

Monitoring the air fungal contamination load in two educational hospitals in Sanandaj, Iran

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ABSTRACT

Opportunistic fungal pathogens are known to increase infection in the health care workers and patients with immune deficiency. This study aimed to investigate the qualitative and quantitative airborne fungal contamination load in two Sanandaj hospitals. In this cross-sectional study, 112 biological samples from 15 different hospital wards were collected for 6 months by using the single-stage Anderson sampler in Sabouraud's dextrose agar. Data were analyzed for the descriptive and analytical tests using IBMSPSS V.21 software and the air fungal contamination load (AFCL) was calculated (cfu/m³). The average hospital AFCL in Besat and Tovhid was 21.13 and 14.51 cfu/m³, respectively. No statistically significant differences were observed between AFCL and relative humidity (RH) in the two hospital samples ($p = 0.495$) according to independent t-test, whereas this difference in the average temperature in the hospital wards was significant ($p < 0.001$). Highest AFCL was observed in the surgical ward for females (AFCL = 35.34 cfu/m³) and the lowest was observed in the surgical and urology wards (AFCL = 2.2 cfu/m³) in the two hospitals. The frequency of *Penicillium* was the maximum with 77.6 and 65.25% in the Besat and Tovhid hospitals, respectively. In wards such as oncology, gynecological surgery, and operating room, further studies are needed to better control environmental conditions and fungal contamination. Thus, utilizing highly efficient air purification systems and regular monitoring of the biological risk for both patient and health care employees is recommended.

Keywords: Fungal infections, Hospitals, Aerosols, Environmental pollution, Biological monitoring

Introduction

Indoor air quality (IAQ) is an important factor in the hospital facilities for preventing infections. Poor hospital IAQ can cause hospital-acquired infections, sick hospital syndrome, and various occupational hazards.¹ Biological aerosols (bioaerosols) are suspensions of airborne particles that contain living organisms such as viruses (0.01–0.3 μm),

bacteria (0.1–10 μm), fungal and fern spores (1–30 μm), plant pollen (5–100 μm), and fragments of animal and plant matter.^{2–4} Particles with aerodynamic diameter less than 10 μm are crucial in the pathogenesis, leading to about 5–34% of indoor air pollutions; these pathogens can enter the human bodies via inhalation, ingestion, and skin contact and may lead to adverse health conditions including infection, respiratory diseases, chronic toxic effects, allergies, and cancer. The saprophytic fungi play a significant role in the development of allergic disease.^{3–6} Nosocomial and employment infection is increasing in people via increased exposure to the infectious agents due to the presence of several patients, inadequate ventilation, and

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improper disinfection of medical equipment in various wards of the hospital and in patients with immunocompromised system including those with AIDS, cancer, and transplant recipients, causing serious adverse health effects.^{7,8} Multiple studies have reported that invasive pulmonary aspergillosis is a life-threatening factor for patients with hematologic malignancies as well as for recipients of bone marrow transplantation.³ In order to prevent such infections, these category pollutants should be constantly monitored both quantitatively and qualitatively. Bioaerosols are harmful for people who work in the hospitals and medical centers, and could lead to occupational problems such as decreased efficiency, absenteeism from the workplace, etc.^{9, 10} For such pollutants, the occupational exposure limit has not been provided by the relevant organizations; hence, in order to evaluate and compare the density in the workplace exposure limits, these organizations have applied the results of similar studies.¹¹

Several studies have investigated the fungal air quality in the hospital environments. Azimi et al. assessed various rooms in a hospital in Tehran and found that the total mean concentration of the detected fungi in the hospital rooms was 55 ± 56 cfu/m³.¹² This study revealed that the highest concentrations (97 ± 217 cfu/m³) of fungi were reported in the Orthopedics Operating Room and the most common fungal genera found were *Penicillium* (70%) and *Aspergillus* (14%). A study by Park et al. demonstrated that the average levels of the airborne fungi in 6 hospital lobbies were 7.7×10 cfu/m³; thus, all hospital lobbies were generally contaminated.¹³

Microbial profile of air contamination in some hospital wards was determined by Abdollahi et al.¹⁴ The microbial profile of the air samples revealed *Micrococcus* to be the most commonly found bacteria. *Cladosporium* was the most frequently found fungi, whereas *Aspergillus niger* and *Alternaria spp.* were the least frequent ones.

