 03.02.2005

Introduction

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 [1]. 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* [1,2]

The presence of microorganisms in the patients' environment has a direct influence on the condition of these people. 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 [3-6]. 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 [7]. 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.

The pathogenic process depends on the quality and the condition of the building as well as on the time of exposure to fungi spores present in the air [1,2].

Air filters of various types, selected on the basis of discoloration, were collected from the primary and secondary filter banks of the heating, ventilating, and air-conditioning systems

in seven hospitals in the eastern United States and examined with direct microscopy for fungal colonization [8]. 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 purpurescens* and *Rhinocladiella* had not been previously reported to colonize air filters [6,8,9].

We studied the fungi contamination of the social welfare home in Czerewki in relation to season. We wanted to investigate whether is some relationship between incidence of the fungi in air and the isolated from the residents of the social welfare home.

Material and methods

Investigations were carried out in a social welfare homes in Czerewki. The home was loacted in the village of Czerewki, about 20 km from the city. The building was built 15 years ago. Air was sampled at the entrance to building, corridors, patients' rooms, nurses' rooms, dining rooms, bathrooms, store rooms, recreation rooms and reading room. Total number of tested rooms in Czerewki was 34. Swabs were taken from the walls, and the feet, hands, and nails of the residents of the social welfare home. Air samples were obtained by using SAS-Super 100. The Surface Air System (SAS) encompasses several models which use the same principle. Air is aspirated at a fixed speed for a variable time through a cover which, has been machined with a series of small holes to a special design. The resulting laminar air flow is directed onto the agar surface of a "Contact Plate" containing medium consistent with the microbiological examination to be made. When the present sampling cycle is completed, the plate is removed and incubated. The organisms are then visible to the naked eye and can be counted for an assessment of the level of contamination. SAS – Super 100 possess several references [10-12]. Air samples (1 minutes) were taken with the SAS. The sampler was at the center of the room. The windows were closed for at least 1 h prior to sampling. Samples were taken in the morning between 10 and 11 clock. Next they were shipped to the laboratory within 2 h under cool conditions and incubated at 25°C for 5 days. Colonies of filamentous fungi were identified to genus level on the basis of colonial and microscopic morphology. Swabs were taken from the skin of the interdigital spaces of feet and hands, nails and the oral cavity of the residents. The fungi from the swabs were cultured on Sabouraud medium and incubated at 25°C. The yeast-like fungi strains were identified by using Candi*Select* (Bio-Rad). Moulds and dermatophytes were identified in the direct examination and microculture. Dermatophytes were incubated at 37°C for overnight and next were incubated at 20°C for 7 weeks.

The number of organisms counted on the surface of the "Contact Plate" must first be corrected for the statistical possibility of multiple particles passing through the same hole. The statistical formula is taken from (Correction Tables – from producer) for both the 55 mm standard Contact head and the 84 mm Maxi-Contact head. The probable count (Pr) is then used to calculate The Colony Forming Unit (CFU) per cubic meter of air sampled.

Results were calculated according to the following formula:

$$X = \frac{Pr \times 1000}{V} \text{ CFU per 100 litres of air (1000 litres} = 1 m^3)$$

Where: V – volume of sampled air = 200 l of air, r – Colony Forming Units counted on "55 mm Contact Plates" = 67, Pr – Probable count obtained by positive hole correction = 80, X – Colony Forming Units per 1000 litres (=1 cubic metre) of air

The microbiological environment on the surface of the walls was evaluated by using the Count-Tact of bioMerieux. Materials for testing were collected using a special bioMerieux applicator. The medium was in accordance with the Draft Standard CEN/TC 243/WG2.

Statistical analysis

Statistical analyses are performed using Statistica release 5.0. To approximate a normal distribution were converted to log 10 units, and these transformed numbers were used for t-test. The least significant t-test was used for comparisons among air samples and the walls. Pearson's correlation coefficient was calculated to find correlations between the fungi isolation from air and the walls of the home.

