

## On the nature of indoor airborne bioaerosols at a hospital in Iran

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### ABSTRACT

**Introduction:** Hospitals are sensitive places owing to the contagious nature of diseases transferred by patients to others such as health care workers and staff.

**Materials and methods:** The aim of the present work is to evaluate the type and concentration of bacterial and fungal bio-aerosols in the indoor air of four operating rooms (ORs) and four wards in Khalkhal, Iran during 2019. A total of 192 bacterial and fungal samples were collected.

**Results:** Mean total concentrations of airborne bacteria for ORs and wards were between  $11 \pm 1.2$  to  $48 \pm 3.1$  CFU/m<sup>3</sup>, respectively, while for airborne fungi values ranged from  $95 \pm 5.6$  to  $51 \pm 1.2$  CFU/m<sup>3</sup>, respectively. The predominant genera of airborne bacterial isolated (ORs vs. wards) were Staphylococcus epidermidis (72% vs. 58%), Group D Streptococcus (4% vs. 17%), Group A Streptococcus (13% vs. 3%), and Staphylococcus saprophyticus (6% vs. 4%). In addition, the main fungal species identified (ORs vs. wards) were Cladosporium sp. (37% vs. 38%), Penicillium sp. (28% vs. 22%), and Aspergillus Niger (21% vs. 12%). A statistically significant correlation was observed between the mean concentration of bio-aerosols and population density ( $p < 0.05$ ).

**Conclusion:** Furthermore, a statistically significant difference was observed between the mean concentrations of bio-aerosols and the values recommended by WHO ( $p < 0.05$ ), linked presumably to inadequate disinfection, improper design and operation of standard central ventilation (SCV), and the high density of visitors and patients. Addressing such issues can help reduce airborne fungi and bacteria in hospital.

### Introduction

Indoor air pollution in hospital settings is an important problem especially in developing countries [1-5] as it relates to impacts on human health and the environment [3, 4, 6-8]. Humans

in hospitals are exposed to a wide suite of biological agents [9-12]. Biological contaminants in the form of bio-aerosols are considered as a major source of indoor air pollution of different wards of hospitals, comprised of bacteria, cell

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fragments, fungal spores and by-products of microbial metabolism [10, 11, 13-17]. Bio-aerosols constitute about 6 to 31 percent of indoor air pollution [18, 19].

One of the most important ways for transmitting these microorganisms in hospitals is inhalation, leading to an assortment of issues such as acute toxic effects, allergies, and cancer [10, 20-22]. The aerodynamic diameter of airborne biological particles is between 0.001 to 100  $\mu\text{m}$  [23]. Those particles with aerodynamic diameter less than 10  $\mu\text{m}$ , and especially less than 2.5  $\mu\text{m}$ , are able to penetrate more deeply into the respiratory system to promote deleterious health effects [1, 8, 24-27]. Most vulnerable are medical staff, service staff, patients, and visitors [5, 28, 29]. In addition to endangering the health of those in hospitals, bio-aerosols may result in reduction of productivity in the workplace [6, 10, 14, 30]. Past work has shown evidence of a wide range of biological species in the indoor air of hospitals. One study isolated ten fungal genera from nine different units in a teaching hospital in Ghana, including *Alternaria* sp., *Abida corymifera*, *Rhizopus stolonifer*, *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Epicoccum nigrum*, *Verticillium* sp., *Penicillium* sp., and *Phema glemerata* [31]; furthermore, they identified eight bacterial species, including *Yersinia enterocolitica*, *Vibrio cholera*, *Salmonella enterica*, *Vibrio vulnificus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*.

The presence of airborne fungi and bacteria in hospital wards is dependent on (i) the efficiency of the air-conditioning system and ventilation [5, 32-35], (ii) environmental factors (temperature and relative humidity) [6, 36-39], (iii) population density [5, 6], (iv) inadequate disinfection [5, 6], (v) patient/staff activities [40], and (vi) different seasons [5, 12, 41]. For example, previous works described that patient/staff activities such as talking, walking in wards, sneezing, coughing, vomiting, and diarrhea can directly emit bio-aerosol into the indoor air of hospitals wards [40, 42-45].

The aim of the present work was to 1) determine

the concentration of culturable bacteria and fungi bio-aerosols in indoor air of Imam Khomeini hospital wards in Khalkhal, Iran, during summer and autumn in 2019, 2) identify the genera and percentage of bio-aerosols, and 3) study the effects of environmental factors and population density on the amount of bio-aerosols.

## Materials and methods

### Study area

Ardabil province is located in the northeastern part of Iran, with one of its populated cities being Khalkhal (37°37'08"N 48°31'33"E). This city's population is around 95000 (2019 values) [46]. Sampling focused on bacterial and fungal bio-aerosols in the indoor air of four operating rooms (ORs) and four wards in an educational hospital (Imam Khomeini Hospital) (Fig. 1). This educational hospital was founded in 2008 with a capacity of 130 beds and 15 wards. Specialties in this hospital include: internal medicine, surgery, pediatrics, dialysis, obstetrics and gynecology, coronary care unit (CCU), and post CCU.

### Cleaning and ventilation in ORs and wards

All of the instruments in ORs and wards, including beds, chests, and tables were wiped and disinfected. The floors and walls of ORs and wards were also disinfected by Septicidine-surface. During and after surgery, ORs and wards floor were wiped by damp and wet mopping. Furthermore, ORs were wiped every Wednesday in the following sequence: (a) all rolls, mattresses, logo boards, stretchers, suction canisters, flowmeter, and rims were cleaned with detergent and water and disinfected with Sayasept HP disinfection; and (b) doors and walls were cleaned with detergent and water and disinfected by Septicidine-surface. Furthermore, after following all the steps mentioned, percidin 1% and UV radiation (lower than two hours) were applied for sterilization of ORs. Moreover, the hospital has standard central ventilation (SCV).



Fig. 1. Map of the study area (Ardabil Iran)) and sampling site by an educational hospital (Imam Khomeini Hospital) in Khalkhal.

### *Sampling procedures*

Following the National Institute for Occupational Safety and Health [47] 0800 procedure (Bio-aerosol Sampling (Indoor Air)) [47-49], sampling was conducted in indoor air of four operating rooms: women's surgery and caesarean section operating room (hereafter WSCOP), orthopedic operating room (hereafter OOP), urology operating room (hereafter UOR), and cosmetic operating room (hereafter COR). In addition, sampling was done in the following four wards: internal medicine ward (hereafter IMW), women's ward (hereafter WW), surgical ward (hereafter SW), and pediatric ward (hereafter PW). Sampling was conducted at each location once every 12 days in the summer from 1 August to 22 September and in autumn from 23 September to 12 November. Sampling for each room was

repeated three times. We collected 96 samples in indoor air of ORs and wards in summer (48 bacterial and 48 fungal samples) and 96 samples in indoor air of ORs and wards in autumn (48 bacterial and 48 fungal samples). Samples of bio-aerosols (bacterial and fungal) in indoor air of ORs and wards were collected based on the standard index of microbial air contamination (IMAC for environments at risk) [50, 51] using an active sampling method (QuickTake 30, BioStage Single-stage Impactor, SKC, Inc., USA) at a flow rate of 28.3 L/min for 10 min in the morning. The sampler device includes petri dishes (9 cm-containing a solid nutrient medium) placed at a height of 1.5 m from the floor and at a distance one meter from all four sides of the wall and away from physical barriers [12, 50, 52-54]. Before sampling, disinfection of the sampler was

performed with 70% ethanol (5). After sampling, plates were wrapped with masking tape, stored at 4°C (using portable plastic cooler box) and moved to a laboratory. Fungal samples were put in an incubator at 25°C for five days, whereas the samples of bacterial were stored at 37°C for 24 to 48 h [5, 12, 53, 55]. In order to determine the relationship between concentrations of bio-aerosols and environmental parameters like temperature and humidity, the latter were measured using the heat index WBGT meter (Model No: WBGT-2010SD, Lutron Electronic Enterprise CO., Ltd, Taiwan).

