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Assessment of airborne bacterial and fungal communities in different wards of educational hospitals: A case study in Urmia, Iran

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ABSTRACT

Introduction: Bioaerosols consist of aerosols which are biologically originated and can be present ubiquitously in different environments, including the indoor air of hospitals. The objective of this study was to survey the bioaerosol type and density in various environments of four governmental educational hospitals in Urmia, Iran, namely the intensive care unit (ICU), operating room, the internal medicine room, the infectious diseases room, the infectious diseases corridor, and ambient air.

Materials and methods: Sampling was performed during summer and winter of 2019 at four different day-times using passive (sedimentation plate) and active methods (an Andersen one-stage viable impactor and Quick Take-30 sampling instrument) and by counting plates containing a bacterial and fungus-selective medium.

Results: The results revealed that the highest microbial bioaerosol load was related to the infectious diseases corridor (100 and 150 CFU/m^3 for total bacterial and fungal load, respectively). The highest bacterial and fungal density was observed in the afternoon at 17-18; and the concentration of bioaerosols was higher in summer than winter. A comparison of indoor and outdoor bacterial loads showed that the indoor bacterial concentration mean (49.1±23.8 CFU/m^3) was higher than the outdoor value (47.1±21.5 CFU/m^3), and the indoor levels of fungal contamination (83.3±31.9 CFU/m^3) were significantly lower than outdoor values (182.5±48.0 CFU/m^3). The predominantly isolated bacteria were *Staphylococcus* (95%) spp, and the main isolated fungi belong to the genera *Aspergillus* (50%) and *Penicillium* (32%).

Conclusion: The results of this study can be useful in developing indoor air microbial quality guidelines in hospitals, which has not been done so far.

Introduction

Indoor air quality is one of the most significant factors affecting the health of people, because people spend at least 80% of their time in various indoor spaces. Exposure to indoor particulate matter (PMs) is one of the most significant environmental risks [1]. Bioaerosols are a colloidal particulate with an aerodynamic diameter of 0.01-100 µm and include bacteria, fungus vie ruses, and plant pollens [2]. The amount and variation of bioaerosols can be affected by various factors such as season, weather conditions, tem-

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perature, relative humidity, indoor environment conditions, outdoor microbial load, human density and activities, construction materials, indoor air exchange rate, and the ventilation system [3].
Exposure to airborne microorganisms can be hazardous and cause several health problems such as infections, toxicities, and inflammatory diseases, especially in hospitals where they greatly increase morbidity from different nosocomial diseases [4, 5]. Also, exposure to immunogenic substances or endotoxin (derived from non-viable bacterial remnants) can cause some allergic reactions and pulmonary irritation [4].
The amount of airborne microorganisms in hospitals air can vary not only due to the abovementioned parameters, but also because of various indoor hospital and outdoor environmental sources, i.e. contamination of air ducts; the number of occupants, patients, and visitors; the type of the wards; human activities; flowers brought in by visitors from outdoor environments; an air conditioning and ventilating system (HVAC) without regular replacement; and contamination of the indoor structures because of the age of the hospital [6, 7]. The main airborne pathogen microorganisms known as a potential source to increase hospital-associated infections are Gram-negative bacilli, *Aspergillus flavus*, *Neisseria meningitidis, Staphylococcus aureus, Streptococcus pneumonia, Serratia marcescens, Streptococcus pyogenes, Mycobacterium tuberculosis,* and *Corynebacterium diphtheriae* [4, 7].
Many studies have highlighted the bioaerosols diversity and effects in hospitals and healthcare centers. Some researchers reported that Gram-positive coccus is the dominant bacterial genera (about 88%), followed by *Staphylococcus* (51%) and micrococcus; also, *Penicillium* (41%) and *Aspergillus* (24%) have been determined as the dominant fungus genera in the indoor air of different wards [6]. In a study, it was showed that occupant density is a key factor influencing the level of airborne bacteria in the indoor environment, and humidity is an important factor affecting bioaerosols' diversity within the hospital wards [8]. Other researchers also reported that season is a key factor affecting bioaerosols' diversity in the indoor air of hospitals, and bioaerosol counts during summer are significantly greater than winter in all the wards. They also concluded that other factors such as temperature, humidity, and airflow influence microorganism variety [8].
With respect to the complex hospital environment, it is required to pay special attention to bioaerosols' biodiversity to ensure a healthy indoor air quality to protect healthcare workers and patients from nosocomial infections and occupational diseases [6]. Also, the investigation of airborne microorganism's general profile distributed in different wards could be useful for understanding the nosocomial and opportunistic infections' transmission and proposing preventive alternatives to restrain the spread of nosocomial infectious diseases [7].
Because of the very different conditions of the indoor and outdoor environments of any hospital and healthcare unit worldwide, it is not reasonable to apply foreign data directly to other hospitals. Thus, the present study aimed to characterize the distribution characteristics of the levels of airborne bacteria and fungi at four general governmental hospitals in Urmia, Iran, and to evaluate potential airborne contamination sources. The resulting information can contribute to the development of recommendations for guidelines with the aim of facilitating the control and management of hospital indoor air quality.