Results

A summary of the results for airborne fungi and fungi colonies isolated from walls is presented in *Tab. 1* and *2*. Microbiological studies revealed significant differences in fungal flora between the place of isolation (corridor, rooms, nurses' room, kitchen, dinning room, bathroom etc.) and the season (summer, autumn, winter and spring). In the Czerewki social welfare home a significant ($p<0.001$) increase in fungi colonies isolated from the air during autumn, winter and spring was found compared with summer. Significant ($p<0.001$) differences between winter–spring and autumn–summer in the isolation of fungal colonies from the walls in the Czerewki home were found (*Tab. 2*). During summer in the Czerewki, only a few colonies were isolated from the air (*Tab. 3*). During the mycological examination in autumn a great variety of genera in the samples of air analyzed were observed: *Candida albicans*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Cladosporium*, *Acremonium*, *Alternaria*. The dominant contaminants were *Candida albicans*, *Cladosporium* and *Rhizopus* (more than 200 CFU/m^3) (*Tab. 3*). During winter and spring, *Penicillium* was the dominant contaminant in the air (below 150 CFU/m^3).

During summer in the social welfare home, the dominant colonies isolated from the walls were *Cladosporium* and *Penicillium* (*Tab. 4*). *Alternaria*, *non-Candida albicans*, *Candida albicans* were isolated more often in autumn. The dominant colonies were *non-Candida albicans* and *Cladosporium* during winter. Similar results were obtained in spring. No significant correlations with indoor air fungi were found for fungi isolated from the walls during summer ($r=-0.24$, $p=0.57$ in the Czerewki

Table 1. **Fungi in the air in the social welfare home in Czerewki**

Rooms	Social welfare home in Czerewki n=34 rooms				
	Count (log 10 CFU/m³) of air				
	Summer	Autumn	Winter	Spring	Total
Patients rooms	0.6	2.65	1.53	2.24	2.82
Nurses' room	0	1.14	0	0.47	1.27
Recreation room	0	1.14	0.3	0	1.23
Store room	0.9	1.91	1.27	1.77	2.27
Dining room	0	0.47	0.69	0.3	1
Kitchen	0	1.5	0.69	0.3	1.59
Bathrooms	1	1.88	1.53	1.69	2.23
Corridor	0.3	1.41	0.6	0.77	1.57
Entrance to building	0	1.8	0.9	1.17	1.94
Mean ± SE	0.12 (0.08)	1.35 * (0.20)	0.52 * (0.17)	0.96* (0.26)	1.55 (0.19)

Data are means (standard error) of 34 * P<0.001 vs summer test-t

Table 2. **Fungi colonies isolated from the walls in rooms tested of the social welfare home in Czerewki in relation to the season**

Rooms	Social welfare home in Czerewki n=34 rooms				
	Number of fungi colonies (log 10) per plate Count-Tact				
	Summer	Autumn	Winter	Spring	Total
Patients rooms	1.16	3.13	1.86	2.35	3.33
Nurses' room	0	1.83	0.3	0.84	1.89
Recreation room	0	1.46	0	1	1.59
Store room	0	2.22	1.34	1.82	2.49
Dining room	0	1.23	1.3	1.14	1.7
Kitchen	0	1.14	1.65	0.47	1.79
Bathroom	0	2.43	1.55	1.8	1.56
Corridor	0	1.82	0.6	1	1.9
Mean ±SE	1.16* (0.18)	1.76* (0.20)	0.77 (0.22)	1.07 (0.14)	1.95 (0.21)

Data are means (standard error) of 34 samples * P<0.001 vs winter, spring, test-t

Table 3. **Fungi colonies isolated from the air in rooms tested of the social welfare home in Czerewki in relation to the season**