### ***Quantification and characterization of bio-aerosols***

Tryptic soy agar (TSA) culture media (Hardy Diagnostics Co, USA) with cycloheximide and sabouraud dextrose agar (SDA) culture media (Hardy Diagnostics Co, USA) with chloramphenicol were applied to speciate and quantify bacterial and fungal bio-aerosols, respectively [5, 12]. Bio-aerosol concentrations in air samples were reported in units of CFU/m<sup>3</sup>. Bergey's Manual and biochemical tests were performed for recognition of bacterial species, whereas the slide culture technique in the electronic microscope (Olympus BX60M BF/DF) with a magnification of 100 × 400 was used for diagnosis of fungal species [6, 56].

### ***Quality control (QC)***

#### ***Culture Media***

The batch should be carefully examined for pollution before passing for laboratory use. It is also recommended that the whole batch of the provided media be examined for contamination by keeping the plates at least for 3 days at room temperature. Duplicate plates from the test batch were placed into the incubator at 37 °C for 24 h. If any growth occurred, the process was repeated by getting two plates from the same batch. According to recommendations, if contamination in plates exceeds 10%, all the plates should be discarded [57, 58]. In this work, there was no

growth on duplicate plates at 37 °C for 24 h and on the plates at least for 3 days at room temperature.

### ***QC of samples***

Field blanks and shipping blanks were collected to go through proper QC procedures [58, 59]. Blank sample values for bio-aerosols were less than ten percent of the post sampling values for whole samplers. Sterility of the plates was controlled by having one un-exposed shipping blank of each medium (tryptic soy agar and sabouraud dextrose agar). The shipping blank was supplied by getting an unused plate (without opening the Petri dish) and moving it to the laboratory with the other collected plates including bio-aerosols [59]. Contamination was not observed on shipping blanks. The repeatability (precision) of sampling and analysis was evaluated by sample triplicates [59]. Reported concentrations for each sampling location was the average of triplicate samples.

### ***Statistical analysis***

Statistical analysis of this work was performed using SPSS analytical (Version 22.00). The Kolmogorov-Smirnov test is used to check the normality of data. The one-sample t-test compares the mean concentrations of bacterial bio-aerosols with World Health Organization (WHO) recommendation (10 CFU/m<sup>3</sup> for ORs and 100 CFU/m<sup>3</sup> for wards) [60]. In addition, the one-sample t-test compares the mean concentrations of fungal bio-aerosols with the WHO recommendation (10 CFU/m<sup>3</sup> for ORs and 50 CFU/m<sup>3</sup> for wards) [60]. Moreover, multiple regression analysis was applied to determine whether environmental factors (temperature and relative humidity) or population density have the most effect on concentrations of bio-aerosols in ORs and wards. Relationships were quantified with the Pearson's correlation coefficient.

## Results and discussion

### *Concentration of bio-aerosols aerosols in different operating rooms and wards*

#### *Bacterial aerosols*

The mean ( $\pm$  SD) concentrations of airborne bacteria isolated in indoor air of four ORs and four wards (CFU/m<sup>3</sup>) are shown in Table 1. The total mean concentrations (CFU/m<sup>3</sup>) of airborne bacteria in ORs and wards were  $48\pm 3$  for WSCOP,  $48\pm 3$  for OOP,  $27\pm 2$  for UOR,  $38\pm 2$  for COR,  $45\pm 4$  for IMW,  $28\pm 2$  for WW,  $11\pm 2$  for SW, and  $16\pm 3$  for PW. Nine species of culturable bacteria were isolated in ORs and wards. *Staphylococcus epidermidis* was isolated at all sampling locations. Past work showed that the most frequent bacteria species identified in indoor hospital air in Rio de Janeiro city (Brazil) were *Staphylococcus epidermidis* (27 CFU/m<sup>3</sup>) and *Staphylococcus haemolyticus* (17 CFU/m<sup>3</sup>) [61]. Therefore, indoor hospital air is a key pathway for transmission of *Staphylococcus* sp. to patients and visitors [61-63]. *Lactobacillus* sp. ( $2\pm 1$  CFU/m<sup>3</sup>) was identified just in the WSCOP, while *Staphylococcus aureus* ( $8\pm 4$  CFU/m<sup>3</sup>) and *Listeria* sp. ( $1\pm 1$  CFU/m<sup>3</sup>) were measured exclusively in the IMW and SW.

For comparison, past study demonstrated that the mean concentration of culturable bacterial in the surgical ward and in ORs at a hospital in Portugal ranged from 99 to 495 CFU/m<sup>3</sup> and 2 to 170 CFU/m<sup>3</sup>, respectively [64]. Moreover, previous studies reported that the concentrations of airborne bacteria in indoor air of ORs at the university hospital of Parma (Italy) and in Silesian hospitals (Poland) were limited from 0 to 18 CFU/m<sup>3</sup> and 100 to 1000 CFU/m<sup>3</sup>, respectively [65, 66].

The findings of this study show that the WSCOP and IMW sites exhibited the highest mean concentration of bacterial aerosols compared with other sampling sites. Past study showed that the highest mean concentration of bacteria was in the internal medicine ward for women in Kamkar hospital in Qom (Iran) [67]. For comparison, previous work showed that the mean

concentrations of airborne bacteria at the University of Port Harcourt Teaching Hospital in Port Harcourt (Nigeria) ranged from 9.5 CFU/m<sup>3</sup> in the urology ward to 199.33 CFU/m<sup>3</sup> in the HIV clinic, while the mean concentrations of airborne fungi ranged from 10.5 CFU/m<sup>3</sup> in the surgery outpatient clinic (SOC) to 23.5 CFU/m<sup>3</sup> in the anatomical pathology laboratory (APL) [68]. We speculate that concentrations of airborne bacteria in the WSCOP and IMW sites were higher than other sampling locations owing to some combination of ventilation issues and inadequate disinfection, which is consistent with the findings of previous works in hospital operating rooms in Shiraz (Iran) [5] and in ORs at Ayder Referral Hospital in northern Ethiopia [69].

