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Seasonal and spatial variations of bioaerosols and antibiotic resistance bacteria in different wards of the hospital

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ABSTRACT

Introduction: Transmission of bioaerosols through the air is known as an important route for a wide range of nosocomial infections. Therefore, in the present study, we aimed to evaluate the type and diversity of bioaerosols and antibiotic resistance of bacterial bioaerosols in the indoor environments of Sina educational and treatment hospital, Tabriz, Iran.

Methods and materials: 150 samples of bacteria and fungi (75 fungi and 75 bacteria) bioaerosol samples were collected on petri dish containing Sabouraud dextrose agar from February to March and June to July 2020 in three periods of daytime (morning, noon and evening) according to National Institute for Occupational Safety and Health (NIOSH 0-800) standard. After sampling, fungal and bacterial samples were incubated and the disk diffusion agar method (Kirby-Bauer) was used for assessing the antibiotic resistance.

Results: The concentration of bioaerosols varied significantly in different wards. In addition, the concentration of bioaerosols in winter was observed to be higher than in summer. The highest and lowest airborne fungal concentrations were found in burns operating room and men's infectious ward (49 CFU/m^3) and children's burns ward (28 CFU/m^3), respectively. The predominantly isolated bacteria were Streptococcus spp. (38%) and Staphylococcus spp. (37%). Also, the main isolated fungi belonged to the genera Aspergillus (75.9%) and Penicillium (22.5%). The highest rates of antibiotic resistance were observed for colistin (100%) in Gram-negative and penicillin (84.2%) in Gram-positive.

Conclusion: Timely and regular disinfection of hospital wards can affect the density of bioaerosols. Owing to the prevalence of COVID-19 epidemic in the world, the staff and patients often were wearing masks, gloves and special clothing as well as using disinfectants to prevent coronavirus infection in wards during the summer sampling.

Introduction

Indoor air quality is one of the most significant factors affecting the health of people because humans spend more than 90% of their time in closed spaces. In indoor environments, various

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pollutants including airborne Particulate Matter (PM) and microorganisms can exist that enter the human lungs through breathing [1, 2].

There are a wide range of microorganisms in the indoor air, known as bioaerosols [3]. Bioaerosols include dead or living pathogenic or non-pathogenic bacteria, viruses, fungi, molds, high molecular weight allergens, bacterial endotoxin, fungal toxins, residues of bacteria, pollen and fibril(1, 3). These microorganisms can cause allergic reactions, asthma, edema of the nasal mucosa, infections, rhinitis and other respiratory diseases [4, 5].

One of the most important places contaminated with airborne bioaerosols is health centers and hospitals, where the presence of bioaerosols in indoor air causes a public health issue [5]. It is estimated that airborne bioaerosols cause approximately 10 to 20% of hospital pollution and infections [6]. In this regard, World Health Organization (WHO) has offered the maximum hospital guideline values for bacteria 100 CFU/m^3 and fungi 50 CFU/m^3. Therefore, Indoor Air Quality (IAQ) needs special attention to ensure protection of the patients and health care workers against nosocomial infections [7].

The concentration of bioaerosols can vary from one ward to another in a hospital and also from one hospital to another in a city or region [3]. The presence of bioaerosol in hospital wards is associated with several factors including ward type, condition and type of patients, temperature, ventilation, air conditioning systems, humidity, types of surgery, season, disinfectant using, design and operation of indoor as well as microbial load in the ambient air [1, 8].

Widespread use of antibiotics and disinfectants for a long time reduces their effectiveness and leads to antibiotic resistance [9]. WHO has introduced antibiotic resistance as one of the three major public health threats for the 21st century [10]. Centers for Disease Control and Prevention (CDC) estimates that 23,488 deaths occur each year in the United States from antibiotic-resistant infections and 11,000 deaths are attributed to methicillin-resistant Staphylococcus aureus [11]. It is estimated that 10 million people will die from antibiotic resistance by 2050 worldwide, which will impose about 100 trillion dollars to the global economy [12].

As the rate of nosocomial infections can be directly related to the density and type of bioaerosols, it is important to determine the type and density of these microorganisms. The quantitation and qualification of fungal and bacterial bioaerosols in the hospitals indoor air can be used in planning and constructing healthy environment for patients and hospital staffs. In addition, bacterial antibiotic resistance assessment can provide useful information to understand the extent of infection and control antibiotic resistance; specialists can also use antibiotics with better therapeutic properties to treat the patients [13] .

