

Influence of high-efficiency particulate air filtration on indoor air fungal contamination in a hospital in Mashhad, Iran

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ABSTRACT

Introduction: Poor hospital Indoor Air Quality (IAQ) may result in various occupational hazards, hospital-acquired infections, and sick hospital syndrome. Air-control measures are vital to reduce airborne biological particle dissemination in hospitals. This study aimed to evaluate the effectiveness of High-Efficiency Particulate Air (HEPA) filters in decreasing indoor fungal pollution in an organ transplantation hospital in Mashhad.

Materials and methods: In this work, 96 specimens were collected from the air of three operating rooms and the Intensive Care Unit (ICU) ward. Sampling was performed using National Institute for Occupational Safety and Health (NIOSH-0800) instructions in two stages before and after using HEPA filters. Fungal density was reported based on the number of colonies per m³ (CFU/m³).

Results: According to the results before using HEPA filters, the colony frequency of *Aspergillus* was 50%, which was the highest among the detected fungi. *Penicillium* with a frequency of 23% was followed by *Aspergillus*. After using HEPA filters, the frequency of *Aspergillus* and *Penicillium* decreased by 40% and 6% to 10% and 17%, respectively. The mean concentrations of fungi in all three operating rooms and ICU before use and after using HEPA filters were 9.52 and 3.11 (CFU/m³), respectively indicating a reduction of about 67%, which is statistically significant ($P \leq 0.005$).

Conclusion: Hence, using these filters is recommended considering the good performance and high efficiency of HEPA filters in reducing fungal contamination and its consequences.

Introduction

Indoor Air Quality (IAQ) is a vital factor in indoor space [1] since the inappropriate IAQ may result in different occupational risks, infections, and hospital syndrome [2]. Density of bioaerosols such as bacteria, viruses, fungi,

and pollens is a key factor affecting on IAQ [3]. Airborne bioaerosols exposure is the main issue for medical centers and hospitals for reducing inpatient morbidity. There are numerous reports on higher concentrations of different bioaerosols in hospitals [4, 5]. Bioaerosols have been related to adverse health effects

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such as infectious disease transmission, acute toxic effects, as well as respiratory symptoms of allergy and asthma [6]. The existence of bioaerosols in healthcare settings is a major main of nosocomial infections, mainly for patients with immune deficiencies [7]. Within various kinds of bioaerosols, fungi have a key role in human health [8], particularly in hospital environments, in which patients are susceptible to these infections [9]. Former studies revealed that fungi like *Candida albicans*, *Aspergillus* species, *Cladosporium*, *Fusarium*, *Penicillium*, and *Mucorales* promote the hospital infections [10, 11]. Bioaerosol pollution in ORs was assessed by researchers in France. They found *Cladosporium* spp., *Aspergillus fumigatus*, *Penicillium* spp., and *Aspergillus* spp. as the leading species [12]. Microbe levels were evaluated in another study in various parts of a hospital revealing the fungal concentrations range of 0 to 7.33 CFU/m³ in ORs [13]. Concentrations of bacteria and fungi in a hospital in Taiwan was within the range of 0-319 and 1-423 CFU/m³, respectively [14]. Furthermore, fungal bioaerosols can cause colonizing syndromes like allergic bronchopulmonary aspergillosis and nosocomial aspergillosis [11, 13]. The existence of fungi in hospital wards, mainly ORs, is related to several factors including patients' activities, existence, and conditions, temperature, insufficient ventilation, humidity, poor air-conditioning systems, Organic Matter (OM) available in walls materials, kinds of surgery, the season, and insufficient disinfection [2, 10, 12]. Nosocomial infections lead to considerable economic and health problems [5, 16]. Generally, it is approximated that at least 1.4 million people suffer from such afflictions at any considered time [17]. High-Efficiency Particulate Air (HEPA) filtration systems present an effective tool to obtain higher air quality for at-risk patients' with this infection [18]. This study aimed to study the efficiency of HEPA filters in reducing fungal pollution in the air inside an organ transplantation hospital in Mashhad.