The results of this work show that the total mean concentrations of airborne bacteria in ORs were 1.63 times higher than wards; therefore, ORs exhibit poorer air quality in terms of bacterial bio-aerosol levels. The results of this work showed that the main bacterial species detected at all sampling locations were *Staphylococcus epidermidis*, Group A, B, and D *Streptococcus*, *Staphylococcus* sp., *Lactobacillus* sp., and *Listeria* sp.. Previous work reported that the most common airborne bacteria in private and government owned hospitals in Benin (Nigeria) were *Staphylococcus aureus*, *Staphylococcus aeroge epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella aerogens* [70]. In addition, the most frequent species of airborne bacteria identified in ValieAsr Hospital in Tehran (Iran) by pervious work were *Stenotrophomonas* sp. (0-2 CFU/m<sup>3</sup>), *Micrococcus* sp. (3-10 CFU/m<sup>3</sup>) and *Staphylococcus epidermidis* (3-10 CFU/m<sup>3</sup>). Furthermore, the most frequent species of airborne bacteria detected in different hospital sites in Setubal (Portugal) were airborne gram-positive cocci (88%), *Staphylococcus* sp. (51%) and *Micrococcus* sp. (37%), which they speculated are released into the air either via clothing of visitors and medical staff or transport of personal and medical materials [71].

Table 1. The mean concentrations of airborne bacteria and fungi isolated indoor air of four ORs and four wards (CFU/m<sup>3</sup>).

The average ( $\pm$ SD) airborne bacteria concentrations								
Type of organisms	Four operating rooms (CFU/m <sup>3</sup> )				Four wards (CFU/m <sup>3</sup> )			
Bacteria	WSCOP	OOP	UOR	COR	IMW	WW	SW	PW
<i>Staphylococcus epidermidis</i>	33 $\pm$ 4	36 $\pm$ 2	15 $\pm$ 2	31 $\pm$ 3	20 $\pm$ 5	17 $\pm$ 3	8 $\pm$ 1	13 $\pm$ 2
Group D <i>Streptococcus</i>	-	-	7 $\pm$ 2	-	4 $\pm$ 3	11 $\pm$ 1	2 $\pm$ 1	-
Group A <i>Streptococcus</i>	12 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1	4 $\pm$ 1	-	-	-	3 $\pm$ 1
<i>Staphylococcus saprophyticus</i>	-	7 $\pm$ 4	2 $\pm$ 1	-	4 $\pm$ 3	-	-	-
<i>Staphylococcus pneumoniae</i>	-	3 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	5 $\pm$ 2	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	8 $\pm$ 4	-	-	-
Group B <i>Streptococcus</i>	-	-	-	2 $\pm$ 1	4 $\pm$ 3	-	-	-
<i>Lactobacillus</i> sp.	2 $\pm$ 1	-	-	-	-	-	-	-
<i>Listeria</i> sp.	-	-	-	-	-	-	1 $\pm$ 1	-
Total	48 $\pm$ 3	48 $\pm$ 3	27 $\pm$ 2	38 $\pm$ 2	45 $\pm$ 4	28 $\pm$ 2	11 $\pm$ 2	16 $\pm$ 3
WHO recommendation	10	10	10	10	100	100	100	100
The average ( $\pm$ SD) airborne fungi concentrations								
Type of organisms	Four operating rooms (CFU/m <sup>3</sup> )				Four wards (CFU/m <sup>3</sup> )			
Fungi	WSCOP	OOP	UOR	COR	IMW	WW	SW	PW
<i>Cladosporium</i> sp.	24 $\pm$ 2	19 $\pm$ 4	30 $\pm$ 3	19 $\pm$ 2	28 $\pm$ 3	30 $\pm$ 4	20 $\pm$ 2	25 $\pm$ 2
<i>Penicillium</i> sp.	11 $\pm$ 2	13 $\pm$ 2	18 $\pm$ 1	32 $\pm$ 6	7 $\pm$ 1	14 $\pm$ 2	20 $\pm$ 2	19 $\pm$ 2
<i>Aspergillus Niger</i>	8 $\pm$ 1	11 $\pm$ 2	6 $\pm$ 1	31 $\pm$ 6	7 $\pm$ 2	13 $\pm$ 2	7 $\pm$ 2	6 $\pm$ 2
<i>Rhodotorula</i> sp.	8 $\pm$ 1	7 $\pm$ 1	-	-	4 $\pm$ 1	4 $\pm$ 2	23 $\pm$ 3	-
<i>Aspergillus flavus</i>	-	2 $\pm$ 1	3 $\pm$ 1	7 $\pm$ 1	-	-	-	-
<i>Curvularia</i> sp.	-	-	-	2 $\pm$ 1	-	5 $\pm$ 2	-	-
<i>Alternaria</i> sp.	-	-	-	-	2 $\pm$ 1	-	-	5 $\pm$ 2
<i>Rhizopus</i> sp.	-	-	-	2 $\pm$ 1	-	-	5 $\pm$ 1	-
<i>Drechslera</i> sp.	-	-	-	-	-	-	7 $\pm$ 1	-
<i>Aspergillus fumigatus</i>	-	-	-	-	5 $\pm$ 1	1 $\pm$ 1	-	6 $\pm$ 2
<i>Stofelim</i> sp.	-	-	-	-	5 $\pm$ 2	-	-	-
<i>Oculanium</i> sp.	-	-	-	-	-	5 $\pm$ 2	-	-
<i>Mucor</i> sp.	-	-	-	2 $\pm$ 1	-	-	-	-
Total	51 $\pm$ 3	52 $\pm$ 3	57 $\pm$ 4	95 $\pm$ 6	58 $\pm$ 2	72 $\pm$ 5	82 $\pm$ 5	61 $\pm$ 4
WHO recommendation	10	10	10	10	50	50	50	50

### Fungal aerosols

Thirteen species of culturable fungi were isolated in ORs and wards, with concentration statistics summarize in Table 1. The total average concentrations of airborne fungi in ORs and wards based on CFU/m<sup>3</sup> were 95 $\pm$ 6 for COR, 82 $\pm$ 5 for SW, 72 $\pm$ 5 for WW, 61 $\pm$ 4 for PW, 58 $\pm$ 2 for IMW, 57 $\pm$ 4 for UOR, 52 $\pm$ 3 for OOP, and 51 $\pm$ 3 for WSCOP. *Cladosporium* sp., *Penicillium* sp., and *Aspergillus Niger* were isolated at all locations. Former work showed that the most frequent fungal

species measured in indoor air of ORs in Shiraz (Iran) were *Aspergillus* sp., *Penicillium* sp., and *Alternaria* sp. [5]. *Mucor* sp. (2 $\pm$ 1 CFU/m<sup>3</sup>) was identified just in the COR, whereas *Oculanium* sp. (5 $\pm$ 2 CFU/m<sup>3</sup>), *Drechslera* sp. (7 $\pm$ 1 CFU/m<sup>3</sup>) and *Stofelim* sp. (5 $\pm$ 2 CFU/m<sup>3</sup>) were measured exclusively in the WW, SW and IMW, respectively.

For comparison, Former work demonstrated that the mean concentration of culturable fungi in Farshchian hospital in wards and ORs at Hamadan (Iran) varied from 15.6 to 16.7 CFU/m<sup>3</sup> and 7.8

CFU/m<sup>3</sup>, respectively [62]. Past study reported that the concentrations of airborne fungi in indoor air of a hospital in Portugal were less than one CFU/m<sup>3</sup> for ORs and from 1 to 32 CFU/m<sup>3</sup> for a surgical ward [64]. Previous work reported concentrations of airborne fungi in different wards of private and government hospitals in Benin (Nigeria) between 10 to 53 CFU/m<sup>3</sup>; the most frequent fungal species they detected were *Penicillium* sp. and *Aspergillus* sp.