As opportunistic fungal infections are increasing, the risk of infection in the immunocompromised patients is high; since

fungal spores are present almost everywhere in the hospital environment, this study was conducted with an aim to investigate the qualitative and quantitative airborne fungal contamination in the two educational hospitals of Sanandaj, Iran.

Materials and Methods

In the present cross-sectional research, the air fungal contamination load (AFCL) in 15 different wards of 2 hospitals (Tovhid and Besat) of Sanandaj was studied for 6 months from January to June 2015. The air biological samples ($n = 112$) were collected from males and females in the different hospital wards including Hematology and Oncology, ICU, General Surgery, men's surgery and gynecological surgery, Pediatric Infectious, and Gastroenterology, for an interval of 6 months from January to June 2015. In order to collect the air samples, one of the most accurate and the most important microorganisms sampling method (0800 method) from the National Institute of Occupational Safety and Health and the best microbial air samplers (one stage Anderson sampler), were used.¹⁵ The pump used for passing polluted air from the absorbent medium (culture medium) was Quick Take 30 (SKC, USA) that calibrated the flow rate of 28.3 L/min for 2 min (the sampling train and the sample plate are presented in Figure 1).



Fig. 1. Airborne fungal spores plates and related air sampling equipment's

Fungi in the air were sampled directly onto plates containing Sabouraud's dextrose agar (Merck, Germany) with chloramphenicol (SC). Chloramphenicol was added to control the bacterial growth in the culture. In order to disinfect the Anderson sampler plate, cotton gauze pad and 70% isopropanol alcohol were used. After of the plates were dried and the

sampler pores were open, the sample was collected at a height of 130 cm above the floor and 1 m away from the walls and other surfaces, following which the plates were closed and blocked with a tape. In order to determine the impact of environmental conditions on the concentration of bioaerosols, dry bulb temperature and relative humidity (RH) during sampling were measured by using a calibrated digital thermo hygrometer (model TES 1360). Samplings at fixed distinctive locations in hospitals during morning shifts (09:00–15:00) were done as the crowd was less at that time. Samples were transported to the Mycology laboratory section of Kurdistan University of Medical Sciences and were incubated at 26–28°C for 48 h in an incubator (Model Memmert); thereafter, the type of fungi were identified by lactophenol cotton blue staining procedure and the number of fungal colonies were counted and recorded. By calculating the total number of colonies and dividing them by

the volume of sampled air (air volume corrected according to temperature and pressure), AFCL and colony forming unit/cubic meter (cfu/m³) were obtained. Collected data were entered in Microsoft Excel Worksheet and IBM SPSS V.21 software, were analyzed by the descriptive and analytical tests such as independent sample t-test and one-way ANOVA.

Results and Discussion

According to the results, the minimum and maximum temperatures measured in the wards were 22.1 °C and 30.7 °C, respectively, with an average of 26 ± 1.76 °C, and the percentage of RH was $24.2 \pm 4.5\%$ and barometric pressure was 615 mmHg. Moreover, the simultaneously temperature and relative humidity of the outside environment were 22.9 ± 4.4 °C and $22.7 \pm 4.3\%$, respectively. The average AFCL in Besat and Tohvid hospitals was 21.13 cfu/m³ and 14.51 cfu/m³, respectively.