Several studies have been performed on bioaerosols in hospitals, both indoors and outdoors, and of course, the pattern of antibiotic resistance [1, 13, 14]. To the best of our knowledge, there is no published study about concentration and type of bacterial and fungi bioaerosols and antibiotic resistance of bacterial bioaerosols at Sina hospital, as one of the large hospitals in Tabriz.

Therefore, in the present study, we aimed to evaluate the type, density and diversity of bacterial and fungi aerosols in indoor environments of Sina educational and treatment hospital, Tabriz, Iran. Then, antibiotic resistance of the identified bacterial bioaerosols was examined.

Name of hospital: Sampling season:										
No of sample	Sampling Data	Sampling time	Name of ward	Number of bed	Number of staff	Number of visitors	Type of air conditioner	Life of the building (year)	Temperature (T^{oC})	Humidity (%)

Materials and methods

Sampling area

Sina educational and treatment hospital is a specialized and sub-specialized hospital with 510 beds affiliated to Tabriz University of Medical Sciences, Tabriz, Iran. This hospital has 333 active beds along with 23 active wards (inpatient wards, preclinical and specialized and sub-specialized wards) and 7 intensive care wards. The prepared table to collect information on wards, buildings and environmental conditions at the sampling time is given below.

In this study, bacterial and fungal bioaerosols were evaluated from February to March (winter) and June to July (summer) 2020, during 6 am to 5 pm in three periods of daytime (morning, noon and evening) in the mentioned wards of this hospital.

Sampling method

In total, 150 samples of bacteria and fungi (75 fungi and 75 bacteria) were collected from different wards including Men's Infectious Ward (MIW), Women's Infectious Ward (WIW), Skin Ward (SW), Burn Operating Room (BOR), Men's Burn Ward (MBW), Women's Burn Ward (WBW), Children's Burn Ward (CBW) and Burn Intensive Care Unit (BICU) .It should be noted that in order to evaluate and compare active and passive sampling results, active and passive sampling was performed simultaneously in the BOR.

Active sampling was performed according to National Institute for Occupational Safety and Health (NIOSH 0-800) standard [13]. In this method, the samples were collected by a Quick Take sample pump -30 equipped with standard bio-stage impactor containing 400 holes (Bio Stage Single-stage Impactor, SKC, Inc., USA) for 5 min with the flow rate of 28.30 L/min. One 100 mm diameter Petri dish containing Sabouraud dextrose agar (Merck, Germany) containing 50 mg/L chloramphenicol for fungi and blood agar (Merck, Germany) for bacterial colonies, and eosin methylene blue (EMB) (Merck, Germany) for Gram-negative bacteria were employed. Air samples were collected approximately 1.5 m from the floor and 1 m distance from walls and barriers [15].

Passive sampling was performed as the standard pattern of 1,1,1 [16]. Briefly, Petri dishes, having all 3 types of culture medium, were placed in the sampling site at the distance of 1 m above the floor and 1 m away from walls or major barriers for 1 h. For determining the number of bioaerosol in passive sampling method, Koch deposition method was used according to Polish standard PN 89 / z-04008/08 and bioaerosol was reported as CFU/m^3. Briefly, in this method, the bioaerosol settled directly from the air of sampling area on the surface of the prepared plate, having suitable nutrient, for the time of 5-10 min [17, 18].

Before each sampling, the sample was sterilized with 70% ethanol to remove any contamination; after sampling, all the sampled plates were sealed with the Para-film to prevent secondary contamination and immediately transferred to

the microbiology laboratory using the cooled box. To investigate the effect of temperature and humidity on the concentration of bioaerosols, relative humidity (%) and temperature (°C) were measured by Lutron WBGT 2010SD portable device during the sampling time.

Incubation and identification of bacteria and fungi

To identify the organisms, the fungi samples were incubated for 24-96 h at 25-28 °C; the bacterial samples were incubated at 35°C for 24-48 h [5, 19]. The number of colonies was counted and reported as CFU/m^3. Airborne fungal colonies transferred to SDA plates were morphologically classified according to the color and shape of the spores. The fungi colonies were identified according to their microscopic shape. Identification of dominant colonies up to their genera was performed through preparing wet–mount slides with lacto phenol blue solution and observation under microscope. The bacterial colonies were identified through routine bacteriological tests including Gram's stain, oxidase, catalase and tubular coagulase. Diagnostic gallery test protocols (NCCLS1) were used to identify Gram-negative (NCCLS1) (20, 21).