The results of this work reveal that the main fungi species detected in indoor air of ORs and wards were *Cladosporium* sp., *Penicillium* sp., *Aspergillus Niger*, and *Rhodotorula* sp. Previous work showed that the main airborne fungal species in Valiasr hospital in Zanzan were *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., and *Alternaria* sp. [72]. The total average concentrations of airborne fungi in ORs were similar to wards in contrast to bacterial levels that showed more of a difference.

### ***Comparison of bio-aerosol concentrations with proposed guidelines***

#### ***Bacteria***

According to Table 1, the total airborne bacteria concentrations in all ORs (WSCOP, OOP, UOR, and COR) were higher compared to the values suggested by WHO (10 CFU/m<sup>3</sup>), while the total airborne concentrations of bacteria in all wards (IMW, WW, SW, and PW) were lower than the values recommended by WHO (100 CFU/m<sup>3</sup>). The one-sample t-test was used to compare the mean concentrations of bacterial bio-aerosols and the values suggested by WHO (10 CFU/m<sup>3</sup> for ORs and 100 CFU/m<sup>3</sup> for wards). The results of one-sample t-test indicate that there was a significant difference between the mean concentrations of bacterial bio-aerosols in the ORs and the values suggested by WHO ( $p = 0.037$ ). In addition, the findings of the same analysis showed that a significant difference was observed between the mean concentrations of bacterial bio-aerosols in the wards and the values suggested by WHO ( $p = 0.002$ ).

#### ***Fungi***

Based on Table 1, the total airborne fungi concentrations in hospital wards (IMW, WW, SW, and PW) and ORs (WSCOP, OOP, UOR, and COR) were higher compared to the values suggested by WHO (10 CFU/m<sup>3</sup> for ORs and 50 CFU/m<sup>3</sup> for wards). The one-sample t-test was applied to contrast the average concentrations of fungal bio-aerosols and the values proposed by WHO (10 CFU/m<sup>3</sup> for ORs and 50 CFU/m<sup>3</sup> for wards). The findings of one-sample t-test suggested that there was a significant difference between the mean concentrations of fungal bio-aerosols in the ORs and the values recommended by WHO ( $p = 0.011$ ). Furthermore, the t-test results displayed that a significant difference was found between the average concentrations of fungal bio-aerosols in the wards and the values proposed by WHO ( $p = 0.048$ ).

### ***Interrelationships between bio-aerosol concentrations, population density and environmental factors***

#### ***Bacteria***

Environmental parameters such as temperature and relative humidity and population density are parameters potentially influencing bio-aerosol concentrations in different parts of a hospital [36, 73]. The mean  $\pm$  standard deviation of the temperature, relative humidity, and population density (ORs versus wards) were as follows, respectively:  $13.41 \pm 3.27$  °C versus  $14.23 \pm 2.18$  °C,  $40.05 \pm 4.91\%$  versus  $41.12 \pm 3.82\%$  and  $8 \pm 3$  number of people per ORs versus  $12 \pm 2$  number of people per wards. Multiple regression analysis (MRA) was applied to determine which of these three parameters have the most effect on concentrations of bio-aerosols in ORs and wards. The results of MRA indicated that population density for ORs and wards had a significant correlation with the concentration of bacteria bio-aerosols ( $p=0.002$  and  $\text{Beta}=0.790$  for ORs and  $p=0.004$  and  $\text{Beta}=0.820$  wards). In addition, the results of MRA showed that temperature for ORs and wards did not have a significant

correlation with the concentration of bacterial bio-aerosols ( $p=0.823$  for ORs and  $p=0.682$  for wards). Furthermore, the findings of MRA showed that relative humidity for ORs and wards did not exhibit a significant correlation with the concentration of bacterial bio-aerosols ( $p=0.868$  for ORs and  $p=0.870$  for wards).

Past work showed that a significant correlation was not observed between the concentration of bacteria with either temperature or humidity ( $p>0.01$ ) in indoor air of hospital areas, which is consistent with the results of this study [74, 75]. However, Past study found that a significant correlation between bacterial concentration with either population density or relative humidity ( $p<0.01$ ) in a Singapore hospital [76]. Past work reported that temperature had a significant positive correlation with the concentration of bacteria bio-aerosols in various university indoor environments in Changan and Xian (China) [32, 77].

### Fungi

The findings of MRA illustrated that population density for ORs and wards exhibited a significant correlation with the concentration of fungi bio-aerosols ( $p=0.003$  and  $\text{Beta}=0.821$  for ORs and

$p=0.004$  and  $\text{Beta}=0.876$  wards). Temperature did not exhibit a significant correlation with the concentration of fungi bio-aerosols in ORs and wards ( $p=0.622$  for ORs and  $p=0.723$  for wards). Relative humidity for ORs and wards also did not have a significant correlation with the concentration of fungi bio-aerosols ( $p=0.561$  for ORs and  $p=0.718$  for wards). Similarity, past study showed that a significant correlation was not observed between the concentration of fungi bio-aerosols with either temperature or humidity ( $p\geq 0.05$ ) in ORs of an educational hospital in Shiraz (Iran) [5].

For comparison, former work demonstrated that environmental factors such as temperature and humidity were significantly correlated with fungi bio-aerosols in various hospitals in Yangzhou, Shenzhen, Ningbo, Guangzhou, and Fenghua (China) [36]. Temperature and humidity can impact the growth of fungi bio-aerosols, which is opposite with the results of this study and others [36-38].

Lastly, in terms of interrelationships, Table 2 describes Pearson's correlation coefficient between fungal and bacterial concentrations in ORs and wards based on average concentrations. There were no significant correlations between bio-aerosols in ORs and wards.

Table 2. Pearson's correlation coefficient (r) between fungi and bacteria concentrations in ORs and wards.

Bio-aerosols		Bacteria (ORs)	Fungi (ORs)	Bacteria (wards)	Fungi (wards)
Bacteria (ORs)	r	1			
	P-value				
Fungi (ORs)	r	.084	1		
	P-value	.874			
Bacteria (wards)	r	.008	.262	1	
	P-value	.987	.361		
Fungi (wards)	r	.065	.138	.021	1
	P-value	.838	.716	.940	



## Frequency of occurrence of bio-aerosols

### Bacterial species

The percentage of isolated bacterial species in ORs and wards based on frequency in different sampling sites is summarized in Fig. 2. In this study, the predominant genera of airborne bacterial isolated in the indoor air of ORs and wards were *Staphylococcus epidermidis* (72% for ORs and 58% for wards), Group D *Streptococcus* (4% for ORs and 17% for wards), Group A *Streptococcus* (13% for ORs and 3% for wards), *Staphylococcus saprophyticus* (6% for ORs and 4% for wards), and *Staphylococcus pneumoniae* (3% for ORs and 5% for wards).

The lowest percentage of isolated bacterial species in different sampling sites were *Lactobacillus* sp. (1% for ORs and 0 for wards) and *Listeria* sp. (0 for ORs and 1% for wards). As shown in Fig. 2,

the main species of bacteria identified in the indoor air of ORs and wards was *Staphylococcus* sp., which is part of the normal flora of skin, hair, and the respiratory tract [62, 78, 79]. For comparison, former work described that the dominant bacterial species in different sampling sites in a teaching hospital in Kandy (Sri Lanka) were *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Exiguobacterium* sp., *Enterobacter* sp., *Escherichia* sp., *Sphingomonas* sp., *Massilia* sp., and *Kocuria* sp. [79]. Former study reported that the most common airborne bacteria in Alavi hospital in Ardabil (Iran) were *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Enterococcus* sp. [80]. In addition, the most common bacteria at the University of Port Harcourt Teaching Hospital in Port Harcourt (Nigeria) were *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Escherichia coli*, and *Bacillus* sp. [68].