Materials and methods

Subjects and hospital environments

This study was conducted from February to September 2019. Four general governmental hospitals located in Urmia, namely Motahhari (MOT), Taleghani (TAL), Imam Khomeini (IKH), and Seyyed-al-Shohada (SAS), which can accommodate 150–550 patients were selected since they were deemed sufficient for representing the large scale of general hospitals. More detailed information related to these hospitals is given in Table 1.
The study sites and sampling locations were the

Table 1. General characteristics of the studied general hospitals

General hospitals	IKH	MOT	SAS	TAL
Building age (year)	24	91	10	39
Area (m^2)	30000	16000	10000	17500
Total number of beds	635	400	153	450
The number of active beds	523	205	153	226
The number of staffs	1500	750	440	700
The number of patients	1500	850	600	780
Cleaning times of the day	17-19			
Visiting times of the day	14:30-16:30	14:30-16:30	14:30-16:30	15-17
Ventilating and Air Conditioning system	Natural Ventilation and Mechanical Air Conditioner			
Type and usage of around environment	Street, green zone and residential areas	Street and residential areas	Street and residential areas	Street, river and green zone

internal medicine and infectious diseases ward and lobby, intensive care unit (ICU), main lobby, operating room, and internal medicine room, and outdoor ambient air. Each hospital was visited 2-3 times during summer and winter. As visits were allowed on all weekdays, all the samples were taken in seven sites per hospital on visiting days. Air sampling was performed at four times (at 7-8, 12-13, 17-18, and 23-24). At each sampling stage from each hospital, one sample from the outdoor air or the air inlet of the hospital building was obtained, by taking into account a 20m distance between the main entrance, and sampling was performed in non-rainy weather conditions in order to compare the measurement results inside the hospital with the outdoor results [9-11].

In total, 224 air samples were obtained during the study period and their average concentration was used in order to determine the quality of the hospitals and different wards. Table 2 presents the number and percentage of sampling from each site in all hospitals.

Table 2. Number and percentage of sampling from each ward

Part	Frequency	Percent
ICU	32	14.3
Operating room	48	21.3
Internal medicine room	32	14.3
Internal medicine corridor	32	14.3
Ambient air	32	14.3
Infectious diseases corridor	24	10.7
Infectious diseases room	24	10.7
Total	**224**	**100**

Sampling strategy and method

Passive sampling was implemented for bioaerosol sampling during summer, and active sampling was used during winter. The passive sampling was performed with a 1,1,1 standard pattern. The sampling plates were placed a minimum 1 m away from the wall and 1 m above the floor, and remained 1 h exposed to the ambient air.

Blood Agar and eosin methylene blue (EMB) were used for bacterial bioaerosol samples, and Sabouraud Dextrose with Chloramphenicol (SDAC) was applied for fungus bioaerosol samples [12, 13]. The microbial and fungal counts were expressed in terms of colony-forming units (CFU) per unit volume of air (m^3).

In passive sampling, Kouch's method was adopted for the calculation of the number of cultured colonies per cubic meters of air (CFU/m^3) as Eq. 1 [14]:

$$\frac{CFU}{m^3} = \frac{a.10000}{p.t.0.2} \quad (1)$$

where

a = Number of observed colonies on a plate

p = Plate surface (cm^2)

t = Plates' contact time (minutes)

Active sampling was performed in respiratory height (about 1.5 m) for 3-5 min to avoid the collection of unaccountable colonies, by using an Anderson single-stage cascade sampler (quick take-30 impactor, SKC, USA) at an airflow rate of 28.3 L/min [6] and a Biostage single-stage viable cascade impactor equipped with 100-mm-diameter Petri dishes. On sampling days, indoor air temperature and relative humidity were simultaneously measured using a digital PHB-318. Before each sampling, the inside of the sampler and the cap of the cascade were cleaned with a 70% ethanol solution to prevent cross-contamination [15].

The concentration of airborne bacteria and fungi (CFU/m^3) in the active method was calculated by dividing the value obtained from counting the colonies formed in the culture medium by the sampling air volume.

Incubation and identification of bacteria and fungi

After sampling, the culture media were immediately closed, carried to the laboratory, and were cultured in the incubator for 1–2 days at 35-37 ˚C for bacteria and for 5–7 days at 25–27 ˚C for fungi [16]. During the culturing period, the plates were investigated daily for bacterial and fungal growth. The genera of all the cultured airborne bacteria were identified according to the classification method of Bergey's manual.

Also, when suspect fungal colonies were detected, they were isolated with plates containing Sabouraud Dextrose with Chloramphenicol medium. The airborne fungal genera were identified using the classification method of Ainsworth (1976) by observing the microscopic and macroscopic form, shape, and color of the colony and spore [17]. The values of air bio-burden were presented in CFU/m^3 and the limit quantification for airborne bacteria and fungi was 1 CFU/m^3.

Data analysis

The results were analyzed in SPSS (version 23) with $p_{value} < 0.05$. T-test, independent t-test, and analysis of variance (F) were performed to assess the concentration differences of airborne bacteria and fungi among the sites.

Results and discussion

Variation of the bioaerosols in different hospitals

As mentioned previously, bioaerosol samples were taken in February (winter) and September (summer) in seven environments of four hospitals to characterize airborne microbial concentrations and to assess the contamination from outside sources and potential seasonality effect. The results revealed that IKH had the highest

microbial bioaerosol load, ranging from 24.8 to 99.5 CFU/m^3 for total bacterial aerobic counts, while MOT had the highest airborne fungal load, ranging from 16.5 to 149.2 CFU/m^3 (see Fig. 1 and Table 4).