Determination of antibiotic resistance

To evaluate antibiotic resistance, 40 bacterial bioaerosol isolates were randomly selected, including 34 strains of Staphylococcus aureus, 2 strains of Pseudomonas aeruginosa, 3 strains of Acinetobacter spp. and 1 strain of Enterobacter aerogenes. The disk diffusion agar method (Kirby-Bauer) was used for assessing the antibiotic resistance according to the Clinical and Laboratory Standards Institute's (CLSI) guidelines.

The antibiotic discs included tetracycline (30 µg), erythromycin (15 µg), amikacin (30 µg), rifampin (5 µg), gentamicin (10 µg), ampicillin-sulbactam (10 µg), clindamycin (2 µg), cefoxitin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), colistin (10 µg), trimethoprim-sulfamethoxazol (25 µg), oxacillin (1 µg), penicillin (10 µg), vancomycin (30 µg), cefotaxime (30 µg), cefazolin (30 µg) and piperacillin-tazobactam (100/10 µg). The inoculated antibiotic discs were incubated at 35 °C for 18-24 h.

Data analysis

SPSS software (ver. 26; Chicago, IL, USA) was used for data analysis by the descriptive statistics. Data normality was evaluated using one-sample Kolmogorov-Smirnov test. Spearman correlation coefficients were used for investigating the relationship between bioaerosols with temperature and relative humidity. The relationship between wards, type of antibiotic and antibiotic resistance was analyzed using Chi-square test. The significance was set at $p \leq 0.05$.

Results and discussion

Airborne bacterial and fungal concentrations

The results of the present study showed that the average total density of fungal bioaerosols in winter and summer was (21.2 ±11.6 CFU / m^3 and 10.4±11.5 CFU/m^3, respectively). Moreover, the average total bacterial bioaerosol density in winter and summer was 64.3 ± 136 CFU/m^3 and 14.9 ± 25.3 CFU/m^3, respectively. In general, the highest counts of bacterial and fungal bioaerosols were 5112 CFU/m^3 and 1876 CFU /m^3, respectively. Based on the data obtained, the density and concentration of airborne bioaerosols in different wards of the hospital were significantly different. Similar to our study, another study reported the average amount of airborne bacterial bioaerosols was higher than the level of fungal bioaerosols in all the study ward.

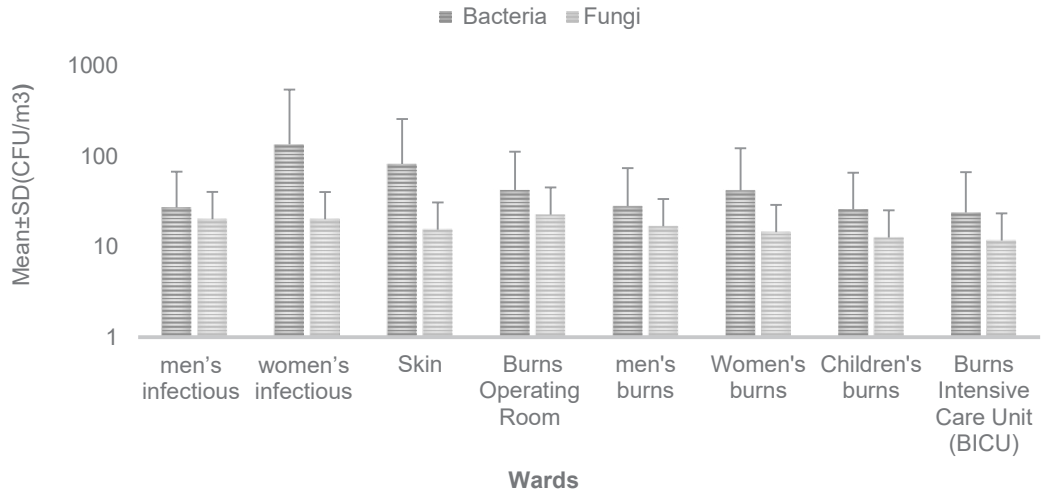

Fig. 1. Concentration of bacteria and fungi (mean±SD) in the air of hospital wards according to logarithm