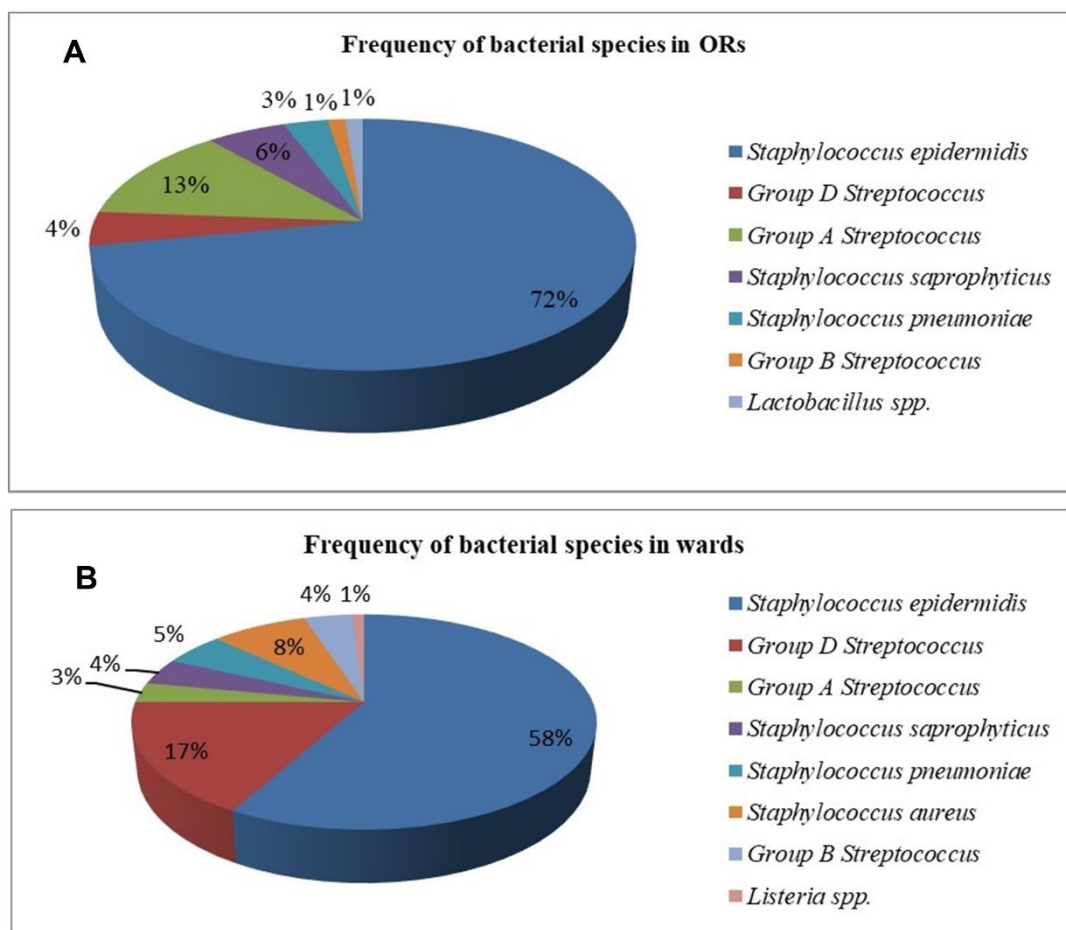


Fig. 2. The percentage of identified bacteria based on frequency in ORs (A) and wards (B).

### Fungal species

The percentage of isolated fungal species in ORs and wards based on frequency in different sampling sites is shown in Fig. 3. Accordingly, the predominant genera of airborne fungal identified in indoor air of ORs and wards were *Cladosporium* sp. (37% for ORs and 38% for wards), *Penicillium* sp. (28% for ORs and 22% for wards), *Aspergillus niger* (21% for ORs and 12% for wards), *Rhodotorula* sp. (6% for ORs and 11% for wards), *Rhizopus* sp. (1% for ORs and 2% for wards), and *Curvularia* sp. (1% for ORs and 2% for wards).

For comparison, past study found that the dominant species of fungi in a teaching hospital in Kandy (Sri Lanka) were *Fusarium* sp. and *Aspergillus* sp. [79]. Former work reported that the dominant fungal types in three hospital wards in Roma (Italy) were *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., and *Aspergillus* sp. [33]. In addition, Sautour and co-authors reported that the predominant fungal species identified in indoor air of Dijon Hospital in Burgundy (France) were *Penicillium* sp. (23-

25%), *Aspergillus* sp. (15-23%) and *Bjerkandera adusta* (11-13%) [81]. Furthermore, former study isolated seven fungal genera in Trakya university hospital in Edirne (Turkey), including *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., *Scopulariopsis* sp., *Paecilomyces* sp., *Aspergillus* sp., and *Trichothecium* sp. [82].

Differences in the frequency and diversity of fungi in this study compared with other studies can be explained potentially by sampling in different seasons (summer and autumn), ventilation defects and inadequate disinfection, types of patients admitted, and open windows, which is consistent with the findings of previous works in hospital operating rooms in Shiraz [5], in the different areas of Trakya university hospital in Edirne (Turkey) [82], in general indoor hospital air [83], and in indoor air of surgical rooms in Shariati hospital in Karaj (Iran) [84]. Generally, owing to growth of different types of fungi in various hospitals around the world, especially in our study, we conclude that hospitals have the ability to create various levels of infection and therefore corrective actions are necessary in these places.

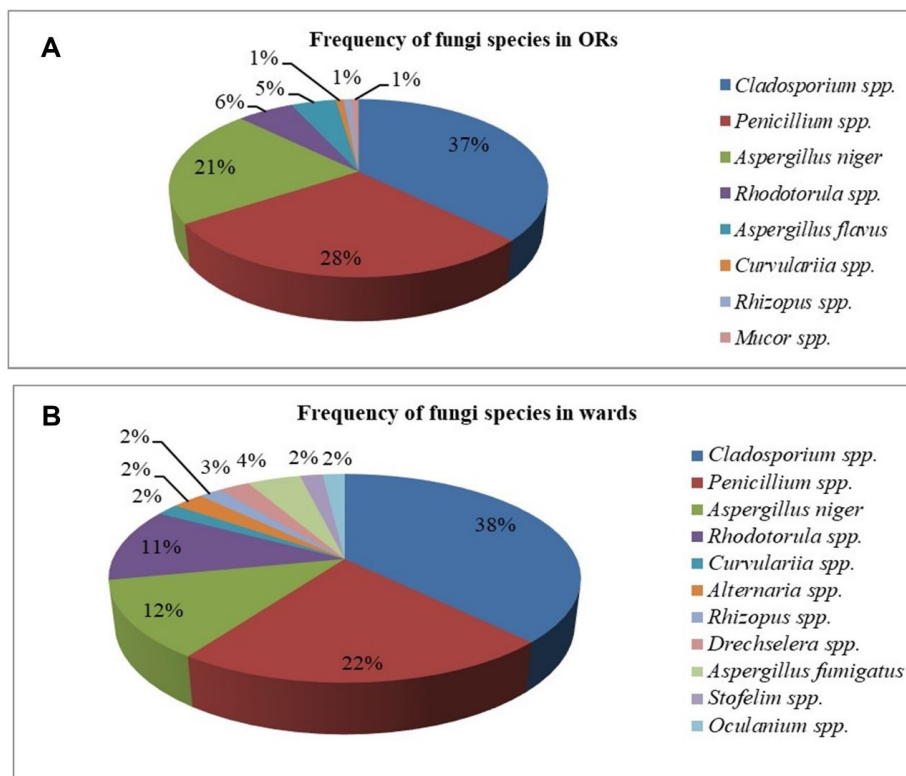


Fig. 3. The percentage of identified fungal based on frequency in ORs (A) and wards (B).

