Environmental risk of mycosis in patients treated at an acquired immunodeficiency ward

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Abstract

Purpose: Patients with acquired immunodeficiency are particularly predisposed to fungal infections. The purpose of the study was evaluation of the presence of fungi in the environment of a ward where Human Immunodeficiency Virus (HIV) positive and Acquired Immune Deficiency Syndrome (AIDS) patients were treated.

Materials and methods: The evaluation of fungal presence in the indoor air and on the room walls at an acquired immunodeficiency ward in the University Hospital in Cracow was carried out in December 2006. Indoor air specimens were sampled using an aspiration method (a MAS 100 device) while imprints from the walls using Cont-Tact method (bioMèrieux) in the morning ad in the evening during five consecutive days. A total of sixty air specimens and thirty imprints from the walls were obtained. The fungi cultured from those specimens were analysed using standard mycological procedures.

Results: It was found out that the numbers of fungi sampled from the indoor air in the morning were significantly higher than those sampled in the evening. The average numbers of fungi isolated in the rooms inhabited by the patients varied from 55 c.f.u (colony forming units)×m^{-3} to 490 c.f.u×m^{-3} as calculated for the entire testing period. Fungi potentially pathogenic for persons with impaired immunity were found in all of the rooms: *Aspergillus* sp., *Mucor* sp., and yeast-like fungi *Candida* sp.

Conclusion: Reduction of the numbers of potentially pathogenic bacteria, viruses and fungi in the indoor air should be a standard in the practice of medical staff (mainly epidemiological nurses).

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Introduction

Acquired Immune Deficiency Syndrome (AIDS) was diagnosed in 1798 persons in Poland between the year 1985 and August 31, 2006. Out of them, 825 have died. However, it is estimated, that the real number of persons infected with human immunodeficiency virus (HIV) and/or suffering from AIDS is 15 000-20 000 [1].

The stages of HIV infection, according to Center for Disease Control and Prevention (CDC) make the basis for evaluation of the disease progression and are commonly applied in the clinical practice. The following criteria are used in the above classification: immunological i.e. the number of CD4 cells, and clinical – concerning concomitant diseases accompanying HIV infections and/or suggesting the presence of AIDS. Fungal infection very often accompany HIV infections. Clinical data give evidence that oral candidiasis develops in 100% of HIV positive patients when the number of CD4 cells is below 200 in one millilitre of blood [1,2].

Opportunistic fungi may cause infections in otherwise healthy persons, but those infections recover spontaneously due to natural defence mechanisms. However, similar infections in immunocompromised patients result in severe invasive infections. Such infections comprise aspergillosis, candidiasis cryptococcosis, mucormycosis, fusariosis. The mortality due to fungal infections in immunocompromised patients is high [3,4].

Immunodeficiency is the primary factor predisposing to fungal infections in AIDS patients, however, the risk of invasive infection depends also on the exposure, infecting dose, pathogenicity of the fungus, the form of immunosuppressive treatment and fungal infections undergone in the past [5,6].

Evaluation of the extent of fungal infection risk is very important from the clinical point of view, however, the expo-

sure intensity and the quantity of infectious material can hardly be determined because there are no non-pathogenic fungi for immunocompromised patients.

Therefore, it appears reasonable environment cleanness monitoring in the rooms where patients under immunosuppression are present. Identification of pathogenic and/or biochemically active fungi (e.g. *Candida* sp., *Aspergillus* sp., *Mucor* sp.) in the hospital indoor air should be an imperative to undertake proper safety measures. Spores of those fungi can colonise the patients' airways and, in presence of predisposing factors, to cause fungal infection [7-9].

The purpose of this study was determination of the presence of fungi in the environment of an acquired immunodeficiency ward.

Materials and methods

The study was carried out at the Acquired Immunodeficiency Ward in the Gastroenterology, Hepatology and Infectious Diseases Department of the University Hospital in Cracow in December 2006. Patients infected with HIV and suffering from AIDS were treated at the Ward. The ward included six rooms: two bays, two corridors, patients' bathroom and nurse's station. The presence of fungi was tested in all of those rooms by simultaneous sampling indoor air specimens and imprints from the walls. The materials were sampled during five consecutive days between 11 and 15 December 2006. The samples were taken twice daily, at approximately 7 a.m. and 7 p.m. Additional air specimens were sampled in front of the ward entrance and in front of the Department building entrance door once daily in the morning.

Sixty indoor air specimens were taken using aspiration method (MAS 100 device, Merck), while thirty imprints from the walls were taken using Count-Tact method (bioMèrieux).