The highest bacterial density was observed in WIW (835 CFU/m^3), which could be due to the high number of patient, companions and nurses, lack of air conditioning and closed windows for several hours [19, 22]. The lowest bacterial density was detected in MIW (119 CFU/m^3). On the other hand, the highest concentration of fungal bioaerosols was found at BOR and MIW (49 CFU/m^3) and the lowest was observed at CBW (28 CFU/m^3), which can be explained by the less number of patients and staff, air conditioning system and meeting from behind the glass enclosure in these wards. In a study it was found the lowest microbial load in the isolation room, where visitors meet patients behind glass enclosure or using monitor [23], and indicated the importance of the presence of visitors in the wards. Results of this study revealed that the total density of fungal bioaerosols to the separation of wards was not higher than the maximum standard allowed by the WHO's guidelines (50 CFU/m^3) [7]. Our results showed that the total concentration of fungal and bacterial bioaerosols in different wards and different sampling times did not have a normal distribution; thus, Kolmogorov-Smirnov test was used. Bacterial and fungal bioaerosols were negatively correlated (not significantly) with relative humidity (Spearman correlation coefficients were -0.099 and -0.103, respectively) ($p> 0.05$). Also, the results showed no significant relationship between the temperature and density of bacterial aerosols; the results of a study contradicted with our results [14]. Finally, the results revealed a significant correlation between temperature and fungal bioaerosol concentration ($p<0.05$), while in a study it was found that indoor temperature and humidity did not significantly affect the bacterial and fungal loads [23].

Effect of the hospital characteristics on concentration of bioaerosols

The physical condition of the building is one of the main factors affecting the concentration of bioaerosols in different wards of the hospital. It should be noted that Sina hospital has two buildings for the treatment of patients. At this hospital, men's infectious ward, women's infectious wards and skin wards were located in the old building and the other wards were located in the new building.

The highest levels of bacterial bioaerosols were found in WIW and the SW (mean: 84.8

CFU/m^3 and 81.8 CFU/m^3, respectively), which could be due to the lifespan of the building, non-standard flooring, consumable materials, seams in the walls, high number of old beds in the wards, natural ventilation and high density of patients in the wards. Lower concentrations of bioaerosols were found in other wards located in the new building. Many studies have stated that one of the main factors increasing production and release of airborne bioaerosols is the number of patient beds [3, 23]. In addition, some studies have shown that artificial air conditioning could reduce the number of bacteria in the indoor environment [19]. It should be noted that the particles produced in any place can be quickly transferred to other places, even if the rooms are separate [22, 24].

Sina Hospital is located near the main street and is very crowded. Since there is a large volume of traffic in the hospital yard, it can cause bioaerosols to penetrate into the hospital building, especially in the older building. Finally, the source of contamination may have originated from sources around the hospital [25]. Therefore, factors such as dust, sterilization, biological contaminants, cleaning, physical condition of the building, hospital staff, visitors and sick building syndrome can contribute to the spread of diseases and infection in the hospital. In addition, air conditioning could be used to prevent the spread of diseases and infection in the hospitals [8].

Identification of airborne bacterial and fungal species

Table 1 presents the frequencies of the bacteria isolated from different wards of this hospital. The results of our study showed that the predominant bacterial species included Streptococcus spp. (38%), Staphylococcus epidermidis (36%), Bacillus cereus (11%), Micrococcus spp. (10%) and Staphylococcus aureus (0.01%). In addition, a fewer number of Acinetobacter spp., Enterobacter aerogenes and Pseudomonas aeruginosa were found, which could be dangerous for humans and patients.

Staphylococci are a group of Gram-positive bacteria that are present in all open organs of the body including the skin and organs with static mucus. Important species of Staphylococcus include S. Epidermidis, S. Saprophyticus, S. hemolyticus and S. aureus, which are important factors for infection in high-risk groups of patients and, sometimes, cause death [26]. S. aureus is one of the most important species of staphylococci, which can cause diseases by colonization and producing exoproteins in the host [27]. Staphylococcal infections are transmitted through contact with an infected person or private belongings including clothing, bedding and sheets. Hospital staff also act as carriers of these microorganism [28]. S. aureus can cause superficial skin lesions, from deep abscesses to sepsis, pneumonia, osteomyelitis and endocarditis [29]. P. aeruginosa is a Gram-negative bacterium that can adapt to different environments [30]. This bacterium is one of the most common causes of opportunistic infections and burn wound [31], which can be one of the causes of sepsis leading to burn death [32]. The infectious diseases caused by this bacterium are limited and depend on the defense and safety of the host [33]. They cause 10-15% of nosocomial infections worldwide [34]. In the present study, this opportunistic pathogen was found in children's burns and men's infections wards, which can be dangerous.