## Conclusion

The present work characterizes the nature of airborne fungi and bacteria at Imam Khomeini hospital in northeastern Iran, including a comprehensive look at type and concentration profiles in various parts of the hospital. Despite disinfection, 13 species of culturable fungi and 9 species of culturable bacteria were identified in different parts of the hospital. The results of this study indicate that the predominant genera of airborne bacterial isolated in both of ORs and wards were *Staphylococcus epidermidis*, Group D *Streptococcus*, Group A *Streptococcus*, *Staphylococcus saprophyticus*, and *Staphylococcus pneumoniae*. The predominant genera of airborne fungal identified in both of ORs and wards were *Cladosporium* sp., *Penicillium* sp., *Aspergillus Niger*, *Rhodotorula* sp., *Rhizopus* sp., and *Curvularia* sp. Results of this work emphasize that hospitals have the potential to spread disease via airborne bio-aerosols and therefore corrective actions are necessary to mitigate the negative public health effects. The role of different potential influential factors (ventilation system, environmental factors, type of patients, used disinfectants, density of visitors and patients, and management of infectious waste) should be investigated. Appropriate monitoring systems should be considered to reduce the type and concentration of bio-aerosols in the air of ORs and wards.

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## Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

## Authors' contributions

“Conceptualization, Hadi Sadeghi and Maryam Dashti; methodology, Hadi Sadeghi and Abbas Norouziyan Baghani; software Seyed Ahmad Mokhtari and Abbas Norouziyan Baghani;

validation, Hadi Sadeghi, Maryam Dashti and Abbas Norouziyan Baghani; formal analysis, Seyed Ahmad Mokhtari; investigation, Maryam Dashti and Seyed Ahmad Mokhtari; data curation, Maryam Dashti; writing—original draft preparation, Abbas Norouziyan Baghani; writing—review and editing, Armin Sorooshian and Abbas Norouziyan Baghani; visualization, Mehdi Vosoughi; supervision, Hadi Sadeghi; project administration, Hadi Sadeghi and Maryam Dashti; funding acquisition, Hadi Sadeghi. All authors have read and agreed to the published version of the manuscript.”

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## Ethical considerations

Authors are aware of, and have complied with, best practices in ethics, specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. The authors have adhered to the publication requirements that the submitted work is original and has not been published elsewhere in any language.

## References

1. Pérez N, Pey J, Castillo S, Viana M, Alastuey A, Querol X. Interpretation of the variability of levels of regional background aerosols in the Western Mediterranean. *Science of the total environment*. 2008;407(1):527-40.
2. Saini J, Dutta M, Marques G. A comprehensive review on indoor air quality monitoring systems for enhanced public health. *Sustainable*

- Environment Research. 2020;30(1):6.
3. Nabizadeh R, Sorooshian A, Delikhoon M, Baghani AN, Golbaz S, Aghaei M, et al. Characteristics and health effects of volatile organic compound emissions during paper and cardboard recycling. *Sustainable cities and society*. 2020;56:102005.
  4. Nabizadeh R, Sorooshian A, Delikhoon M, Baghani AN, Golbaz S, Aghaei M. Dataset on specifications, carcinogenic and non-carcinogenic risk of volatile organic compounds during recycling paper and cardboard. *Data in brief*. 2020;29:105296.
  5. 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.
  6. Chegini FM, Baghani AN, Hassanvand MS, Sorooshian A, Golbaz S, Bakhtiari R, et al. Indoor and outdoor airborne bacterial and fungal air quality in kindergartens: seasonal distribution, genera, levels, and factors influencing their concentration. *Building and environment*. 2020:106690.
  7. Nazmara S, Sorooshian A, Delikhoon M, Baghani AN, Ashournejad Q, Barkhordari A, et al. Characteristics and health risk assessment of polycyclic aromatic hydrocarbons associated with dust in household evaporative coolers. *Environmental pollution*. 2020;256:113379.
  8. Baghani NA, Bahmani Z, Sorooshian A, Farzadkia M, Nabizadeh R, Delikhoon M, et al. Characterization of polycyclic aromatic hydrocarbons associated with PM<sub>10</sub> emitted from the largest composting facility in the Middle East. *Toxin reviews*. 2020:1-15.
  9. Jaffal A, Banat I, El Mogheth A, Nsanze H, Bener A, Ameen A. Residential indoor airborne microbial populations in the United Arab Emirates. *Environment International*. 1997;23(4):529-33.
  10. Baghani NA, Sorooshian A, Delikhoon M, Nabizadeh R, Nazmara S, Bakhtiari R. Pollution characteristics and noncarcinogenic risk assessment of fungal bioaerosol in different processing units of waste paper and cardboard recycling factory. *Toxin Reviews*. 2020:1-12.
  11. Naddafi K, Nabizadeh R, Baghani AN, Fazlzadeh M. Bioaerosols in the waterpipe cafés: genera, levels, and factors influencing their concentrations. *Environmental Science and Pollution Research*. 2019;26(20):20297-307.
  12. Dehghani M, Sorooshian A, Ghorbani M, Fazlzadeh M, Miri M, Badiie P, et al. Seasonal Variation in Culturable Bioaerosols in a Wastewater Treatment Plant. *Aerosol and air quality research*. 2018;18(11):2826-39.
  13. Stetzenbach LD. Airborne Infectious Microorganisms. In: Schaechter M, editor. *Encyclopedia of Microbiology (Third Edition)*. Oxford: Academic Press; 2009. p. 175-82.
  14. Su C, Lau J, editors. Review of air cleaning technologies in ventilation system for bio-aerosols. 12th International Conference on Indoor Air Quality and Climate 2011; 2011.
  15. Soleimani Z, Goudarzi G, Sorooshian A, Marzouni MB, Maleki H. Impact of Middle Eastern dust storms on indoor and outdoor composition of bioaerosol. *Atmospheric environment*. 2016;138:135-43.
  16. Mosalaei S, Amiri H, Rafiee A, Abbasi A, Baghani AN, Hoseini M. Assessment of fungal bioaerosols and particulate matter characteristics in indoor and outdoor air of veterinary clinics. *Journal of Environmental Health Science and Engineering*. 2021.
  17. Chegini FM, Baghani AN, Hassanvand MS, Sorooshian A, Golbaz S, Bakhtiari R, et al. Indoor and outdoor airborne bacterial and fungal air quality in kindergartens: Seasonal distribution, genera, levels, and factors influencing their concentration. *Building and Environment*. 2020;175:106690.
  18. Kim KY, Kim CN. Airborne microbiological characteristics in public buildings of Korea.