Each of the air specimens consisted of 200 litres of air aspirated on the Petri dish. The air sampler was positioned in the middle of the room, 1.5 metre above the floor level. The doors and windows of the rooms were closed during the air sampling. The Petri dishes used in the study were filled with commercial Sabouraud Glucose Selective Agar medium, with gentamicin and chloramphenicol added. After material sampling, the Petri dishes were incubated at 27°C. After three days of incubation the colonies were counted, and their morphology evaluated. The Petri dishes were further incubated up to 14 days, the exact time of incubation depended on the fungal genus. After incubation, the real number of colonies was corrected and the number of colony forming units in one cubic metre of air was calculated using the formula:

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

where: α – number of fungal colonies grown on the medium from the air sample; V – volume of the air sample in liters; X – the number of fungi present in the air expressed in terms of number of colony forming units in one cubic meter of air (c.f.u.×m^{-3})

The imprints from the walls were taken using the Count-Tact technique. The applicator (bioMèrieux) was used to make the imprint from the dry wall surface. The imprints were taken on the dish with Sabouraud glucose medium with chloramphenicol added. The imprints were taken from the walls 1.5 metre above the floor level, while in the bays just above the patients' beds. The dishes with material samples were then incubated, first at 37°C for 3 days, then at 27°C for next 3 days. The colonies were counted, and the number of c.f.u. on one square centimeter was calculated using a formula:

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

where: α – number of fungal colonies in the dish, r – radius of the dish in centimeters, X – number of colony forming units on one square centimeter of the wall (c.f.u.×cm^{-2})

The fungi were identified using routine procedures of mycological diagnostics. The moulds were evaluated macroscopically and microscopically on the basis of the culture appearance and their morphological features in direct preparations stained with lactophenol and methyl blue (Merck). In doubtful cases, slide microcultures were made and preparations made of them were identified. Yeast-like fungi were Gram-stained and cultured on starvation medium.

In the statistical analysis, maximum, minimum, median and mean values were found and the standard deviation was calculated. The data were processed using the t-test. The value of $p<0.05$ was accepted as the threshold of significance.

Results

The mean numbers of fungi isolated from the indoor air samples taken at the acquired immunodeficiency ward in the morning and in the evening during five consecutive days are presented in *Tab. 1* in terms of c.f.u×m^{-3}. Fungi were present in all of the sixty air specimens. The numbers of c.f.u×m^{-3} varied from 12 in the bathroom to 710 in the corridor No 1. The highest numbers of fungi were detected in the corridors while the lowest in the nurse's station during the entire resting period. The mean number of fungi in the bays varied between 55 and 490 c.f.u×m^{-3}; it was considerably lower in the evening. The fluctuations of the number of fungi in the particular rooms during the entire testing period were highest in the morning sampling at the corridor No 1 (standard deviation 247.52) and the lowest in the evening sampling at the bay No 2 (standard deviation 57.73). Little differences in the mean numbers of fungi were observed both in the morning and evening samplings at the nurse's station (standard deviation 75.61 and 62.55 respectively).

The comparison of median, mean, minimum and maximum numbers isolated from all of the rooms in the particular sampling days revealed that higher numbers of fungi were isolated in the morning than in the evening (*Tab. 2*). Those observations were confirmed by the t-test which revealed significance on the third ($p<0.001$), the second and fourth ($p<0.01$) as well as on the first day of sampling ($p<0.05$). It was also observed that the numbers of fungi in the indoor air decreased during the consecutive days, as compared with those on the first day, however, they increased on the last day.

Table 1. **Mean numbers of fungi (in terms c.f.u. [colony forming units]×m^{-3}) isolated from the indoor air in the morning and the evening in the rooms during entire assay period**

Samplings time	Statistical analysis	Sampling site					
		corridor 1 (n=10)	**bay 1** (n=10)	**corridor 2** (n=10)	**bay 2** (n=10)	**bathroom** (n=10)	**nurse's station** (n=10)
Morning (07:00)	arithmetic mean	279	238	269	212	189	184
	standard deviation	247.52	161.73	167.01	118.77	90.37	75.61
	median	210	205	235	190	185	200
	maximum	710	490	470	340	335	265
	minimum	75	70	55	55	90	60
Evening (19:00)	arithmetic mean	176	126	148	128	153.4	140
	standard deviation	90.99	58.67	59.85	57.73	143.10	62.55
	median	155	115	130	115	130	130
	maximum	325	225	240	225	390	240
	minimum	85	70	85	80	12	70

n – number of days in which samples were collected

Table. 2. **Maximum, minimum, medians, and arithmetic mean of fungi (c.f.u [colony forming units]×m^{-3}) isolated from the indoor air in the morning and evening in the rooms during entire assay period**