To the best of our knowledge, there is no defined national legislation for indoor bacterial and fungal concentrations. Usually, standards and guidelines have been set for the hospital indoor airborne bioaerosol levels to protect high-risk, sensitive, or fragile-immunity populations, such as children, the elderly, and pregnant women, against exposure to airborne microorganisms. Despite environmental guidelines/criteria for bioaerosols in working and residential indoor environments proposed by several researchers, no uniform international standard has been set to date about the allowable levels bioaerosol loads. This is due to the variations in the human body's reaction to exposure, the complexity of microorganisms' composition, and difficulties in gathering bioaerosol that can be hazardous during sampling; however, some countries have developed national and local standards for this purpose.

The guidelines/standards for bioaerosols that have been suggested by different private organizations and countries are summarized in Table 3. To prevent the health risks of bioaerosols, the World Health Organization (WHO) suggested that the total amount of bioaerosols should not exceed 1000 CFU/ m^3 in indoor environments. If the bioaerosols' load is higher than this, the studied environment is considered as a polluted environment [18]. Some authors have proposed that 750 CFU/m^3 and 300 CFU/m^3 should be respectively the limits for bacteria and fungi [7]. The Europe Commission presented hygiene standards for non-industrial buildings in 1993, in which a pollution load >500 cfu/m^3 has been declared to be a high level for bacteria and fungi [19].

Analysis of the airborne bacterial and fungal concentration in various wards of hospitals showed that the infectious diseases corridor has the highest bacterial aerobic count, ranging from 49.7 to 99.5 CFU/m^3, followed by internal medicine corridor and internal medicine room ranging from 49.7 to 99.5 CFU/m^3. Also, the results of the fungal load measurement demonstrated that the infectious diseases corridor has the highest fungal load, ranging from 82.9 to 149.2 CFU/m^3, followed by infectious diseases room, ranging from 78.7 to 136.7 CFU/m^3, and internal medicine room ranging from 91.1 to 128.4 CFU/m^3 (Fig. 1).

A comparison of bacterial and fungal density in the wards showed that the fungal density was the highest in the operating room of TAL, SAS, MOT, and IKH in that order, and there was no significant difference in the other wards. A low level of fungal airborne load in the operating room of TAL could be due to the location of this operating in a separate building. Therefore, the low density of personnel and patients and a good natural ventilation system in this area have reduced the microbial pollution load.

Due to the existence of a mechanical air conditioner in the IKH operating rooms, it was expected that the bacteria and fungal densities should be low in this hospital compared to the other hospitals with a natural ventilation system; but the findings showed that the microbial pollution load in IKH operating rooms is higher than the rest. This can be due to the lack of proper maintenance, routine cleaning, and control of air-conditioning functioning. This result is in agreement with findings reported by Alves Simoes et. al. in two university hospitals of Mato Grosso, Brazil. In this study, the efficiency of the installed ventilation systems for reducing fungal bioaerosols was investigated in the ICU. *Aspergillus* spp, *Penicillium* spp, and *Cladosporium* spp were detected in both hospitals, and the colony units' density was higher than the allowable limit [30]. As reported by other authors, the number of patients, occupants, and visitors, and human activities are the other important factors that could influence microbial growth in hospitals [31].

Table 3. Summary of quantitative guidelines and standards for bioaerosols in indoor air by different private and governmental organizations

Organization	Residential indoor air quality	Guidelines/standards/limitation				Notes	Reference
		Airborne bioaerosols (CFU/m^3)	Bacteria level (CFU/m^3)	Fungia level (CFU/m^3)	other		
The European database	Residential indoor air quality		5000	5000	bacterial endotoxin less than 5 ng/m^3	limit values	[20, 21]
WHO	Indoor environments	<1000				limit values	[19, 22]
ACGIH [a]	Work environments	<100				Low	[23]
		100-1000				Intermediate	
		>1000				High	
AIHAb	Work environments	There is no safe level of an uncontained pathogenic organism					[24]
CEC [c]	Residential indoor environments	<50				Very Low	[25]
		<200				Low	
		<1000				Intermediate	
		<10000				High	
		>10000				Very High	
IAQ [d]	Indoor environments	<300		Common fungi is OK		limit values	[19]
		<150		Mixed fungi other than pathogenic orexigenic is OK		limit values	
OSHAA [e]	Work environments	>1000		106 fungi/g of dust		Indicates contamination	[26]
EC [f]	Indoor environments			>50		One species should be investigated	[27]
				<150		OK if mixture of species	
				<500		OK if Cladosporium or other common phylloplane	
				Presence of the pathogenic and toxigenic fungi		Unacceptable in indoor air	
Ministry of environment, Republic of Korea	Indoor environments	<800				limit values	[19]
				<500		clean indoor air	[28]
suggested guidelines for passive sampling of bioaerosols in India	Hospital wards	<20				Low contaminated wards	[29]
		20-50				Intermediate contaminated wards	
		>50				High contaminated wards	
	Surgical wards		<10	<1		limit values	