- Building and Environment. 2007;42(5):2188-96.
19. Henderson TJ, Ibrahim S, Eisenkraft A, Adler M, Salem H, Santarpia J, et al. *Aerobiology: The Toxicology of Airborne Pathogens and Toxins*: Royal Society of Chemistry; 2016.
  20. Hoseinzadeh E. Evaluation of bioaerosols in five educational hospitals wards air in hamedan. *Jundishapur Journal of Microbiology*. 2013;6(6)
  21. Jensen PA, Schafer MP. Sampling and characterization of bioaerosols. *NIOSH manual of analytical methods*. 1998;1(15):82-112.
  22. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and health impacts of air pollution: A review. *Frontiers in public health*. 2020;8.
  23. Baron P. Generation and behavior of airborne particles (aerosols). PowerPoint Presentation. US Department of Health and Human Services, Centers ...; 2010.
  24. D'amato G. Environmental urban factors (air pollution and allergens) and the rising trends in allergic respiratory diseases. *Allergy*. 2002;57(s72):30-3.
  25. Fan Z, Lin L. Exposure Science: Contaminant Mixtures. In: Nriagu JO, editor. *Encyclopedia of Environmental Health*. Burlington: Elsevier; 2011. p. 645-56.
  26. Youn J-s, Csavina J, Rine KP, Shingler T, Taylor MP, Sáez AE, et al. Hygroscopic properties and respiratory system deposition behavior of particulate matter emitted by mining and smelting operations. *Environmental science & technology*. 2016;50(21):11706-13.
  27. Sorooshian A, Csavina J, Shingler T, Dey S, Brechtel FJ, Sáez AE, et al. Hygroscopic and chemical properties of aerosols collected near a copper smelter: implications for public and environmental health. *Environmental science & technology*. 2012;46(17):9473-80.
  28. Hamzavi SS, Amanati A, Badiie P, Kadivar MR, Jafarian H, Ghasemi F, et al. Changing face of *Candida* colonization pattern in pediatric patients with hematological malignancy during repeated hospitalizations, results of a prospective observational study (2016–2017) in shiraz, Iran. *BMC infectious diseases*. 2019;19(1):759.
  29. Ghanizadeh F, Godini H. A review of the chemical and biological pollutants in indoor air in hospitals and assessing their effects on the health of patients, staff and visitors. *Reviews on environmental health*. 2018;33(3):231-45.
  30. Gamero WM, Agudelo-Castañeda D, Ramirez MC, Hernandez MM, Mendoza HP, Parody A, et al., editors. *Hospital admission and risk assessment associated to exposure of fungal bioaerosols at a municipal landfill using statistical models*. International Conference on Intelligent Data Engineering and Automated Learning; 2018: Springer.
  31. Larrey EK, Laryea JNA, Kpordze SW, Saba CKS. Microbial load of indoor airborne bacteria and fungi in a teaching hospital in Ghana. *African Journal of Microbiology Research*. 2020;14(3):100-5.
  32. Li A, Liu Z, Zhu X, Liu Y, Wang Q. The effect of air-conditioning parameters and deposition dust on microbial growth in supply air ducts. *Energy and Buildings*. 2010;42(4):449-54.
  33. Perdelli F, Sartini M, Spagnolo AM, Dallera M, Lombardi R, Cristina ML. A problem of hospital hygiene: the presence of aspergilli in hospital wards with different air-conditioning features. *American journal of infection control*. 2006;34(5):264-8.
  34. Mirhoseini SH, Didehdar M, Akbari M, Moradzadeh R, Jamshidi R, Torabi S. Indoor exposure to airborne bacteria and fungi in sensitive wards of an academic pediatric hospital. *Aerobiologia*. 2020:1-8.
  35. Borrego S, Molina A. Behavior of the Cultivable Airborne Mycobiota in air-conditioned environments of three Havanan archives, Cuba *Journal of Atmospheric Science Research*. 2020;3(1):65881289.
  36. Gao X-L, Shao M-F, Wang Q, Wang L-T,

- Fang W-Y, Ouyang F, et al. Airborne microbial communities in the atmospheric environment of urban hospitals in China. *Journal of hazardous materials*. 2018;349:10-7.
37. Bowers RM, McCubbin IB, Hallar AG, Fierer N. Seasonal variability in airborne bacterial communities at a high-elevation site. *Atmospheric Environment*. 2012;50:41-9.
38. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al. Architectural design influences the diversity and structure of the built environment microbiome. *The ISME journal*. 2012.1469:6(8).
39. Onmek N, Kongcharoen J, Singtong A, Penjumrus A, Junnoo S. Environmental Factors and Ventilation Affect Concentrations of Microorganisms in Hospital Wards of Southern Thailand. *Journal of Environmental and Public Health*. 2020;2020.
40. Hathway E, Noakes C, Fletcher L, Sleigh P, Clifton I, Elliott M. The role of nursing activities on the bioaerosol production in hospital wards. *Indoor and Built Environment*. 2013;22(2):410-21.
41. Balyan P, Ghosh C, Das S, Banerjee B. Comparison of Efficiency of Active and Passive Methods of Bioaerosols' Estimation. *Indoor Environmental Quality: Springer*; 2020. p. 85-94.
42. Okhuoya J, Okaraedge S. Microflora of road side air and leaf surfaces of selected vegetables. *Nigerian J Pure Appl Am Sci*. 1992;12:42-8.
43. Marcelou Kinti U. Study of the Mycological Flora of the Air Role in Mycosis of the Conjunctiva. *Del Ellen Microbial Etai*. 1977;22(3):159-63.
44. Pepper IL, Gerba CP. *Aeromicrobiology. Environmental Microbiology: Elsevier*; 2015. p. 89-110.
45. Borrego S, Molina A. Fungal assessment on storerooms indoor environment in the National Museum of Fine Arts, Cuba. *Air quality, atmosphere & health*. 2019;12(11):1373-85.
46. tatistical Centre of Iran (SCI) I. 2016 [Available from: Available from: <http://irandataportal.syr.edu/census/census-2016> (Accessed on 11/22/2017)
47. NIOSH NfOSaH. NIOSH method 0800 - BIOAEROSOL SAMPLING (Indoor Air)- NIOSH Manual of Analytical Methods (NMAM), Fourth Edition- Culturable organisms: bacteria, fungi, thermophilic actinomycetes. 1998.
48. Madureira J, Pereira C, Paciência I, Teixeira JP, de Oliveira Fernandes E. Identification and levels of airborne fungi in Portuguese primary schools. *Journal of Toxicology and Environmental Health, Part A*. 2014;77(14-16):816-26.
49. Ashley K. NIOSH Manual of analytical methods 5th edition and harmonization of occupational exposure monitoring. *Gefahrstoffe, Reinhaltung der Luft= Air quality control/ Herausgeber, BIA und KRdL im VDI und DIN*. 2015;2015(1-2):7.
50. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. *Journal of hospital infection*. 2000;46(4):241-56.
51. Tolabi Z, Alimohammadi M, Hasanvand MS, Nabizadeh R, Soleimani H, Zarei A. The investigation of type and concentration of bioaerosols in the air of surgical rooms: A case study in Shariati hospital, Karaj. *MethodsX*. 2019.
52. Faridi S, Hassanvand MS, Naddafi K, Yunesian M, Nabizadeh R, Sowlat MH, et al. Indoor/outdoor relationships of bioaerosol concentrations in a retirement home and a school dormitory. *Environmental Science and Pollution Research*. 2015;22(11):8190-200.
53. Naddafi K, Nabizadeh R, Baghani AN, Fazlzadeh M. Bioaerosols in the waterpipe cafés: genera, levels, and factors influencing their concentrations. *Environmental Science and Pollution Research*. 2019:1-11.
54. Bolookat F, Hassanvand MS, Faridi S, Hadei M, Rahmatinia M, Alimohammadi M. Assessment of bioaerosol particle characteristics at different hospital wards and operating theaters: A case study in Tehran. *MethodsX*. 2018;5:1588-