Fungi	CFU/m³ of air				
	Summer	Autumn	Winter	Spring	Total
Acremonium spp.	1	6	5	12	24
Alternaria spp.			9	11	20
Aspergillus spp.	9	32	22	32	95
Candida albicans		179	3	46	228
Chrysosporum spp.		7			7
Cladosporium spp.	7	203	12	73	295
Geotrichum			1	3	4
Monilia sitophila					
Mucor spp.				1	1
Mycelia sterola				8	8
Non-C.albicans		2	8	92	102
Penicillium spp.	5		35	88	128
Rhizopus spp.		364	4	4	372
Rhodotorula rubra		22	10	3	35
Scopulariopsis spp.		9			9
Trichophyton mentagrophytes v. interdigitale					
Trichosporon spp.					
Verticillium spp.					
Total	25	824	109	373	1331

Table 4. **Fungi colonies isolated from the walls in rooms tested of the social welfare home in Czerewki in relation to the season**

Fungi	Number of fungi colonies				
	Summer	Autumn	Winter	Spring	Total
Acremonium spp.		16	3	2	21
Alternaria spp.	78	586	2	29	695
Aspergillus spp.	150	30	12	14	206
Candida albicans		278	5	83	366
Chrysosporum spp.					
Cladosporium spp.	94	46	48	39	227
Geotrichum					
Monilia sitophila	21		1	3	27
Mucor spp.			14	4	18
Mycelia sterola			2		2
Non-C.albicans	82	289	49	83	503
Penicillium spp.	120	191	42	61	414
Rhizopus spp.					
Rhodotorula rubra	18			10	28
Scopulariopsis spp.			19		19
Trichophyton mentagrophytes v. interdigitale					
Trichosporon spp.					
Verticillium spp.					
Total	563	1436	197	328	2526

home). Significant ($p<0.05$) correlations with indoor air fungi were found for fungi isolated from the walls during the other three seasons.

In the Czerewki home mainly *Candida albicans* and *non-Candida albicans* were isolated from the patients (nails, feet, hands) during autumn. In the other seasons, only a few colonies were isolated from the patients in the home (*Tab. 5*).

Discussion

Our results show a significant increase the fungi isolated from the air during autumn. During autumn in the Czerewki home, there was significant increase of fungi colonies isolated from the air and the walls as compared with the summer. The identified genera suggests a mix contamination, originated from field and air. The dominant colonies isolated from the air and

***Table 5*. Total number of different types of fungi isolated from different ontocenoses of patients in the social welfare home in Czerewki**

Fungi	Social welfare home in Czerewki n=34 rooms				
	Number of fungi colonies				
	Summer	Autumn	Winter	Spring	Total
Acremonium spp.					
Alternaria spp.			1		1
Aspergillus spp.			1	2	3
Candida albicans	4	35			39
Chrysosporum spp.					
Cladosporium spp.	1		3		4
Geotrichum					
Monilia sitophila					
Mucor spp.					
Mycelia sterola					
Non-C. albicans		24			24
Penicillium spp.	1	1	6	4	12
Rhizopus spp.					
Rhodotorula rubra					
Scopulariopsis spp.					
Trichophyton mentagrophytes v. interdigitale					
Trichosporon spp.					
Verticillium spp.					
Total	6	60	11	6	83

the walls were: *Alternaria*, *Cladosporium Penicillium*, *Rhizopus*, *non-Candida albicans*, *Candida albicans*, during autumn and summer. *Candida albicans*, *non-Candida albicans* and *Penicillium* were isolated from the air and the walls more frequent than other fungi. Similar results we have noticed during winter, therefore the number of fungal colonies and CFU/m^3 were two fold lower. *Candida albicans*, *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* were isolated from the toe and finger nails and the interdigital spaces of the hands and feet during autumn. A similar study [13] carried out using both sedimentation and aspiration methods. These authors evaluated the occurrence of fungi in the indoor air, on the room walls and in certain sites on the skin of the inhabitants of the two social welfare homes in Cracow. The mean number of fungi in the rooms of the renovated ward amounted to 192.3/m^3. The mean number – 1243.5/m^3 of fungi in the old ward was significantly higher compared with the renovated one. The same mould genera and species were detected in both wards. The following mould genera (in decreasing order of frequency) were found: *Penicillium*, *Rhizopus*, *Alternaria*, *Mucor*, *Cladosporium*, *Chaetomium*. The same fungal genera isolated from the air, walls and the skin of residents in both the renovated and the old ward.