Another important detected pathogen in the present study was Acinetobacter spp. This Gram-negative bacterium is known as another important cause of nosocomial infections and is one of the most common colonizing pathogen found in burn patients in the world [35]. Acinetobacter spp. can grow on the skin, throat, sputum, urine and feces. Therefore, it can cause pneumonia, meningitis, bacteremia, soft tissue

infection, surgical site infection, peritonitis, endocarditis, catheter-related infections and urinary tract infections [36]. The mortality rate of patients infected by Acinetobacter spp. is twice as high as that of P. aeruginosa [36].

Table 2 presents the frequencies of the fungi isolated from different wards of this hospital. The frequency of fungi isolated from the air of different wards was Aspergillus niger (32.7%), Aspergillus flavus (26.5%), Penicillium olsonii (13%), Penicillium corylophilum (9.5%), Aspergillus fumigatus (9.2%), Aspergillus versicolor (7.5%), Alternaria spp. (0.7%), Nocardia spp., Cladosporium spp. and Trichophyton mentagrophytes (0.3%). The most common fungi isolated from the hospital environment in one of the recent studies included Penicillium spp., Cladosporium spp., Aspergillus niger, Aspergillus flavus and sterile micelles [3]. In general, the genera Aspergillus and Penicillium were the most common fungal bioaerosols that have high growth ability in different climatic conditions and remain in the air by producing small and light spores [37]. It should be noted that presence of Aspergillus spp. in the indoor air of hospitals was considered as a risk factor for patients due to their ability to cause nosocomial infections and allergies [19]. Penicillium spp. are one of the most common fungi in the environment and are usually considered as non-pathogenic to humans. However, they can cause opportunistic infections and even death in immunocompromised hosts [38].

Table1. The max and mean ± SD of isolated bacteria from different wards (CFU/m^3)

Ward		*S. aureus*	*S. epidermidis*	*Streptococcus spp.*	*Bacillus cereus*	*Micrococcus spp.*	*Pseudomonas aeruginosa*	*Acinetobacter spp.*	*E. aerogenes*
MIW	max	7	77	35	21	56	7	ND	7
	Mean ± SD	0.9±2.4	13.5±23.4	6.5±12.8	2.3±6.2	3.7±14.4	0.9±2.4	ND	0.9±2.4
WIW	max	7	98	688	14	42	ND	7	7
	Mean ± SD	0.4±1.8	18.3±31.6	51±176.9	6.5±6.7	6.6±12.7	ND	0.9±2.4	0.9±2.4
SW	max	7	259	350	56	28	ND	ND	ND
	Mean ± SD	0.4±1.8	32.3±68.9	35.5±91.5	8.4±15.9	5.1±9.7	ND	ND	ND
BOR	max	7	70	92	14	35	ND	7	ND
	Mean ± SD	0.7±2.3	14.7±24.8	13.3±30.9	4.6±6	8±15.8	ND	1.5±3	ND
MB	max	7	84	56	28	14	ND	7	ND
W	Mean ± SD	0.4±1.8	13.5±26.2	7±15.4	5.1±8.1	1.4±3.9	ND	0.9±2.4	ND
WB	max	7	127	99	28	28	ND	ND	ND
W	Mean ± SD	0.4±1.8	21±42	10.4±27.6	6±9.8	3.7±9.8	ND	ND	ND
CBW	max	7	56	35	35	42	7	ND	ND
	Mean ± SD	1.4±2.8	9.3±16.2	5.1±11.3	5.1±10.4	4.7±11.6	0.9±2.4	ND	ND
BIC	max	7	42	56	7	35	ND	ND	ND
U	Mean ± SD	0.4±1.8	7.9±13.9	7.4±16.8	1.8±3.2	6±12	ND	ND	ND

ND: not detected

Table 2. The max and mean±SD of isolated fungi from different wards (CFU/m^3)