Statistical analysis	**Date of sampling**									
	11.12.2006		**12.12.2006**		**13.12.2006**		**14.12.2006**		**15.12.2006**	
	Morning (07:00)	Evening (19:00)	Morning (07:00)	Evening (19:00)	Morning (07:00)	Evening (19:00)	Morning (07:00)	Evening (19:00)	Morning (07:00)	Evening (19:00)
arithmetic mean	389.17	139.17	210.83	85	67.5	119.17	190	124.17	293.33	274.17
median	370	135	200	82.5	65	115	195	125	275	240
maximum	710	170	335	115	90	155	210	185	470	390
minimum	185	110	140	70	55	85	150	80	195	225
Test t-student	*p<0.05*		p<0.01		*p<0.001*		*p<0.001*		NS	

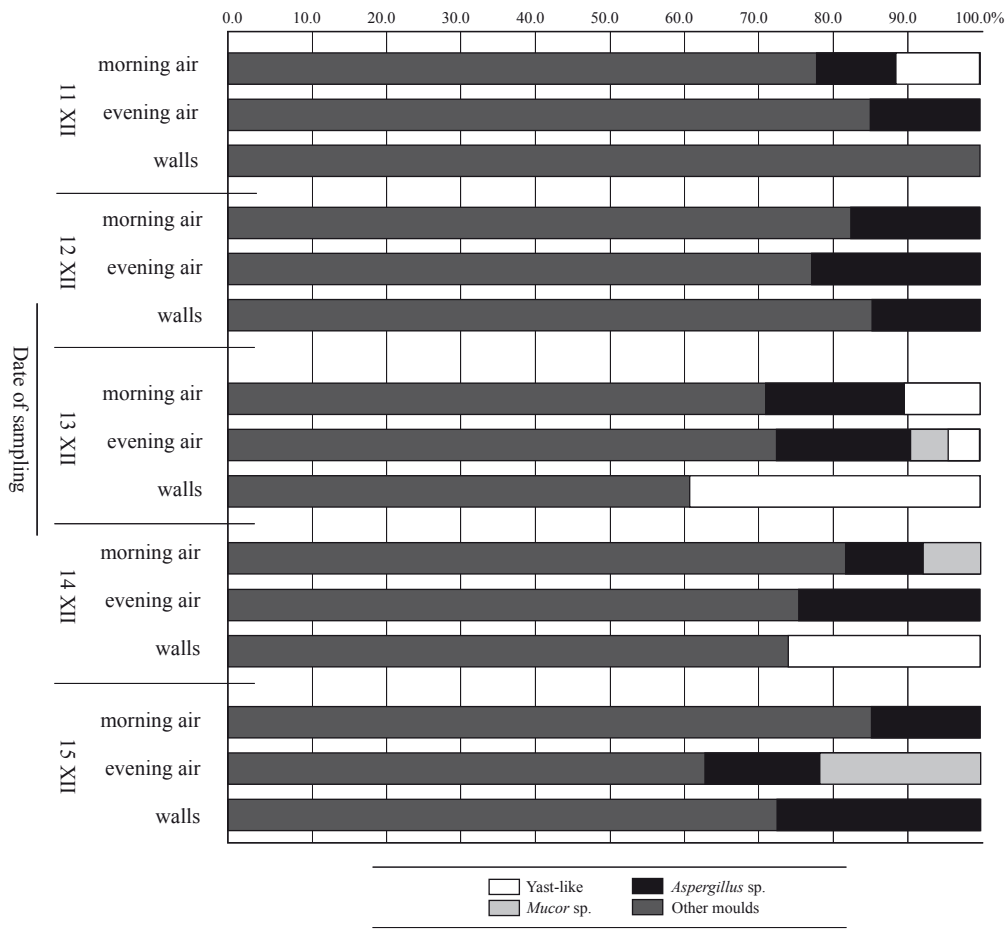
Table 3. **Mean values of fungal c.f.u [colony forming units]×cm^{-2} isolated from the walls in the rooms tested**

Sampling site	**Sampling date**				
	11.12.2006	12.12.2006	13.12.2006	14.12.2006	15.12.2066
Corridor 1	0.04	0.08	0	0	0
Bay 1	0.34	0.51	0.21	0	0.17
Corridor 2	0	0.21	0.04	0.13	0
Bay 2	0	0	0.04	0.21	0
Bathroom	0	0	0	0.13	0
Nurse's station	0	0	0.04	0.4	0.04

A quantitative analysis of the thirty imprints from the walls revealed presence of fungi only in half of them (*Tab. 3*). The mean numbers of fungi varied between 0.04 and 0.51 c.f.u.×cm^{-2}. Most frequently fungi were isolated from the walls in the bay No 1 and in the nurse's station on the third and fourth day of sampling. Least frequently fungi were isolated from the wall in the bathroom: only once on the fourth day.