Table 1. Air fungal contamination load (AFCL) in cfu/m³ in various wards and conditions

Hospital	Sample No./Ward ^a	Ward	Mean AFCL. cfu/m ³ (±SD)	Inside		Outside	
				Temp. ^b (°C)	RH ^b (%)	Temp. ^c (°C)	RH ^c (%)
Besat	7	Pediatric Oncology	17.66 (±26.98)	25.6	25.5		
		Intensive Care Unit (ICU)	25.23 (±40.61)	25.4	23.6		
		Operation Room	10.09 (±26.71)	28	24.9		
		gynecological surgery	35.34 (±33.82)	26.7	23.9	23.3	22.3
		Surgery of male	10.09 (±17.24)	27.01	22.7	(±4.2)	(±4)
		Pediatric Infectious	32.81 (±47.21)	26.4	22.4		
		Female Gastroenterology	32.81 (±46.10)	27.04	25.6		
	Male Gastroenterology	5.04 (±13.35)	27.05	22.8			
56	Total	21.13 (±33.42)	26.66	23.9			
Tohvid	8	Hematology and Oncology	24.29 (±31.23)	25.2	25		
		Intensive Care Unit (ICU)	15.45(±19.89)	25.7	23.6		
		Operation Room	24.29(±49.01)	25.06	24.4		
		Surgery and Urology	2.2(±6.24)	25.78	24.6	22.6	23.1
		Infectious	2.2(±6.24)	25.82	23.1	(±6.2)	(±4.6)
		Female Gastroenterology	13.25(±18.28)	25.38	25.1		
	Male Gastroenterology	8.83(±13.35)	24.52	25.8			
56	Total	12.93(±25.09)	25.36	24.5			

^a Number of samples per each ward

^b Temperature and relative humidity at inside of wards

^c Temperature and relative humidity at outside of wards (outdoor ambient)

Differences observed between the two hospitals's AFCL and RH% were not significant according to the independent sample t-test ($p = 0.495$), whereas the difference in the average

temperature in the hospital wards was significant ($p < 0.001$). Total AFCL differences between the two hospitals were not significant ($p = 0.241$). Moreover, one-way ANOVA

results demonstrated that the differences between the ward's AFCL between the two hospitals were not statistically significant ($p = 0.38$). Highest AFCL level in Besat hospital was observed in the gynecological surgery ward (AFCL = 35.34 cfu/m³), Pediatric Infectious ward (AFCL = 32.81 cfu/m³), and female Gastroenterology ward (AFCL = 32.81 cfu/m³), respectively, and the lowest AFCL was observed in male Gastroenterology ward (AFCL = 5.04 cfu/m³). These findings revealed that the AFCL levels varied in different wards of Tovhid hospital, where maximum pollution rate was observed in the Hematology and Oncology wards and operation rooms (AFCL = 24.29 cfu/m³). Air filtration systems in the operation rooms were inefficient due to the international sanctions and difficulty of providing high-

efficiency HEPA filters. The lowest AFCL level was observed in the Pediatric Infectious ward, Surgical ward, and Urology ward (AFCL = 2.2 cfu/m³), respectively. AFCL of the other wards is summarized in Table 1. The fungal majority observed in both hospitals was that of *Penicillium* (Fig. 2); the infection rate of *Penicillium* was 77.6 and 65.25% in the two hospitals of Besat and Tovhid, respectively. The second most prevalent fungus was *Aspergillus flavus* and the infection rate was 8.9 and 13% in the Besat and the Tovhid hospitals, respectively (Fig. 3). The least amount of fungi frequency in the hospital wards was that of *Rhizopus* and *Ulocladium* (others). The distribution of other types of fungi by type, ward, and hospitals is summarized in Table 2.