	Ward	*A. flavus*	*A.niger*	*A.vercicolor*	*P. Olsonii*	*P.corylophilum*	*A.fumigatus*	*Cladosporium*	*Trichophyton mentagrophytes*	*Alternaria spp*
MIW	max	21	14	21	7	ND	14	ND	ND	ND
	Mean ± S. D	6.5±7.2	6±3.6	2.8±5.7	2.3±3.4	ND	1.8±4.1	ND	ND	0.4±1.8
WIW	max	21	14	ND	14	7	7	ND	ND	ND
	Mean ± S. D	6.5±5.5	6.5±4.1	ND	2.8±4.4	1.4±2.8	2.8±3.5	ND	ND	ND
SW	max	14	14	ND	14	7	14	ND	ND	ND
	Mean ± S. D	3.2±4.4	7±2.6	ND	1.4±3.9	1.4±2.8	2.3±4.3	ND	ND	ND
BOR	Max	21	21	7	14	7	ND	7	ND	ND
	Mean ± S. D	4.6±7.8	7.7±8.1	1.5±3	7±4.9	0.7±2.3	ND	0.7±2.3	ND	ND
MBW	max	21	14	7	7	7	7	ND	7	ND
	Mean ± S. D	4.6±6.8	7.4±1.8	1.4±2.8	0.9±2.4	0.9±2.4	0.9±2.4	ND	0.4±1.8	ND
WBW	max	7	14	ND	7	7	21	ND	ND	7
	Mean ± S. D	1.4±2.8	4.6±4.3	ND	.9±2.4	2.3±3.4	4.6±7.3	ND	ND	0.4±1.8
CBW	max	14	7	7	7	7	ND	ND	ND	ND
	Mean ± S. D	5.1±5.5	0.4±1.8	1.4±2.8	2.8±3.5	2.8±3.5	ND	ND	ND	ND
BICU	max	14	7	7	ND	28	ND	ND	ND	ND
	Mean ± S. D	2.8±4.4	4.2±3.5	0.9±2.4	ND	3.7±9.8	ND	ND	ND	ND

ND: not detected

Variations of bioaerosols during daytime and seasons

The mean of total bacterial bioaerosols concentration was measured as 102.9, 25.7 and 33.4 CFU/m^3; and the mean of total fungal bioaerosols was 23.3, 15.4 and 13.5 CFU/m^3 in the morning, afternoon and evening, respectively. The high amount of bioaerosol in the morning (6-8 AM) may be due to the presence of many students, staff and more visits to the emergency department. Researchers demonstrated that due to the high density of people, the amount of bioaerosols in the afternoon shift was higher than other times during the day.

Evening sampling (3-5 PM) was performed during and after families' visit. The results revealed that the amount of fungal bioaerosols in the evening shift was more than that at noon. In a study researchers reported that the fungal bioaerosols were increased at evening shift; this could be due to the number of people in wards, which has a significant effect on the density of bioaerosols. Astudy showed that in the indoor air of different wards of hospitals, the microbial population changed during daytime and the highest density of bacterial and fungal bioaerosols was observed from 5 AM to 6 PM [39].

The results of this study showed that the density of bioaerosols was significantly higher in winter than summer. While other studies have reported that the density of bioaerosols is significantly higher in summer than in winter [19], many researchers reported that the average concentration of total bioaerosols in different seasons did not differ significantly [40]. This study revealed that seasonal changes affected the concentration of bioaerosols, use of masks for patients and staff, absence of visitors and companions' routine and continuse disinfection of surfaces and equipment, etc. in the summer due to the spread of coronavirus and decreased the concentration of bioaerosols in summer. It should be noted that during winter, the sample was collected under routine

and normal working conditions, but in summer, due to the pandemic, many restrictions such as family visit were imposed and the movement of people (staff, visitors, patients, etc.) inside the hospital was very low and limited.

The results showed that the percent of some bioaerosols was higher in summer than in winter, but the average of total bacterial and fungal bioaerosols was higher in winter than in summer. Among the various wards of the hospital, the operating room (especially the burn operating) is very important because susceptible patients are exposed to infection during surgery [41].

The amount of bacterial and fungal species identified during different seasons is shown in Fig 2. The results demonstrated that the important bacterial bioaerosols such as Acinetobacter spp. and E.aerogenes were identified in the burn operating room and burn wards, which may be due to the high percent of burn patients in the burn ward and operating room. More particularly, S.aureus which is identified in the operating room, could be one of the causes of hospital infection in this ward (19).