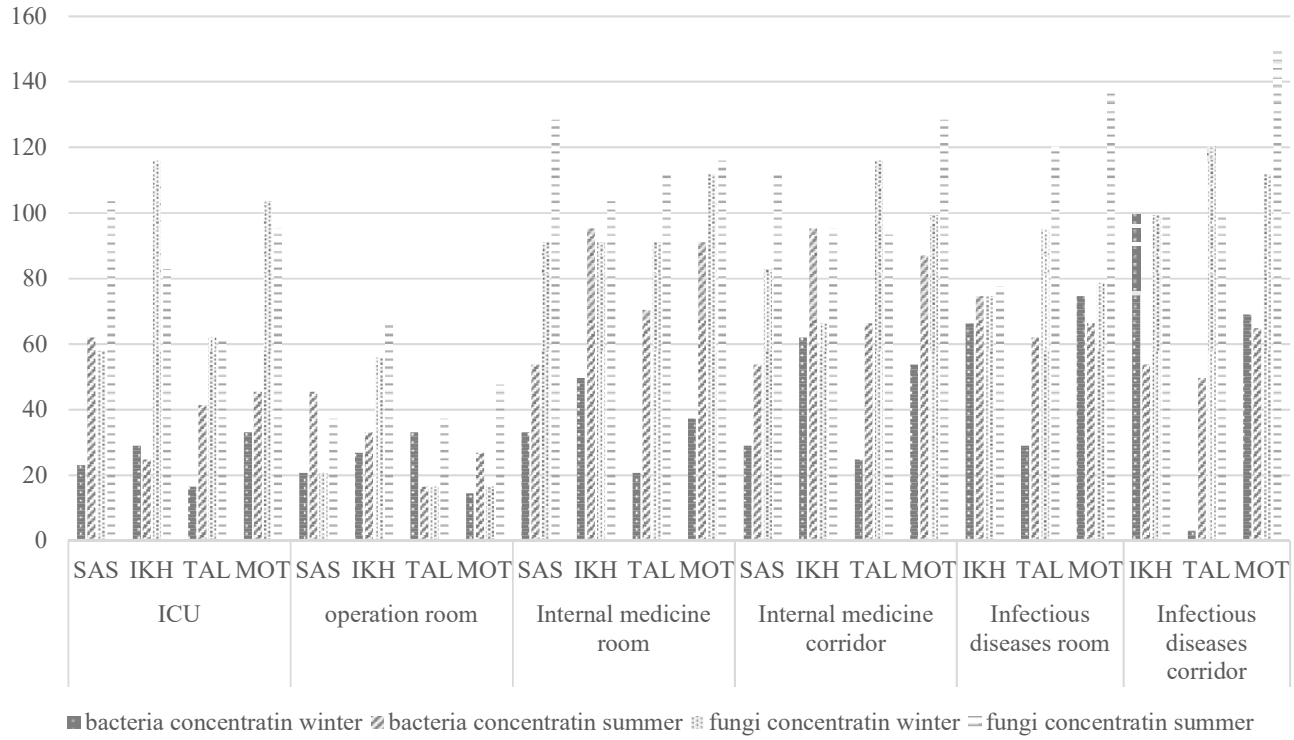

Fig. 1. The concentrations of bacteria and fungi in the air of the studied wards of different hospitals in two seasons, summer and winter

The results of the air quality in different wards of hospitals were evaluated based on the suggested limits for indoor environments and indoor work environments formulated by WHO and ACGIH, respectively. According to the WHO limitation, all the wards included in the study were had hygienic conditions. Also, with respect to the ACGIH classification, almost all the wards had a low contaminated condition, and very few wards belonged to an intermediate contaminated classification. Although all the wards were at their maximum capacity at the time of this study, and despite the high density of patients, the large number of visitors, and the presence of many health and medical sciences students in the wards, it was shown that the concentration of bioaerosols in all the wards was in a suitable condition.

Because of many differences in hospital buildings' age and area, as well as the number of wards, patients, staff, visitors, and ventilating and air conditioning systems, the findings of our study cannot be compared directly with the results from other studies. However, our findings are consistent with some studies and inconsistent with others. For example, the results of Yan Gilbert et. al. on the concentration of airborne bacteria in hospital rooms revealed that airborne bacterial concentration ranged from 14 to 74 CFU/m^3 and that of fungi ranged from 50 to 600 CFU/m^3 [32]. Investigation of the level of fungal contamination in Shariati Hospital rooms in Tehran, Iran, revealed the total mean concentration of detected fungi in the hospital rooms was 55 ± 56 CFU/m^3; the lowest mean counts (37±17 CFU/m^3) were observed in Nursing Stations, and the highest (21797± CFU/m^3) were reported in orthopedics operating room [15]. Also, a study on the level of airborne bacteria in five general hospitals located in Seoul, South Korea, revealed that the concentration of detected airborne bacteria ranged from 202 to 307 CFU/m^3. In some European hospitals, airborne bacteria counts have been found from <10 to >100 CFU/m^3.

Table 4. The summary of the mean density of bacterial and fungal bio-aerosols, indoor temperature, relative humidity, and the number of beds in the studied hospitals during summer and winter