Table 2. Number of fungal colonies and species in the air sampled from various wards

Hospital Ward	Colony of Fungi (%)			Penicillium	Alternaria	Others	
	Aspergillus						
	<i>Flavus</i>	<i>Fumigatus</i>	<i>Niger</i>				
Besat		1 (14.3)	0	5 (71.4)	0	1 (14.3)	
		0	0	0	0	0	
		0	0	4 (100)	0	0	
	Gynecological surgery	2 (14.3)	1 (7.14)	0	9 (64.26)	2 (14.3)	0
	Surgery of male	2 (50)	0	0	0	0	2 (50)
	Pediatric Infectious	0	0	0	13 (100)	0	0
	Female Gastroenterology	0	2 (15.4)	0	11 (84.6)	0	0
Male Gastroenterology	0	0	0	2 (100)	0	0	
Total	6 (8.9)	4 (6)	0	52 (77.6)	2 (3)	3 (4.5)	
Tovhid	Hematology and Oncology	0	0	0	10 (90.9)	1 (9.1)	0
	Intensive Care Unit (ICU)	2 (28.6)	0	1 (14.2)	2 (28.6)	0	2 (28.6)
	Operation Room	1 (8.3)	0	1 (8.3)	7 (58.4)	2 (16.7)	1 (8.3)
	Surgery and Orology	0	0	0	1 (100)	0	0
	Infectious	0	0	0	1 (100)	0	0
	Female Gastroenterology	1 (14.3)	0	0	4 (57.13)	2 (28.57)	0
	Male Gastroenterology	2 (28.6)	0	0	5 (71.4)	0	0
Total	6 (13)	0	2 (4.35)	30 (65.25)	5 (10.9)	3 (6.5)	

The fungal majority observed in both hospitals was that of *Penicillium* (Fig. 2); the infection rate of *Penicillium* was 77.6 and 65.25% in the two hospitals of Besat and Tovhid, respectively. The second most prevalent fungus was *Aspergillus flavus* and the infection rate was 8.9 and 13% in the Besat and the Tovhid hospitals, respectively (Fig. 3). The least amount of fungi frequency in the hospital wards was that of *Rhizopus* and *Ulocladium* (others). The distribution of other types of fungi

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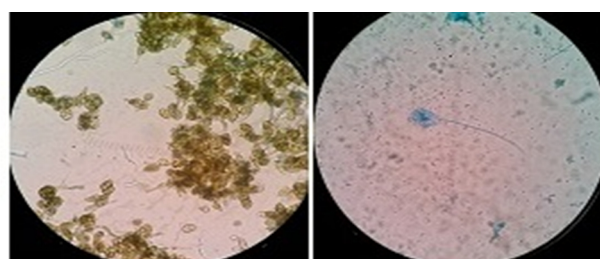


Fig. 2. *Penicillium* (right) and *Alternaria* (left) seen in the following in samples taken from different wards of hospitals

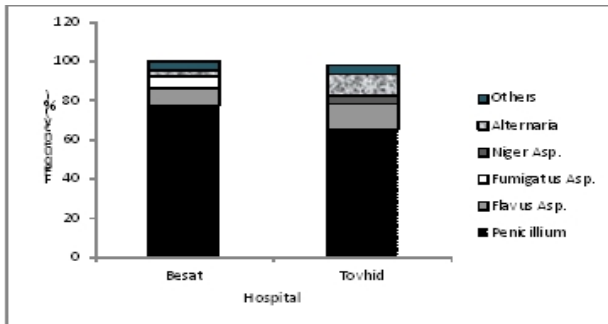


Fig. 3. Percentage of Hospitals Contaminated by Various Genera of Fungi during the study

Table 2.

The average AFCL level in Besat and Tovhid hospitals were 21.13 cfu/m^3 and 14.51 cfu/m^3 , respectively. The threshold limits value available in the scientific literature for bioaerosols in the occupational environments for fungi: $1.0 \times 10^1 - 1.0 \times 10^4 \text{ cfu/m}^3$ for nonindustrial workplaces and $<1.0 \times 10^2 - 1.0 \times 10^7 \text{ cfu/m}^3$ for manufacturing and industrial premises; for the pathogenic microorganisms, there is no safety level (the threshold limit should be 0 cfu/m^3).¹⁶ Hence, in the best case scenario no fungal contamination should be present in the hospital wards; however, as zero contamination is not possible, the levels should be minimum.