Material and methods

Air sampling

The present work was performed in the organ transplantation hospital in Mashhad on 96 specimens from 3 Operating Rooms (ORs) and one ICU ward in two stages. In this study, HEPA filters are installed in the inlet air path and ceiling. Also, air enters through the HEPA filter and exits the bottom of the room. The airflow in the ORs is laminar and from top to bottom. The first step was performed before using HEPA filters and the second phase was after it. Air specimens were gathered from the ICU ward and ORs once a week for six months. Table 1 represents the number of samples in each stage. The sampling was conducted using an activate air sampling technique in terms of the national institute for NIOSH-0800 instruction utilizing an Andersen bio-sampler and a Quick Take SKC sampling pump, with a flow of 28.3 L/2 min [19, 20]. By sterilizing bio-sampler before sampling, any interference of germs or other pollutions were avoided utilizing disposable sterile gas as well as 70% ethanol. This was a device catalog and then located under a UV lamp for 20 min. After sterilizing, bio-sampler was put within a sterilized cold box to avoid pollution until reaching the sampling setting. The box was then opened in the sampling location. A 90-mm plate comprising medium was put into the bio-sampler over sampling. The sampling circuit was made at a distance of 120-150 cm from the respiratory tract of the patient and any other obstacle as well as 100-150 cm from the floor [21, 22].

Identifying the fungi

In this work, plates comprising chloramphenicol antibiotic and Saboro dextrose medium were utilized for sampling. The plates were moved to the laboratory via cold boxes and incubation was performed for 7 to 10 days at 32°C. To early differential diagnose of fungal genera, macroscopic examination (color, colonies morphology, texture, colony apparent

diameter and shape) and microscopic mycology experiments were used (existence of mycelium, shape, and Conidia structure, presence of specific productive structures) [23]. The slide culture technique was also utilized since a more accurate diagnosis is required for fungal agents. Sampling was conducted in air pressure circumstances and standard temperature. Thus, based on the number of cultured colonies and the volume of sampled air, the fungal density of the indoor air of various sections of the hospital was determined in m^3 of air and it was reported via Colony Forming Unite (CFU/ m^3) according to the Eq. 1:

$$(CFU/m^3) = (Number\ of\ colonies/VS) \times 1000 \quad (1)$$

Where VS is the volume in liters passed through the bio-aerosol sampler.

Statistical analysis

Data were assessed through SPSS16 and Microsoft Office Excel 2016. Next, they were analyzed using descriptive statistics, including

minimum, maximum, mean, and standard deviation. Kolmogorov-Smirnov test was performed to determine the normality of data distribution. Since the data distribution was non-normal, the Wilcoxon statistical test was used at a significance level of less than 0.05.

The noteworthy point is that the study hospital was equipped with air conditioning. An air conditioning system is a mechanical ventilation method with the main components of a heating system, cooling system, and air discharge. Hence, the four factors of temperature, humidity, speed, and air purity are simultaneously controlled. Therefore, since the humidity and temperature were the same in all ORs and the ICU, the environmental interference (i.e., temperature and humidity) are ignored.

Results and discussion

The colonies developed over the sample plates are represented in Fig. 1.

Table 1. The number of samples in each phase

Status of using HEPA filters	Number of wards		Number of months sampled		Number of samples per month		Total number of samples in each phase		Total number of samples in the study
	OT*	ICU	OT	ICU	OT	ICU	OT	ICU	
Before	3	1	3	3	4	4	3*3*4=36	1*3*4=12	36+12+36+12=96
After	3	1	3	3	4	4	3*3*4=36	1*3*4=12	