Hospital's name	Studied location	Indoor Temperature ^{0}C	Indoor Relative humidity %	Bacterial density mean $\frac{cfu}{m^3}$		Fungal density mean $\frac{cfu}{m^3}$	
				winter	summer	winter	summer
MOT	ICU	23-25	35 -37	33.1	45.5	103.6	95.3
	Operating room	25-26	35 -36.9	14.5	26.9	16.5	47.6
	Internal medicine room	24-26	38-40	37.3	91.1	111.9	116.0
	Internal medicine corridor	24-26	38-40	53.8	87.0	99.4	128.4
	Infectious diseases room	25-27	40-45	74.6	66.3	78.7	136.7
	Infectious diseases corridor	25-27	40-45	69.0	64.9	111.9	149.2
	Ambient air	-	-	33.1	91.1	261.1	207.2
TAL	ICU	23-25	37-52	16.5	41.4	62.1	62.1
	Operating room	24-27	39-50	33.1	16.5	16.5	37.3
	Internal medicine room	23-25	31-55	20.7	70.4	91.1	111.8
	Internal medicine corridor	23-25	31-55	24.8	66.3	116.0	93.5
	Infectious diseases room	23-25	38-55	29.0	62.1	95.3	120.2
	Infectious diseases corridor	23-25	38-55	33.1	49.7	120.2	99.4
	Ambient air	-	-	37.3	62.1	190.6	186.5
IKH	ICU	22-25	35 -37	29.0	24.8	116.0	82.9
	Operating room	23-26	37-55	26.9	33.1	55.9	66.3
	Internal medicine room	23-26	39-45	49.7	95.3	91.1	103.6
	Internal medicine corridor	23-26	39-45	62.1	95.3	66.3	95.3
	Infectious diseases room	23-27	45-52	66.3	74.6	74.6	77.5
	Infectious diseases corridor	23-27	45-52	99.5	53.8	99.4	99.4
	Ambient air	-	-	41.4	41.4	215.5	165.7
SAS	ICU	23-25	40-52	23.1	62.1	58.0	103.6
	Operating room	24-26	45-54	20.7	45.5	20.7	37.3
	Internal medicine room	23-26	23-45	33.1	53.8	91.1	128.4
	Internal medicine corridor	23-26	23 -45	29.0	53.8	82.9	111.9
	Ambient air	-	-	20.7	49.7	111.9	128.4

The results of this study confirmed that there is a direct relationship between bacteria density and the number of hospital beds and temperature, and an inverse relationship between bacteria density and humidity. The results of a study from China showed no significant relationship between humidity and bacteria count in the hospital indoor air [33].

Based on the results of the present study, fungal density has a direct relationship with the number of hospital beds and an inverse relationship with the amount of humidity. The inverse relationship between relative humidity and fungal concentration can be due to the slight fluctuations in relative humidity in the sampling place (38-48%), which is 40-60% lower than the suggested standard [34].

Seasonal variations of bioaerosols

As mentioned previously, the density of bacterial

and fungal contamination was measured in different wards during summer and winter by using passive and active sampling methods, respectively. Based on Table 4 and Fig. 2, the concentration of bioaerosols was higher in summer than in winter.

These data are presented only for the investigation of bioaerosols' level in two seasons without any comparison. It is impossible to directly compare the results of studies which have used passive and active methods. The results of a study on sampling of bioaerosols using two passive and active methods revealed a significant difference in the type and number of bioaerosols collected by these two methods [28]. Some researchers explained that this difference could be due to the different mechanisms of bio-aerosol trapping in the two methods; in the passive sampling method, only those bioaerosols which have a sufficiently high a gravitational sedimentation rate to be deposited in the sampling plates are trapped. They also concluded that the passive sampling method may be suitable for the determination of relative bioaerosol contamination in hospitals [35].

Different studies have confirmed the effect of season on microbial airborne contamination in hospitals. For example, a study by Sandra Cabo Verde in a hospital ward of Setúbal, Portugal, found seasonal variations in total microbial loads, which were markedly higher in summer than in winter [6]. The results of Dong-Uk Park et al.'s study in six Korean hospitals revealed that airborne bacterial concentrations were significantly higher in summer than in either fall or winter [8]. A comparison of indoor and outdoor bacteria loads showed that bacterial concentration mean (49.1±23.8 CFU/m^3) was higher in indoor than outdoor air (47.1±21.5 CFU/m^3), but the difference was not significant ($p<0.05$). Also, a comparison of quantitative values of fungal concentrations found that indoor levels of fungal contamination (83.3±31.9 CFU/m^3) were significantly ($p<0.05$) less than outdoor levels (182.5±48.0 CFU/m^3), suggesting that the fungal contamination resulted from the concentration of fungi from outside to the indoor environment (Table 4). This highlights the inefficiency of hospital ventilation systems for reducing air microbial loads.

Variations of bioaerosols at different times of the day

Variation of microbial airborne contamination in different wards was investigated at different times of the day. The results revealed that midnight [23 – 24] has the lowest concentration of both bacteria and fungi, while the afternoon [17 – 18] had 61 and 120 CFU/m^3 of bacterial and fungal concentration which was the maximum value.

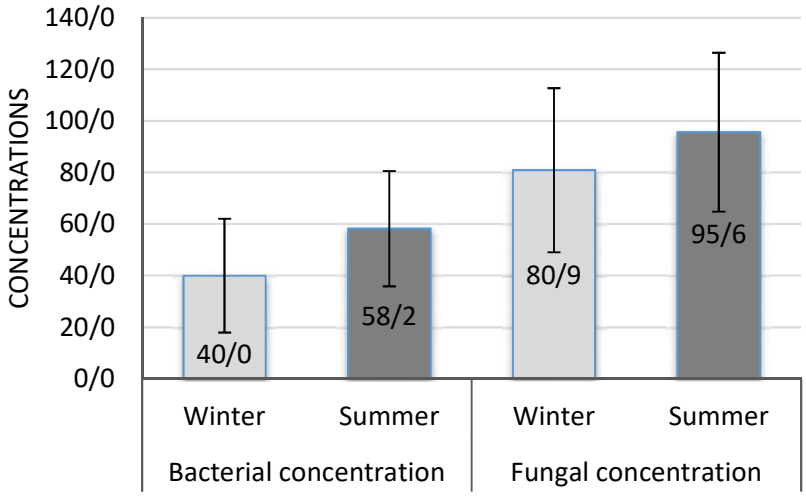

Fig. 2. The concentration of bacteria and fungi in the air of hospitals (mean±SD) during winter (gray) and summer (black)

The large number of visitors and the heavy personnel traffic in the afternoon [17 – 18] could be the main factor affecting bacterial and fungal concentration at this time. Also, reduced operation and/or cleaning activities together with a reduction of personnel and patient traffic may be the main cause of bioaerosol reduction at midnight. In a study it was demonstrated that there were significant differences in bacterial concentration in various sampling times (morning and afternoon) [31].