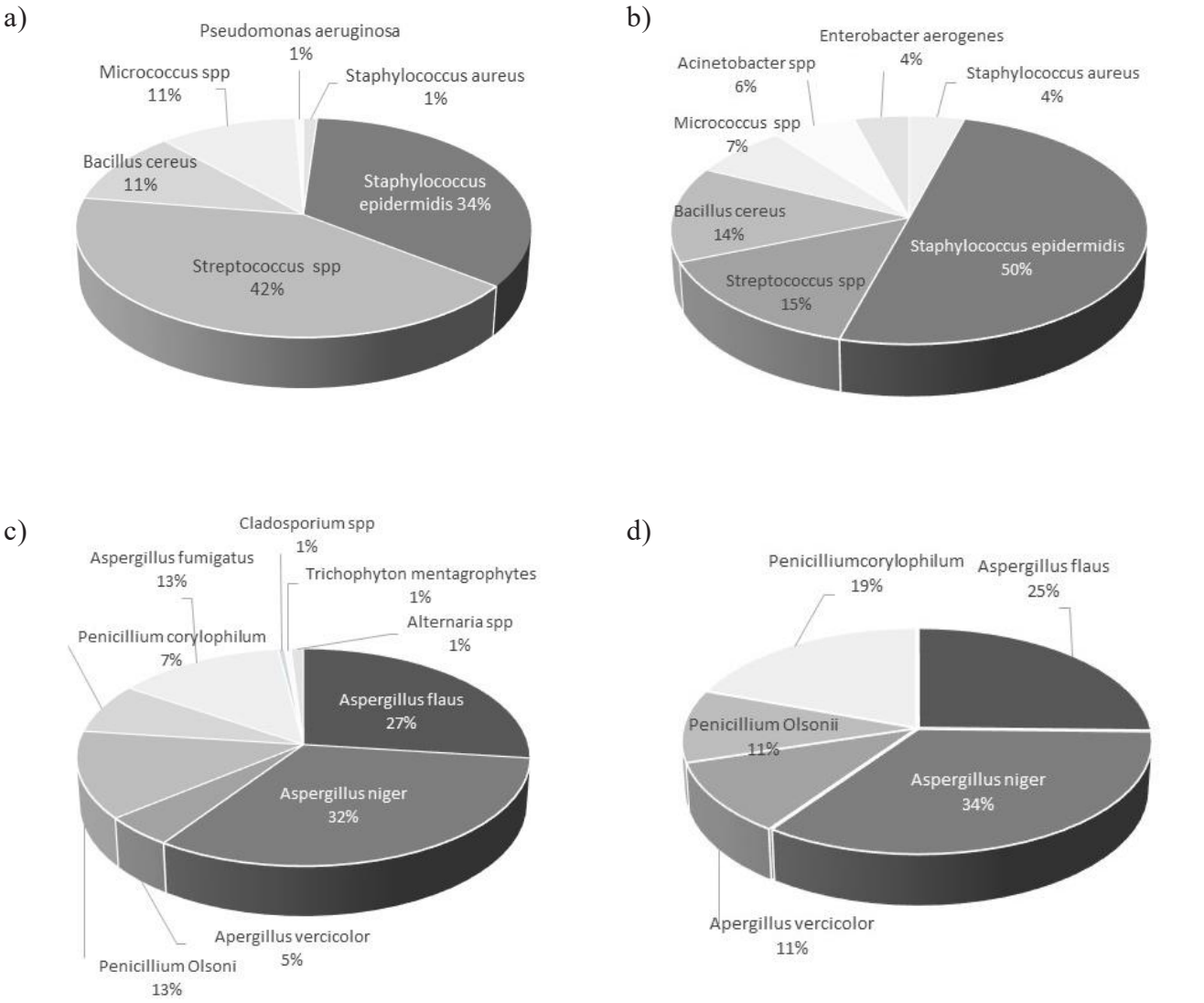

Fig. 2. Amount (%) of bacterial and fungal species identified during different seasons; amount (%) of bacterial species in winter (a) and summer (b); amount (%) of fungal species in winter (c) and summer (d)

Effect of sampling method (active or passive) on bioaerosol concentration

In the present study, active and passive sampling was performed simultaneously in the operating room. BOR is located on the fourth floor and sampling was performed during surgery. The results revealed that the rate of bacterial bioaerosol concentration was 206 and 127 CFU/m^3 in active and passive sampling methods, respectively. On the other hand, the rate of fungal concentration was measured 49 CFU/m^3 and 43CFU/m^3 in the active and passive procedures, respectively.