OT: Operating Theatre

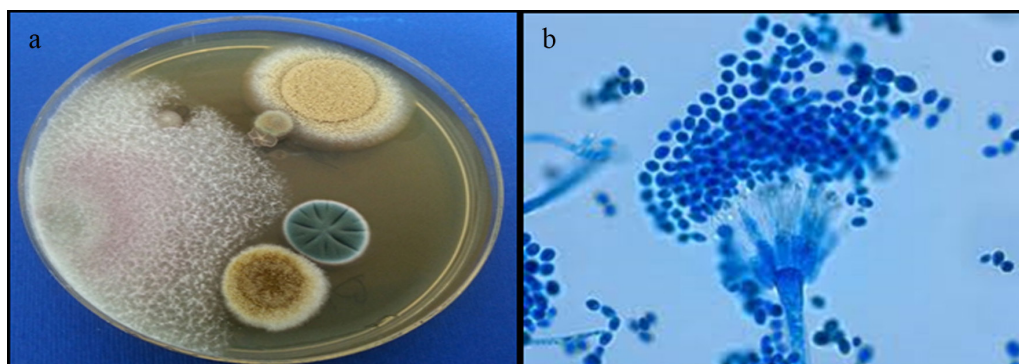


Fig. 1. Colonies developed on the sample plates; a) Macroscopic image b) Microscopic image

Table 2. The average concentrations of fungi in various sampling locations before and after using HEPA filters

Sampling place	Fungi concentration (CFU/m ³), Mean (\pm SD)		p
	Before	After	
Operating theatre 1	9.69 (3.25)	1.38 (3.23)	≤ 0.003
Operating theatre 2	8.3 (0.00)	2.67 (4.08)	≤ 0.005
Operating theatre 3	8.99 (2.44)	3.45 (4.27)	≤ 0.01
ICU	11.09 (4.12)	4.84 (4.2)	≤ 0.01
Total	9.52 (2.98)	3.11 (4.06)	≤ 0.001

SD = Standard deviation

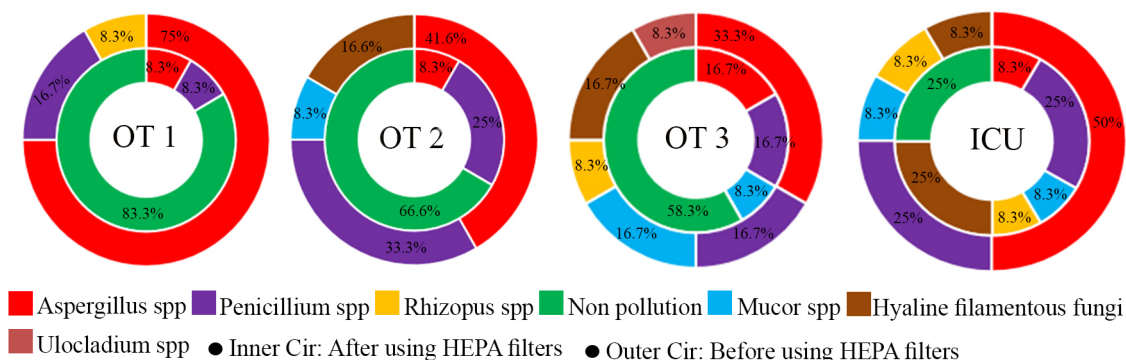


Fig. 2. The frequency of fungi identified in different sampling locations

Table 2 represents the concentrations of fungi in different sampling locations in two stages before and after using HEPA filters.

According to Table 2, the average concentration of fungi in OR 1 was 9.69 before using HEPA filters, which declined to 1.38 after using HEPA filters. This reduction is statistically significant ($P \leq 0.003$). In OR 2, the average concentration of fungi declined after using HEPA filters compared to before ($P \leq 0.005$). As can be seen, the average concentration of fungi in OR 3 was

8.99 and 3.45 before and after using HEPA filters, respectively. This result represents a reduction of about 5.5, which is statistically significant. In the ICU, the average fungi concentration was 11.09 before using the filters, which reached 4.84 with a reduction of about 60% after using the filters. In general, the average concentrations of fungi in all three ORs and ICU before and after using HEPA filters were 9.52 and 3.11, respectively. These values represent a reduction of about 67%, which is statistically significant

($P \leq 0.005$). The frequency of fungi detected at each sampling site before and after using HEPA filters is shown in Fig. 2.