Papavassiliou and Bartzokas [14] assessed mycological flora of the Athenian air. The open plate technique was used, Petri dishes containing Sabourand's agar being exposed for 15 minutes. The genus Alternaria was most frequently isolated, representing 38% of the total colonies. Other genera occurred in the following proportions: *Penicillium* 27%, *Aspergillus* 13%, *Candida* 9%, *Rhodotorula* 6% and *Mucor* 1%. Species of other genera accounted for 6% of colonies, including 4% which were classified as *Mycelia sterilia*. Bartzokas [5] noted that during autumn and winter the number of suspended microfungi was more than double that which occurred during spring and summer. The fungal content appeared to be correlated positively with humidity and negatively with temperature, although during the analysis of the six predominant genera some exceptions were found to the general form of the results. Pei-Chih et al. [15] evaluated the airborne fungal concentrations at urban and suburban areas in Taiwan. In summer, the total fungal concentration, both indoors and outdoors of suburban homes, were significantly higher than those of urban homes. Shelton et al. [16] examined 12026 fungal air samples from 1717 buildings located across The United States; these samples were collected during indoor air quality investigations performed from 1996 to 1998. For all buildings, both indoor and outdoor air samples were collected with an Andersen N6 sampler. The fungal levels were highest in the fall and summer and lowest in the winter and spring. The most common culturable airborne fungi, both indoors and outdoors and in all seasons and regions, were *Cladosporium*, *Penicillium*, nonsporulating fungi, and *Aspergillus*: *Stachybotrys chartarum* was identified in the indoor air in 6% of the buildings studied and in the outdoor air of 1% of the buildings studied. Exposure to some fungi can induce allergic or asthmatic reactions, while other species can cause primary infectious diseases. The inhalation of spores containing mycotoxins has been shown to cause many of the symptoms typically associated with the "sick building syndrome"; for example the spores of *Aspergillus fumigatus* and *Histoplasma capsulatum* can cause hypersensitivity pneumonitis. Affected individuals often experience relief when they leave the building for several days [1]. Su et al. [17] investigated correlations among airborne microorganisms collected with Andersen samplers from homes in Topeka during the winter of 1987 to 1988. The factors derived were used to relate microbial concentrations with categorical, questionnaire-derived descriptions of housing conditions. The common soil fungi *Aspergillus* and *Penicillium spp*. were also separated as a group. Elevated concentrations of the soil fungi were significantly associated with the dirt floor, crawl-space type of basement. Elevated concentrations of water-requiring fungi, such as *Fusarium spp*., were shown to be associated with water collection in domestic interiors. Also, elevated mean concentrations for the group of fungi including *Cladosporium*, *Epicoccum*, *Aureobasidium*, and yeast spp. were found to be associated with symptoms reported on a health questionnaire. Miller et al. [18] studied the extent and nature of fungal colonization of building materials in 58 naturally ventilated apartments that had suffered various kinds of water damage in relation to air sampling done before the physical inspections. The results of air samples from each apartment were compared by rank order of species with pooled data from outdoor air. Approximately 90% of the apartments that had significant amounts of fungi in wall cavities were identified by air sampling. Ren et al. [19] characterized the nature and seasonal variation of fungi inside and outside homes in the Greater New Haven, Connecticut area. There indoor air samples (in the living room, bedroom, and basement) and one outdoor sample were collected by the Burkard portable air sampler. House dust samples were collected in the living room