- 96.
55. Hamzavi SS, Amanati A, Badiee P, Kadivar MR, Jafarian H, Ghasemi F, et al. Changing face of *Candida* colonization pattern in pediatric patients with hematological malignancy during repeated hospitalizations, results of a prospective observational study (2016-2017) in Shiraz, Iran. *BMC infectious diseases*. 2019;19(1):759.
56. Brown AE. *Benson's microbiological applications: laboratory manual in general microbiology*. 10th. Boston: McGraw-Hill Higher Education. xiv; 2005.
57. Basu S, Pal A, Desai P. Quality control of culture media in a microbiology laboratory. *Indian journal of medical microbiology*. 2005;23(3):159.
58. Therkorn J, Thomas N, Scheinbeim J, Mainelis G. Field performance of a novel passive bioaerosol sampler using polarized ferroelectric polymer films. *Aerosol Science and Technology*. 2017;51(7):787-800.
59. EPA US. Base study standard preating procedure for sampling and characterization of bioaerosols in indoor air. Environmental Health & Engineering, Inc 60 Wells Avenue Newton, MA 02459-3210-EH&E Report #11663 September 2000 2000.
60. Bartley JM, Olmsted RN, Haas J. Current views of health care design and construction: Practical implications for safer, cleaner environments. *American Journal of Infection Control*. 2010;38(5):S1-S12.
61. Botelho AMN, das Graça Nunes Z, Asensi MD, Gomes MZR, Fracalanza SEL, Figueiredo AMS. Characterization of coagulase-negative staphylococci isolated from hospital indoor air and a comparative analysis between airborne and inpatient isolates of *Staphylococcus epidermidis*. *Journal of medical microbiology*. 2012;61(8):1136-45.
62. Hoseinzadeh E, Samarghandie MR, Ghiasian SA, Alikhani MY, Roshanaie G. Evaluation of Bioaerosols in Five Educational Hospitals Wards Air in Hamedan, During 2011-2012. *Jundishapur J Microbiol*. 2013;6(6):e10704.
63. Madsen AM, Moslehi-Jenabian S, Islam MZ, Frankel M, Spilak M, Frederiksen MW. Concentrations of *Staphylococcus* species in indoor air as associated with other bacteria, season, relative humidity, air change rate, and *S. aureus*-positive occupants. *Environmental Research*. 2018;160:282-91.
64. Verde SC, Almeida SM, Matos J, Guerreiro D, Meneses M, Faria T, Botelho D, Santos M, Viegas C. Microbiological assessment of indoor air quality at different hospital sites. *Research in microbiology*. 2015 Sep 1;166(7):557-63.
65. Pastuszka J, Marchwinska-Wyrwal E, Wlazlo A. Bacterial aerosol in Silesian hospitals: Preliminary results. *Polish Journal of Environmental Studies*. 2005;14(6):883.
66. Pasquarella C, Vitali P, Saccani E, Manotti P, Boccuni C, Ugolotti M, et al. Microbial air monitoring in operating theatres: experience at the University Hospital of Parma. *Journal of Hospital Infection*. 2012;81(1):50-7.
67. Azizifar M, Jabbari H, Naddafi K, Nabizadeh R, Tabaraie Y, Solg A. A qualitative and quantitative survey on air-transmitted fungal contamination in different wards of Kamkar Hospital in Qom, Iran, in 2007. *Qom University of Medical Sciences Journal*. 2009;3 (3).
68. Emuren K, Ordinioha B. Microbiological assessment of indoor air quality at different sites of a tertiary hospital in South-South Nigeria. *Port Harcourt Medical Journal*. 2016;10(2):79.
69. Tesfaye T, Berhe Y, Gebreselassie K. Microbial contamination of operating Theatre at Ayder Referral Hospital, Northern Ethiopia. *International Journal of Pharma Sciences and Research (IJPSR)*. 2015 Oct;6(10).
70. Osaro EF, Ufuoma IO, Dorcas AO. Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria. *World J Med Sci*. 2008;3(1):19-23.
71. 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.
72. Mehrasbi MR, Mohammadi G, Mohammadian Fazli M, Hajikarim B. Indoor Airborne Bio Aerosols in Valiasr Hospital in Zanjan, Iran. *Journal of Human, Environment and Health Promotion*. 2015;1(1):41-8.
73. Tang JW. The effect of environmental parameters on the survival of airborne infectious agents. *J R Soc Interface*. 2009;6 Suppl 6(Suppl 6):S737-S46.
74. Hoseinzadeh E, Samarghandie M, Ghiasian S, Alikhani M, Roshanaie G, Moghadam Shakib M. Qualitative and quantitative evaluation of bioaerosols in the air of different wards of governmental Hamedan hospitals, during 2011-2012. *Yafteh*. 2012;14(4):29-39.
75. Li C-S, Hou P-A. Bioaerosol characteristics in hospital clean rooms. *Science of the Total Environment*. 2003;305(1-3):169-76.
76. 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.
77. Li Y, Wang W, Guo X, Wang T, Fu H, Zhao Y, Wang W. Assessment of airborne bacteria and fungi in various university indoor environments: a case study in Chang'an University, China. *Environmental Engineering Science*. 2015 Apr 1;32(4):273-83.
78. Favero MS, Puleo JR, Marshall JH, Oxborough GS. Comparison of microbial contamination levels among hospital operating rooms and industrial clean rooms. *Appl Environ Microbiol*. 1968;16(3):480-6.
79. Sivagnanasundaram P, Amarasekara R, Madegedara R, Ekanayake A, Magana-Arachchi D. Assessment of Airborne Bacterial and Fungal Communities in Selected Areas of Teaching Hospital, Kandy, Sri Lanka. *BioMed Research International*. 2019;2019.
80. Hazrati S, Valedeyni asl F. Types and concentration of fungal and bacterial bio-aerosols in hospital indoor air of Imam Khomeini and Alavi hospital in Ardabil city: Ardabil University of Medical Sciences; 2016.
81. Sautour M, Sixt N, Dalle F, L'Ollivier C, Fourquenot V, Calinon C, et al. Profiles and seasonal distribution of airborne fungi in indoor and outdoor environments at a French hospital. *Science of the total environment*. 2009;407(12):3766-71.
82. Sarica S, Asan A, Otkun MT, Ture M. Monitoring indoor airborne fungi and bacteria in the different areas of Trakya University Hospital, Edirne, Turkey. *Indoor and built Environment*. 2002;11(5):285-92.
83. Stockwell RE, Ballard EL, O'Rourke P, Knibbs LD, Morawska L, Bell SC. Indoor hospital air and the impact of ventilation on bioaerosols: a systematic review. *Journal of Hospital Infection*. 2019.
84. 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.