As seen in Fig. 2, in OR 1, the frequency of *Aspergillus* before using the filter was 75%, which declined by 8.3% (from 67%) after using the filter. The frequency of *Penicillium* decreased by 8% before using the filter compared to after using the filter. The frequency of *Rhizopus* before using the filter was 8.3%, which reached 0 after using the filters. This figure shows a growth rate of about 83% in the frequency of non-contaminated samples after using the filters. In OR 2, the largest reduction was related to *Aspergillus*, for which the frequency was 41% before and 8% after using the filter. This result shows a 33% reduction. In this room, the frequency of Hyaline filamentous fungi and *Mucor* spp. before using the filter was 16 and 8%, respectively, which decreased to 0 after using the filter. The frequency of *Penicillium* decreased by about 8% (from 33% to 25%) before using the filter compared to after using the filter. Moreover, the frequency of non-

contaminated samples reached 66% after using the filter.

In OR 3, the frequencies of *Aspergillus*, *Rhizopus*, *Mucor* spp., Hyaline filamentous fungi, and *Ulocladium* spp before using the filter were 33.3%, 8.3%, 16.7%, 16.7%, and 8.3%, respectively. After using the filter, these values were 16.7%, 0%, 8.3%, 0%, and 0%, respectively. As can be noticed, the frequency of non-polluted samples was 58% after using the filter.

In the ICU, the frequency of *Aspergillus* before and after using the filter was 50% and 8%, respectively, with a 42% reduction. The frequency of *Penicillium*, *Rhizopus*, and *Mucor* spp was not changed before and after using the filter. It is observed that the frequency of Hyaline filamentous fungi before using the filter was 8%, which increased to 25% after using the filter. Also, the frequency of non-polluted samples increased to 25% after using the filter.

The frequency of fungal changes before and after using the HEPA filter in all four sampling locations in the hospital is represented in Fig. 3.