Variations of bioaerosol type and genera

Based on the results, the predominant isolated bacteria in summer were Gram-positive cocci such as *Staphylococcus* (95%), *Pseudomonas* (4%), and *Acinetobacter* (1%), whereas the main isolated fungi were *Aspergillus* (50%), *Penicillium* (32%), and *Candidae* (19%). Also, in an assessment of bacteria and fungi in the studied wards, the most commonly identified genera in the collected air samples were *Staphylococcus* (93%), *Pseudomonas* (6%), and *Acinetobacter* (1%) and *Aspergillus* (50%), *Penicillium* (35%), and *Candidae* (15%) in winter (Fig. 4).

Most previous studies focused on the detection of *Aspergillus* genera in different wards' air microbiota due to its effect on nosocomial infections [6, 36, 37]. For example, Dehghani's et al. examined the bioaerosols' type and density and reported that the main fungus genera inside the operating room are *Aspergillus fumigatus* (18.3%), *Aspergillus flavus* (18.5%), *Aspergillus niger* (5.8%), and *Penicillium* (3.3%). The bacterial concentration in 41% of the samples was higher than the suggested levels [38].

Another study on fungal genera of the indoor air of hospital wards revealed that the dominant fungal flora belonged to the *A. fumigatus* complex, corresponding to ~80% of the total isolates. Nevertheless, other fungi that can pose respiratory risks as potential sources of allergens and toxins were isolated from the indoor air in different wards (*Aspergillus flavus* complex, *Aspergillus niger* complex, *Rhizopus nigricans*, etc.) [39]. The study by Sandra Cabo et al. indicated that the frequency of Gram-positive coccus was 88%, and it contained 51% *Staphylococcus* and 37% micrococcus; also, the frequency of fungi included 41% *Penicillium* and 24% *Aspergillus*. They stated that the reason for the frequency of cocci was the existence of dust and improper hospital cleaning [6].

Finally, a survey of fungal load in a Spanish hospital revealed the five most frequent groups of airborne fungi to be *Cladosporium*, *Penicillium*, yeasts, *Aspergillus*, and *Alternaria* [40]. As it was observed, the abovementioned studies support our results and show the spread and persistence of some airborne fungal flora in the indoor air of hospitals.

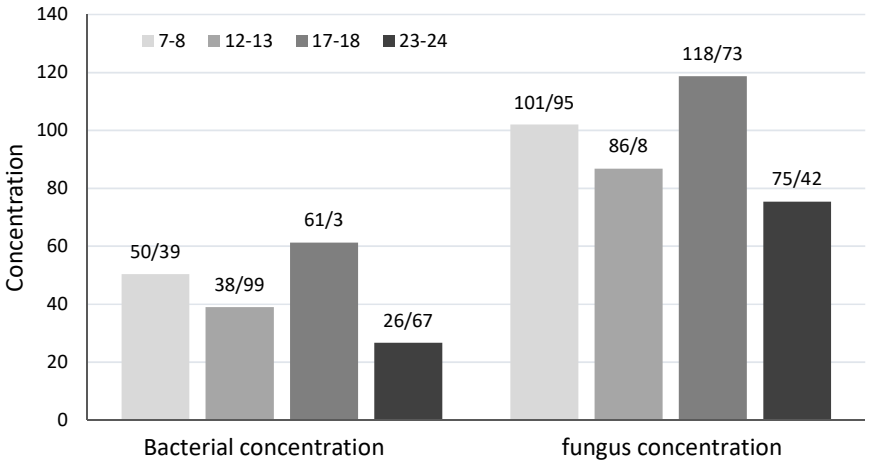

Fig. 3. The concentration of bacteria and fungi in the air of hospitals at different times