by a vacuum cleaner. No significant difference in concentration and type of fungi between living room and bedroom or by season was observed. Both concentration and type of fungi were significantly higher in the basement than other indoor areas and outdoor air in winter. The type of fungi in living room, bedroom, and outdoor air were found to have significant changes among seasons, but there was no significant difference for the basement among seasons. *Cladosporium* species was dominant in both indoor and outdoor air in summer. *Penicillium* and *Aspergillus* were dominant in indoor air in winter, but neither was dominant in any season in outdoor air. The type of fungi and their concentrations in house dust samples were not representative of those isolated in indoor air. In the dust samples, more *Mucor*, *Wallemia*, and *Alternaria* species, but less *Aspergillus*, *Cladosporium*, and *Penicillium* species were found in the all seasons. Air sampling in spring or fall in every suspected house is suggested for year-round fungal exposure assessment.

Conclusions

Microbiological tests of air and walls revealed significant differences in mycological flora in depending on the place isolation (corridor, rooms, reading room, nurses room, kitchen, dining room, bathroom etc.) and season (summer, autumn, winter, spring).

A significant increase in the fungi isolated from the air and walls in the social welfare home was found, depending on the season. *Alternaria*, *Cladosporium Penicillium*, *Rhizopus*, *Non-Candida albicans*, *Candida albicans* were dominant fungi in the air and walls.

References

1. Bush R. Aerobiology of pollen and fungal allergens. J Allergy Clin Immunol, 1989; 84: 1120-4.
2. Hoppe PR Indoor climate. Experientia, 1995; 49: 775-9.
3. Apter A, Bracher A, Hodgson M, Sidman J, Leng WY. Epidemiology of the sick building syndrome. J Allergy Clin Immunol, 1994; 94: 227-88.
4. Bachmann MO, Myers JE. Influences on sick building syndrome symptoms in tree buildings. Soc Sci Med, 1995; 40: 245-51.
5. Bartzokas CA. Relationship between the meteorological conditions and the airborne fungal flora of the Athens metropolitan area. Mycopathologia, 1975; 8: 3-38.
6. Verhoeff A, Van Reenen-Hoekstra E, Samson R, Brunekreef B, Wunen J. Fungal propagules in house dust. Allergy, 1994; 49: 533-9.
7. Croft WA, Jarvis BB, Yatawara CS. Airborne outbreak of trichothecene toxicosis. Atmospher Environ, 1986; 20: 549-52.
8. Simmons RB, Price DL, Noble JA, Crow SA, Ahearn DG. Fungal colonization of air filters from hospitals. Am Ind Hyg Assoc J, 1997; 58: 900-4.
9. Górny RL, Dutkiewicz J, Krysińska-Traczyk E. Size distribution of bacterial and fungal bioaerosols in indoor air. Ann Agricult Environ Med, 1999; 30: 105-13.
10. CEN/TC 243 Norms for Clean Room Technology.
11. FDA – Guideline on Sterile Drug Products produced by Aseptic Process 1987.
12. USP 23-NF – Microbiological Evaluation of Clean Rooms and other Controlled Environments, (1998) 18-8Th Supplement 1116.
13. Macura AB, Gniadek A. Fungi present in the indoor environment of a social welfare home. Preliminary study. Mikol Lek, 2000; 7: 13-7.
14. Papavassiliou JT, Bartzokas CA. The atmospheric fungal flora of the Athens metropolitan area. Mycopathologia, 1975; 7: 31-4.
15. Pei-Chih W, Huey-Jen S, Chia-Yin L. Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. The Science of the Total Environment, 2001; 15: 111-8.
16. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol, 2002; 68: 1743-53.
17. Su HJ, Rotnitzky A, Burge HA, Spengler JD. Examination of fungi in domestic interiors by using factor analysis, correlations and associations with home factors. Appl Environ Microbiol, 1992; 58: 181-6.
18. Miller JD, Haisley PD, Reinhard JH. Air sampling results in relation to extent of fungal colonization of building materials in some water-damaged buildings. Indoor Air, 2000; 10: 146-51.
19. Ren P, Jakun TM, Leaderer BP. Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one North-east American county. J Expo Anal Environ Epidemiol, 1999; 6: 560-8.