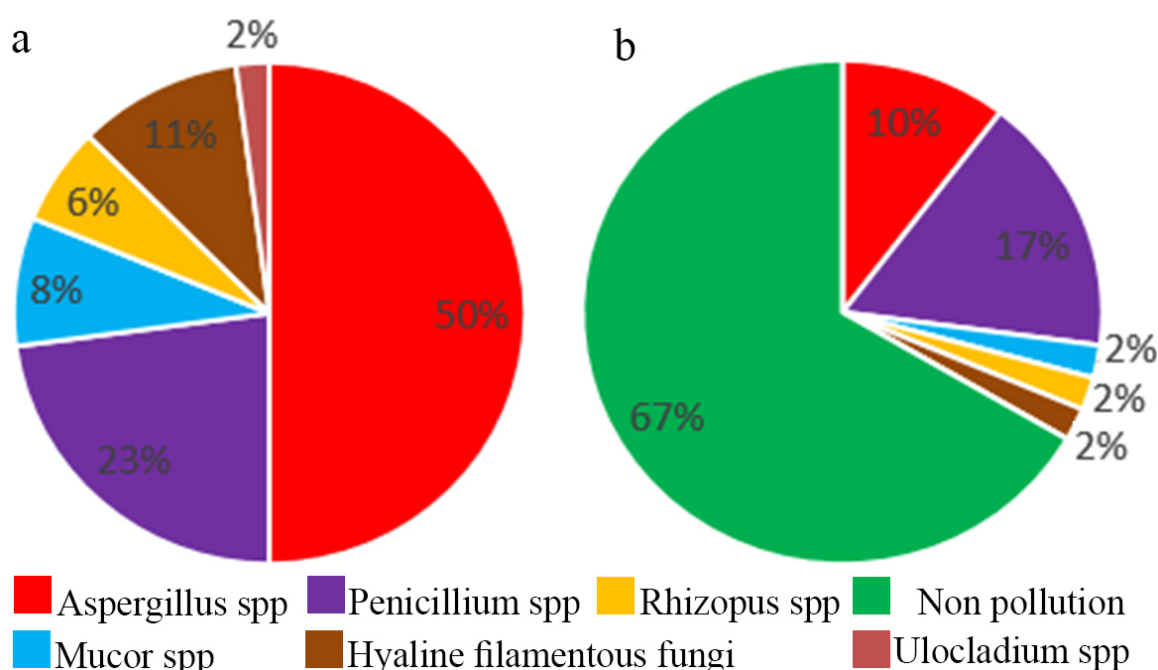


Fig. 3. The changes in the frequency of fungi a) before and b) after using HEPA filters in all four sampling

According to Fig. 3a, the colony frequency of *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, Hyaline filamentous fungi, and *Ulocladium* spp before using HEPA filter were 50%, 23%, 6%, 8%, 11%, and 2%, respectively. The highest frequency before using the HEPA filter was related to *Aspergillus*. The frequency of *Aspergillus* decreased by 40% after using the filter and reached 10%. This value was the highest frequency change before and after using the HEPA filter. In Fig. 3b, the highest frequency is related to *Penicillium*, which is 17%, followed by *Aspergillus*, with a frequency of 10%. Using the filter reduced the frequency of *Rhizopus*, *Mucor*, and Hyaline filamentous fungi by 4%, 6%, and 9%, respectively. Moreover, the frequency of *Ulocladium* spp was 0 after applying the filter. Finally, the frequency of non-contaminated samples after using HEPA filters increased by 67% compared to before. A comparison was made between our results and the guideline values (Fig.4) using Good Manufacturing Practices (GMP). It is an instruction of air cleaning tools for medical device manufacturing. It represents a total aerobic count limit of less than 1 CFU/m³ in class A rooms (very clean), less than 10 CFU/m³ in class B rooms (clean), less than 100 CFU/m³ in class C rooms (medium), and 200 CFU/m³ in class D rooms (contaminated) [24].

According to Fig. 4a, before using HEPA filters, the concentration of fungi in all three ORs is less than 10 CFU/m³, and they are in the clean group. At this stage, the ICU has a fungal concentration of more than 10 CFU/m³, which is not considered in the clean classification based on the standard of the European Code. As shown in Fig. 4b, the average concentrations of fungi in the ORs and ICU decreased after using HEPA filters. The average concentration of fungi in the ICU after using HEPA filters reached 4.84 CFU/m³, which is considered in the clean classification based on the standard of European regulations.

Many researchers isolated *Penicillium* sp., *Mucor* sp., *Aspergillus* sp., and *Candida* sp., *Verticillium* sp. with *Aspergillus* sp. and *Penicillium* from the University of Benin Teaching Hospital. Studying the environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece they found that *Aspergillus fumigatus* and *Aspergillus flavus* were the most prevalent species [25]. *Aspergillus* may be dangerous for high-risk patients although it may be tolerable for healthy people. The airways are invaded by spores readily and could result in aspergillosis in immuno-compromised hosts [26, 27]. In former studies, the existence of *Aspergillus* species in the indoor air of hospitals was regarded as a risk factor for patients as a result of causing allergies and nosocomial infections [24, 28].