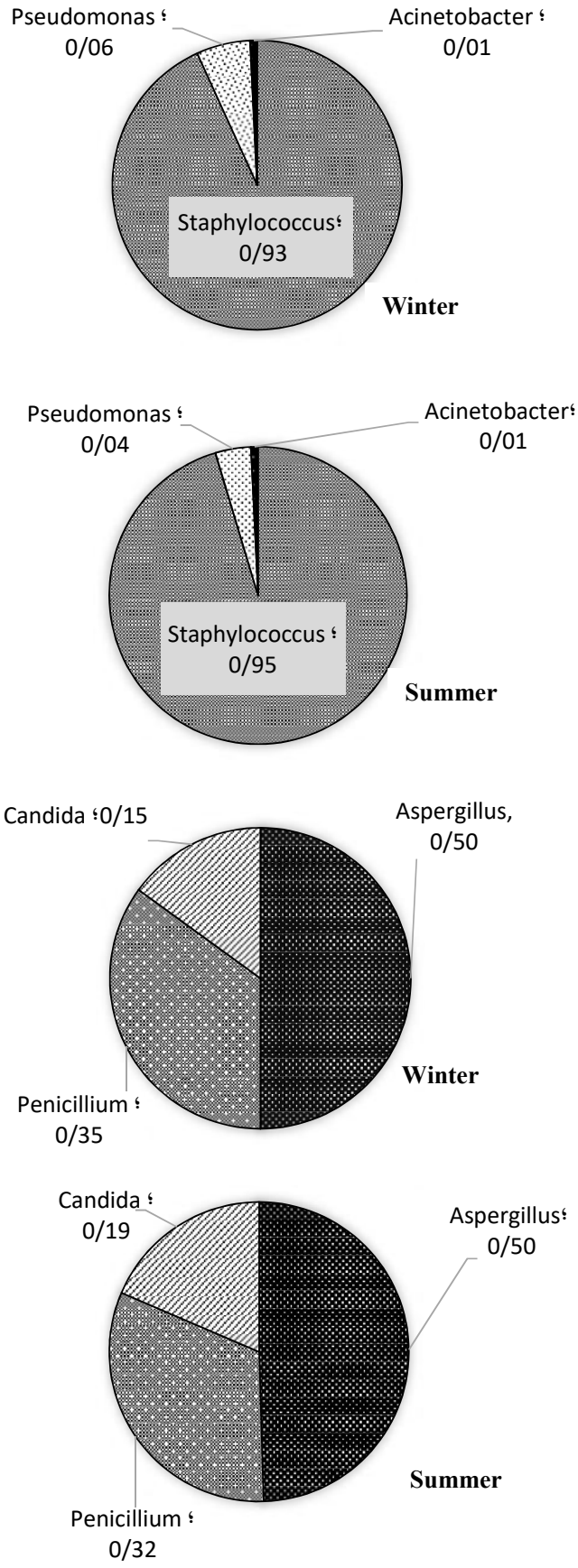

Fig. 4. The percentage of bacterial and fungal spices detected in summer and winter

Conclusion

Bioaerosol characteristics were evaluated in different wards of four hospitals in Urmia. Our results showed that *Staphylococcus* and *Aspergillus* are respectively the most frequently occurring bacteria and fungi in the studied wards.

A better understanding of parameters which affect the load of airborne microorganisms in hospital wards could be effective for setting control strategies and reducing the exposure risk of healthcare workers and patient. To control the verified specific factors, the number of occupants, the functioning of ventilation/filtration systems, the number of hospitalized patients, pollution sources, etc. must receive attention. Proper design and implementation of ventilation systems were performed in the studied hospitals that significantly decreased pollution. Regular and uninterrupted monitoring is necessary for the assessment of air ventilation systems' efficiency and the detection of airborne particles coming from the medical staff, visitors, and/or patients. Furthermore, airborne microbiological investigation data could be used for developing specific air quality guidelines for controlled environments in hospital settings, which has not been done so far.

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Competing interests

The authors declare no competing interest with respect to the publication and authorship of this paper.

Authors' contributions

All the authors contributed to the design, review and revision of the study, and approved the final version of the paper.

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Ethical considerations

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References

1. Morawska L, Ayoko G, Bae G, Buonanno G, Chao C, Clifford S, et al. Airborne particles in indoor environment of homes, schools, offices and aged care facilities: The main routes of exposure. Environment international. 2017;108:75-83.
2. Hsu Y-C, Kung P-Y, Wu T-N, Shen Y-H. Characterization of indoor-air bioaerosols in Southern Taiwan. Aerosol and Air Quality Research. 2012;12(4):651-61.
3. Kalwasinska A, Burkowska A, Wilk I. Microbial air contamination in indoor environment of a university library. Annals of Agricultural and Environmental Medicine. 2012;19(1).
4. Pastuszka J, Marchwinska-Wyrwal E, Wlazlo A. Bacterial aerosol in Silesian hospitals: Preliminary results. Polish Journal of Environmental Studies. 2005;14(6):883.
5. Zorman T, Jeršek B. Assessment of bioaerosol concentrations in different indoor environments. Indoor and Built Environment. 2008;17(2):155-63.
6. Verde SC, Almeida SM, Matos J, Guerreiro D, Meneses M, Faria T, et al. Microbiological assessment of indoor air quality at different hospital sites. Research in Microbiology. 2015;166(7):557-63.
7. Kim KY, Kim YS, Kim D. Distribution characteristics of airborne bacteria and fungi in the general hospitals of Korea. Industrial health. 2010;48(2):236-43.
8. Park D-U, Yeom J-K, Lee WJ, Lee K-M. Assessment of the levels of airborne bacteria, gram-negative bacteria, and fungi in hospital lobbies. International journal of environmental research and public health. 2013;10(2):541-55.
9. Fang Z, Ouyang Z, Hu L, Wang X, Zheng H, Lin X. Culturable airborne fungi in outdoor environments in Beijing, China. Science of the Total Environment. 2005;350(1-3):47-58.
10. Mentese S, Rad AY, Arısoy M, Güllü G. Seasonal and spatial variations of bioaerosols in indoor urban environments, Ankara, Turkey. Indoor and built environment. 2012;21(6):797-810.
11. Obbard JP, Fang LS. Airborne concentrations of bacteria in a hospital environment in Singapore. Water, Air, and Soil Pollution. 2003;144(1-4):333-41.
12. Napoli C, Tafuri S, Montenegro L, Cassano M, Notarnicola A, Lattarulo S, et al. Air sampling methods