In a study conducted by Azimi et al. for 4 months in 2012 in Shariati Hospital of Tehran, the AFCL level was $56 \pm 55 \text{ cfu/m}^3$, with three-fold density that has been observed in this study.¹² In a study conducted by Aboul-Nasr et al. in 2013 in Egypt, the AFCL level in the ICU and surgical room was between 31.13 and 49.61 cfu/m^3 , respectively.¹⁷ In the study conducted in France in the Hematology ward for a year, the average AFCL level was 4.1 cfu/m^3 .¹⁸ In a report by Ekhaïse et al. in five hospitals in Benin City, Nigeria, the AFCL levels were between 10 and 35 cfu/m^3 .¹⁹ In a study in Minnesota, the AFCL levels in the patient care unit were between 11 and 61 cfu/m^3 , and these levels were twice within the hospital when compared to those outside the hospital.²⁰

In the study conducted in the public hospitals of Hamedan to investigate the fungal contamination, the average density of AFCL level in wards was 12.56 cfu/m^3 , which was

relatively similar to the density of AFCL level of the present study.²¹ It seems that the density of fungal contamination in two investigated hospitals were in desirable status.

Based on the results of the independent sample t-test, no significant statistical differences were observed between the two hospitals's AFCL level and RH% ($p = 0.495$), whereas the difference in the average temperature in the hospital wards was statistically significant ($p < 0.001$); hence, the high fungal density may be related to the temperature elevation. At first glance this may seem skeptical but as we observe in Table 1, the temperature was lower in winter than the other seasons and the fungal contamination was high. This is due to the fact that heating systems are active in the winter, which increases the temperature inside the wards.

According to the independent sample t-test, the density of AFCL level between the two hospitals, was not statistically significant ($p = 0.241$), that is, the concentration of the bioaerosols in the two hospitals was different but the difference was not statistically significant. The findings of this study do not match with the results of Zadeh et al., where the density of the AFCL level reveals a significant difference in various wards of the hospital.²¹

Highest AFCL level in Besat hospital was in the female surgical ward (AFCL = 35.34 cfu/m^3), Pediatric Infectious ward (AFCL = 32.81 cfu/m^3), and the female Gastroenterology ward (AFCL = 32.81 cfu/m^3), respectively, and the lowest AFCL was found in the male Gastroenterology ward (AFCL = 5.04 cfu/m^3).

Presumably, the main cause of the high AFCL level in the female wards can be attributed to the presence of some nonmedical and nonsterile equipments as well as the huge crowd in these wards.¹⁸ Hence, high diversity and concentration of bioaerosols in both female Gastroenterology and female surgical wards may be associated with these cases. Certainly, the difficulty in providing high-efficiency HEPA filters due to the cruel sanctions is one of the main causes of pollution in these wards. Notably, similar findings were reported in some other studies including that of Jabari et al. in the

Qom (center of Iran).²² Female ward was one of the most polluted wards in this study, presumably due to the majority of hospitalized patients and large number of visitors.

The most common type of fungus in the hospital air was *Penicillium*, which is consistent with the results of Azizifar M. and et al study.²² AFCL levels varied in different wards of Tovhid Hospital, the highest being in the Hematology and Oncology ward and the surgical room of this hospital (AFCL = 24.29 cfu/m³). The lowest AFCL level was observed in the Pediatric Infectious ward, General Surgery ward, and Urology ward (AFCL = 2.2 cfu/m³), respectively.

Penicillium was majorly found in both hospitals (Fig. 2 and 3); the infection rate of *Penicillium* was 6.77 and 25.65% in the two hospitals of Besat and Tovhid, respectively. The second most prevalent fungus was *Aspergillus flavus*, the infection rate being 9.8 and 13%, respectively, in the Besat and the Tovhid hospitals. The lowest amount of fungi frequency in the hospital wards was related to the other fungal colonies such as *Rhizopus* and *Ulocladium*.