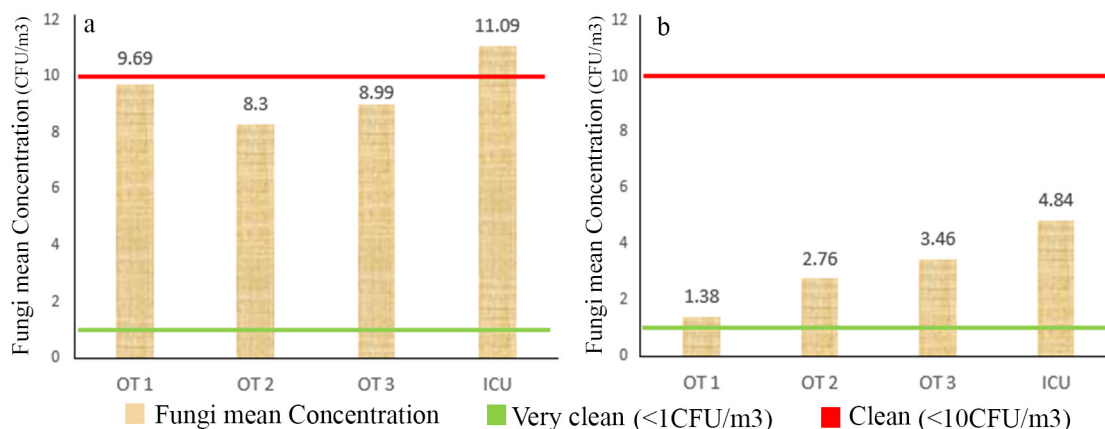


Fig. 4. The Comparison of mean concentrations of fungal colonies isolated in operating wards and ICU a) before and b) after using HEPA filter with standard values

Nonetheless, other fungi were isolated from the air in the ward (*A. flavus* complex, *A. niger* complex, *Rhizopus nigricans*, *Penicillium* spp.), which can also impose respiratory risks as potential sources of toxins and allergens [29, 30]. In a study for measuring the bioaerosols in operating rooms and the ICU of a public hospital in Venezuela, the hospital microbial contamination load was reported within 1 CFU/m³ to 222 CFU/m³. Comparing results with the standards, it was found that in terms of fungal contamination load the air quality in operating rooms was at a clean level (10-100 CFU/m³) and within the acceptable range in the ICU (100-300 CFU/m³). In this study, 12 types and 15 species of fungi were identified, among which, the highest rate of infection was caused by *Penicillium* and *Aspergillus* [31]. In the study of fungi isolated from the air of wards of a hospital, the most common fungi identified from air samples were *Aspergillus flavus*, *Fusarium*, *Penicillium*, *Alternaria*, *Candida*, and *Albicans* [32]. Our findings are supported by all these studies indicating the persistence and prevalence of some fungal species in the hospital environments. *Aspergillus fumigatus* is a well-known hazardous agent possibly causing pulmonary aspergillosis, allergic alveolitis, mycotoxicoses, and asthma [29]. Systemic infection *A. fumigatus* was reported by many researchers [33] in patients 11 days followed by liver transplantation in France. Moreover, it was indicated that lung aspergillosis resultant from the same fungal species was found in two patients at an intensive care unit. *Penicillium* species were occasionally reported to cause pulmonary infection, human penicillosis, and fungemia [34, 35].

In a study, the effects of HEPA filters were investigated to prevent infections caused by *Aspergillus* in the care of immunocompromised patients with blood malignancies. It was found that the installation of HEPA filters has an effective role in controlling and reducing *Aspergillus* *Candida*-caused infections [36].

The results of another study revealed that fungal air pollution in all wards was higher than 100 CFU/m³. Less fungal contamination was found in the wards with a positive air flow HEPA filter [37]. Comparing the results of these studies with our findings confirms the effective role of HEPA filters in reducing the fungal air pollution load.

Conclusion

The results of the present study indicate that after using HEPA filters, the indoor fungal contamination of the operating rooms and the ICU was effectively reduced. Moreover, *Aspergillus*, the main species in the pre-intervention stage changed to *Penicillium* in the post-intervention stage. These results represent the importance of HEPA filters in reducing fungal infection and eliminating dangerous fungal species such as *Aspergillus*. The study hospital is for patients with immune system defects who receive organs. Moreover, the least amount of fungal infection can have irreparable consequences in treating the disease and even lead to the death of patients. Thus, to prevent the risk of transmission of infection caused by airborne bioaerosols and, consequently, to repel the transplanted organ, it is essential to control the indoor air as much as possible and make some modifications in the hospital ventilation system.

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Competing interests

The authors have no competing interests to declare.

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Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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