to evaluate microbial contamination in operating theatres: results of a comparative study in an orthopaedics department. Journal of Hospital Infection. 2012;80(2):128-32.
13. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. Journal of hospital infection. 2000;46(4):241-56.
14. Stryjakowska-Sekulska M, Piotraszewska-Pajak A, Szyszka A, Nowicki M, Filipiak M. Microbiological quality of indoor air in university rooms. Polish Journal of Environmental Studies. 2007;16(4):623.
15. Azimi F, Naddafi K, Nabizadeh R, Hassanvand MS, Alimohammadi M, Afhami S, et al. Fungal air quality in hospital rooms: a case study in Tehran, Iran. Journal of Environmental Health Science and Engineering. 2013;11(1):30.
16. Hoseinzadeh E, Taghavi M, Samarghandie MR. Evaluation of fungal and bacterial aerosols in the different wards of Malayer city's hospitals in 2011-2012. Journal of Hospital. 2014;13(3):99-108.
17. Ainsworth GC. Introduction to the History of Mycology: Cambridge University Press; 1976.
18. Kim K-H, Kabir E, Jahan SA. Airborne bioaerosols and their impact on human health. Journal of Environmental Sciences. 2018;67:23-35.
19. Wanner H, Verhoeff A, Colombi A, Flanigan B, Gravesen S, Mouilleseaux A, et al. Indoor air quality & its impact on man: Report No. 12: biological particles in indoor environments. ECSC-EEC-EAEC, Brussels-Luxembourg, 1993.
20. Dutkiewicz J, Górny R. Biologic factors hazardous to health: Classification and criteria of exposure assessment. Medycyna pracy. 2002;53(1):29-39.
21. Nevalainen A, Morawaska L. Biological agents in indoor environments. Assessment of health risks. Work conducted by a WHO Expert Group between 2000-2003. World Health Organization Geneva. 2009.
22. Macher J. Sampling airborne microorganisms and aeroallergens. Air sampling instruments for evaluation of atmospheric contaminants. 1995:589-617.
23. Association AIH. Biosafety Committee Biohazards Reference Manual. AIHA, Washington, DC, USA. 1986.
24. Wanner H, Verhoeff A, Colombi A, Flannigan B, Gravesen S, Mouilleseaux A, et al. Biological particles in indoor environments. Indoor air quality and its impact on man Commission of European Communities, Brussels. 1993.
25. Administration) OOSaH. Indoor air quality-proposed rule. notice of proposed rulemaking. 1994;Regist. 59 (65): 15968-6039.
26. Canada) EE. Exposure Guidelines for Residential Indoor Air Quality, Environment Canada- Federal-Provincial Advisory Committee on Environmental and Occupational Health, Ottawa, Ontario. 1989;p. 23: (Online at: http://www.bvsde.paho.org/bvsacd/cd16/exposure.pdf).
27. Cho E-M, Hong HJ, Park SH, Yoon DK, Goung N, Ju S, et al. Distribution and influencing factors of airborne bacteria in public facilities used by pollution-sensitive population: a meta-analysis. International journal of environmental research and public health. 2019;16(9):1483.
28. Sudharsanam S, Srikanth P, Sheela M, Steinberg R. Study of the indoor air quality in hospitals in South Chennai, India—microbial profile. Indoor and Built Environment. 2008;17(5):435-41.
29. Commission E. Good manufacturing practices-medicinal products for human and veterinary use (the rules governing medicinal products in the European Union). Office for Official Publications of the European Communities. 2008.
30. Simoes SdAA, Júnior DPL, Hahn RC. Fungal microbiota in air-conditioning installed in both adult and neonatal intensive treatment units and their impact in two university hospitals of the central western region, Mato Grosso, Brazil. Mycopathologia. 2011;172(2):109-16.
31. Fekadu S, Getachewu B. Microbiological assessment of indoor air of Teaching hospital wards: a case of Jimma University specialized hospital. Ethiopian journal of health sciences. 2015;25(2):117-22.
32. Gilbert Y, Veillette M, Duchaine C. Airborne bacteria and antibiotic resistance genes in hospital rooms. Aerobiologia. 2010;26(3):185-94.
33. Li Y, Wang W, Guo X, Wang T, Fu H, Zhao Y, et al. Assessment of airborne bacteria and fungi in various university indoor environments: A case study in Chang'an University, China. Environmental Engineering Science. 2015;32(4):273-83.
34. Holton J, Ridgway GL, Reynoldson AJ. A microbiologist's view of commissioning operating theatres. Journal of Hospital Infection. 1990;16(1):29-34.
35. Tolabi Z, Alimohammadi M, Hassanvand MS, Nabizadeh R, Soleimani H, Zarei A. The investigation of type and concentration of bio-aerosols in the air of surgical rooms: A case study in Shariati hospital, Karaj. MethodsX. 2019;6:641-50.
36. Sudharsanam S, Swaminathan S, Ramalingam A, Thangavel G, Annamalai R, Steinberg R, et al. Characterization of indoor bioaerosols from a hospital ward in a tropical setting. African health sciences. 2012;12(2):217-25.
37. Viegas C, Rosado L. Assessment of fungal contamination in a Portuguese maternity unit. WIT Transactions on Biomedicine and Health. 2011;15:127-33.
38. Dehghani M, Sorooshian A, Nazmara S, Baghani AN, Delikhoon M. Concentration and type of bioaerosols before and after conventional disinfection and sterilization procedures inside hospital operating rooms. Ecotoxicology and environmental safety. 2018;164:277-82.
39. Augustowska M, Dutkiewicz J. Variability of airborne microflora in a hospital ward within a period of one

year. Annals of Agricultural and Environmental Medicine. 2006;13(1):99-106.
40. Tormo-Molina R, Gonzalo-Garijo MA, Fernández-Rodríguez S, Silva-Palacios I. Monitoring the occurrence of indoor fungi in a hospital. Revista iberoamericana de micología. 2012;29(4):227-34.