The finding of this study cleared that the sampling method was able to affect the concentration of identified bioaerosol and the concentration of bioaerosols in active sampling was higher than passive sampling. Many researchers reported a significant relationship between the concentration of bioaerosols in active and passive methods [42].

The factors that can increase the amount of bioaerosols in the operating room can be due to unnecessary conversation during surgery, presence of additional staff in the operating room during surgery, use of shared shoes in the operating room during surgery and in the ward, as well as, use of mobile phones during surgery by surgeons. Studies by many researchers showed that in hospital operating rooms, bioaerosol contamination was mainly dependent on the release of human hair, skin and respiratory system during the operation [43].

Antibiotic resistance

Antibiotic-resistant bacteria, which are difficult or impossible to treat, have become increasingly more in health settings. Gram-positive cocci such as Staphylococcus spp. and Micrococcus spp. are the natural human flora. Staphylococcus spp. and Micrococcus spp. were the main bacteria isolated from airborne samples in the hospital, which was consistent with the study by many researchers [44]. Also, Gram-negative bacteria that grew on the plate surface including, Acinetobacter spp., P. aeruginosa and E.aerogenes can have a near relationship to health-related infections through hospital indoor air [45]. Increasing the pattern of antibiotic resistance by these species reduces the range of therapeutic drugs [46].

The pattern of resistant antibiotics was colistin (100.0%), penicillin (84.2%), ceftazidime (75.0%), cefoxitin (57.9%), erythromycin (47.4%), amikacin (40.0%), cefazolin (26.3%), clindamycin (26.3%), ampicillin-sulbactam (25.0%), cefotaxime (25.0%), ciprofloxacin (24.0%), gentamicin (24.0%), vancomycin (21.1%), piperacillin-tazobactam (20.0%), tetracycline (13.6%), rifampin (10.5%), trimethoprim-sulfamethoxazole (8.7%) and oxacillin (5.3%). The level of colistin resistance can be very worrying because it is used as a 'last resort' against Gram-negative aerobic pathogens such as K. pneumoniae, A. baumannii and P. aeruginosa [47], which can indicate the importance of resistance to this antibiotic [48]. Studies by researchers showed that the highest antibiotic resistance was in cefxime (45.8%), cefazolin (30.2%), gentamicin (12%) and ciprofloxacin (12%). The results of our studies demonstrated that the highest antibiotic resistance was observed in S. aureus to penicillin (84.2%), in P. aeruginosa to, Colistin, Amikacin and Trimethoprim sulfamethoxazol (100%), in Acinetobacter spp. to Colistin (100%) and in E. aeruginosa to Ciprofloxacin, Gentamicin and Ceftazidime (100%). Also, there was a significant relationship between bacterial bioaerosol type and antibiotic resistance ($p<0.05$). Our results showed that the total antibiotic resistance in S. aureus bioaerosol was 37% (sensitive), 36.1% (intermediate) and 26.9 % (resistant); the highest resistance was in the strain isolated from BOR. In P. aeruginosa, 27.3% (inter meditate),

72.7% (resistant) and the highest resistance was observed in CBW. In Acinetobacter spp. 46.7% (sensitive), 16.7% (inter meditate), 36.7% (resistant) and the highest antibiotic resistance was found in WBW and lowest in the BOR. Finally, E. aerogenes in IWW with 57.1% (sensitive) and 9.42% (resistant) was reported.

Conclusion

Bacterial and fungal bioaerosols were isolated from all the samples. Our results showed that the bacterial and fungal bioaerosols in different wards varied in type and concentration. According to our findings, the number of patients, visitors and hospital staff, the ventilation system, site disinfection, season, temperature, building longevity and relative humidity had a significant effect on the number of bioaerosols in the hospital environment. With the expansion of COVID-19 during summer sampling, the total concentration of bacterial and fungal bioaerosols in summer was lower than in winter. Timely and regular disinfection of hospital wards can affect the density of bioaerosols. Owing to the prevalence of COVID-19 epidemic in the world, the staff and patients often wore masks, gloves and special clothing, as well as using disinfectant to prevent coronavirus infection in wards.

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Competing interests

The authors declare that they have no co¬mpeting interests.

Authors' contributions

S.H was the main investigator, who designed and performed the study and drafted the manuscript, while A.G supervised the study. Moreover, H.S.K, S.M, were advisors of the study. All the authors read and approved the final manuscript.

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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 ob-served by the authors."

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