The genera of fungi isolated from both the indoor air and from the walls are presented in (*Fig. 1*). The fungi were divided into four groups: yeast-like fungi, moulds *Aspergillus*, moulds *Mucor* and other moulds. Moulds other then *Aspergillus* and *Mucor* outnumbered other fungi on the particular sampling days and sites (total percentage over 60%). The genus *Aspergillus* was present in the indoor air every day, and comprised from 0 to 30% of all of the fungi isolated. The genus *Mucor* was not isolated from the walls, while in the indoor air it was detected in the evening on the second and fifth days of sampling, and in the morning on the third day. Yeast-like fungi were isolated both from the air and from the walls on the third and fourth day of sampling. They were also present in the indoor air in the morning on the first day and in the evening on the fifth day, The percentage of yeast-like fungi varied from 0 do 40% and depended on the sampling time and site; the majority of them were isolated from the walls. The yeast-like fungi isolated from the indoor air and from the walls belonged mainly to the genus *Candida* and to the species *Rhodotorula rubra*.

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



Discussion

The microorganisms' adherence capacity to the host epithelium is one of their pathogenicity determinants. In healthy individuals, this process is inhibited by physiological mechanisms such as gastrointestinal passage and mucocilliary clearance in the nose, trachea and bronchi [10,11]**.** When those mechanisms are impaired and/or the bacterial flora is scarce, the fungal adherence capacity to the host cells increases. This concerns particularly yeast-like fungi, mainly *Candida albicans* and *Candida tropicalis* as well as moulds *Aspergillus, Mucor,* or *Fusarium.* Moreover, some of those fungi may produce proteolytic and lipolytic enzymes that may damage host cell membranes and enable penetration of hyphe and pseudomycelium to the intercellular space [12-14]. Such an unfavorable situation occurs in patients with acquired immunodeficiency where impaired cell-mediated immunity does not inhibit dissemination of fungal infection. This is confirmed in numerous reports giving evidence that yeast-like fungi *Candida* and moulds *Aspergillus* and *Mucor* produce invasive infections of myocardium, lungs, brain as well as generalized candidiasis, aspergillosis or mucormycosis [15-17]. In many cases, such infections are cause of death or make it necessary to perform surgery. Some studies [9,14,18] suggest that there is a relationship between the number of mould spores in the air and the prevalence of aspergillosis in the patients. It appears unequivocal that opportunistic fungi must not be present in the indoor air at the acquired immunodeficiency ward. Unfortunately, it was not the case in our study. Yeast-like fungi *Candida* as well as moulds *Aspergillus* and *Mucor* were detected in the indoor air on each of the sampling days. The were also isolated from the walls on the second, third, fourth and fifth days. The percentage of the fungi able to cause opportunistic infections varied within the range 0-40% of the total of fungi isolated. The mean number of fungi isolated from the air varied within the range 12 c.f.u×m^{-3} -710 c.f.u×m^{-3} which many times exceeded the standards accepted by Krzysztofik (up to 200 c.f.u×m^{-3}) [19]. If the rooms of the acquired immunodeficiency ward were classified as treatment rooms (standard up to 50 c.f.u×m^{-3}), only the bathroom could meet that criterium, and not on each of the sampling days.

The concentration of the fungi in the indoor air is related to the natural migration of people in the rooms which makes the possibility to transfer the fungal spores on the hands and/or

clothes of the staff and the patients. An acquired immunodeficiency ward, as a rule, is a ward of higher sanitary standards and limited access for visitors [7,9].

Our investigations revealed that higher concentration of fungi was in the morning that in the evening which was significant on four consecutive days: ($p<0.001$, $p<0.01$, and $p<0.05$). Such a situation may be explained by the fact that the rooms were ventilated by day, and the number of patients was 7 on the first day but only four on the last day. The findings are consistent with those obtained at the invasive chest diagnostics ward in the University Hospital in Cracow and at the operating theatre in one of the hospitals in Białystok. In both of those sites, the number of fungi sampled in the morning outnumbered those sampled in the evening [20,21].

Providing conditions conducive to recovery for an inpatient at a hospital ward is an imperative for the medical staff. It is particularly important in case of immunocompromised patients. It appears reasonable that there should not be fungi present in the indoor air in such patients' environment. Reduction of the numbers of potentially pathogenic bacteria, viruses and fungi in the indoor air should be a standard in the practice of medical staff (mainly epidemiological nurses). Proper procedures, including air conditioning, in rooms where immunocompromised patients are present should eradicate the most pathogenic fungi from the environment: *Candida albicans, Candida parapsilosis, Aspergillus* sp., *Mucor* sp. and others potentially causing deep and/or generalised mycoses.

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