These findings are consistent with the study by Azimi et al., in which the frequency of *Penicillium* was 70% and *Aspergillus* was 14%, respectively,¹² followed by *Cladosporium* with 12% frequency. The latest type was not observed in our study, which may be due to the sampling parameters such as season of sampling, temperature, humidity, efficiency of ventilation system, disinfection programs.²¹

In several studies, including those of Hoseinzadeh et al. and Kelkar et al., *Penicillium* and *Aspergillus* had the highest distribution frequency in hospital wards.^{21, 23} In general, these results were similar in type and number of colonies together.

According to the results, the highest amount of AFCL level was observed in March, with 48.58 cfu/m³ for Besat hospital and 42.9 cfu/m³ for Tovhid hospital, respectively, which could be a result of increased morbidity and hospital visits in this season. Moreover, the high moisture level inside the wards could lead to fungal growth. Furthermore, in June, lowest rate of fungal contamination was observed. Monthly distribution of air fungal contamination in two hospitals has been presented in Fig. 4.

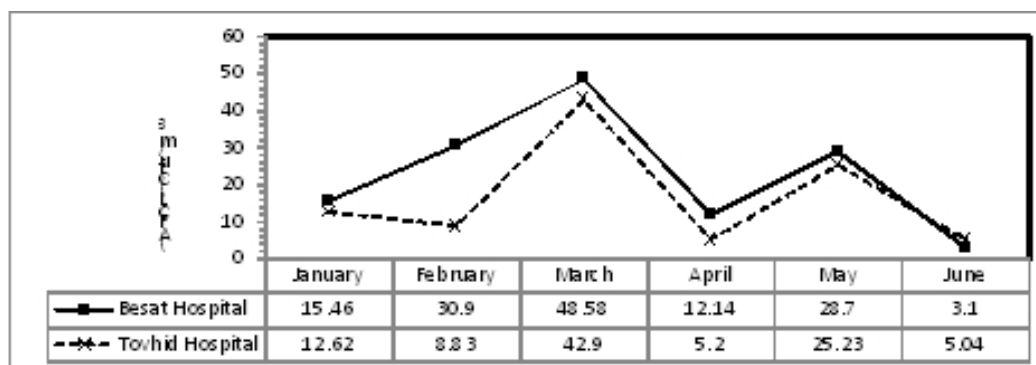


Fig. 4. Air fungal contamination load (AFCL) in two hospitals during the study

The main limitation of this study was its cross-sectional nature (although followed in 6 months), small number of hospitals ($n = 2$), and relatively low number of samples ($n = 112$) due to the time constraint and large AFCL differences between the wards, leading to lesser difference between the mean and standard deviation.

Our results are useful not only for determining the level of AFCL in hospital

wards, but also for identifying the factors that may influence the airborne concentrations of these agents and the importance of continuous monitoring. According to our results, a number of engineering controls (utilizing highly efficient air purification systems), administrative actions, and regulatory monitoring of the biological agents could appropriately be taken to reduce exposure to microorganisms in hospital wards.

Conclusion

In conclusion, compared to similar studies, the level of fungal contamination in the two hospitals of Sanandaj, were nearly desirable. In wards such as Oncology, gynecological surgery and operating rooms, further action is required to control the environmental conditions and fungal infections. Thus, utilizing highly efficient air purification systems and regular monitoring of the biological risk factors for both patient and health care employees is recommended.

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Authors' Contributions

Jamshid Khoubi, Delnia Ahmadnezhad, Shadi Kohzadi, Khorroosh Javan, and Ashkan Faridi were responsible for the study concept and design and acquisition of data. Jamshid Khoubi and Farzad Aala drafted the manuscript and make essential revisions to the article for important intellectual content and English editing. Jamshid Khoubi and Mozhdeh Amiri were responsible for the analysis and interpretation of data as well as statistical analysis. Farzad Aala and Jamshid Khoubi contributed to the administrative, technical, and material support. All authors have given approval to the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interests.

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