



Indoor exposure to airborne bacteria and fungi in sensitive wards of an academic pediatric hospital

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Received: 12 June 2019 / Accepted: 7 January 2020
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Abstract Airborne bacteria and fungi in hospital environments are of great concern due to their potential role as a source of nosocomial infections. The aim of this study was to evaluate concentration and diversity of airborne bacteria and fungi in relation to particle mass concentration in sensitive wards of a pediatric hospital. The study was performed in the cardiac care unit (CCU), the neonatal intensive care unit (NICU), the cancer blood ward (BW), the ENT (ear, nose, throat) operation room (OT1) and the eye operation room (OT2). The air samples were collected by impaction using the single-stage Andersen sampler.

The flow rate and sampling time of the pump were adjusted to 28.3 l/min for 5 min. The mean concentration of indoor airborne fungi and bacteria ranged from 0–63 to 19–356 CFU/m³, respectively. OT2 and CCU wards were the most contaminated wards for airborne bacteria and fungi, respectively (243 ± 77 vs. 30 ± 7 CFU/m³). The airborne Gram-positive cocci (*Staphylococcus* and *Micrococcus*) were the most detected bacterial genera (75%) in all indoor air samples, and the most prevalent genera in indoor environment were *Cladosporium* spp. (19%) followed by *Penicillium* spp. (16%), *Aspergillus* spp. (16%) and *Paecilomyces* spp. (10%). Results showed that the outdoor airborne bacteria and PM concentration at different sizes were significantly higher than indoors, suggesting that the indoor airborne particle may have originated from the outdoor air. There were significant positive relationships between indoor airborne fungi concentrations with indoor PM_{2.5} and PM₁₀.

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Keywords Airborne bacteria · Fungi · Pediatric
hospital · Particle · Nosocomial infection

1 Introduction

Nosocomial or health-care-associated infections (HAIs) are a main cause of morbidity and mortality in hospitalized patients (Armour et al. 2018). According to reports by WHO, about 15% of all hospitalized

patients (1.4 million people) suffer from nosocomial infections (Khan et al. 2017). The rate of total infections in hospitals of developing countries is three times higher than in highly developed countries, while reported rate of infections in neonatal wards is 3–20 times (Nejad et al. 2011). Nosocomial infections are cause for 4–56% of all infant mortality (Khan et al. 2017). The most vulnerable group to HAIs is hospitalized babies and children. Infants are at risk of various infections because they have not yet completely developed their immune systems. Untimely babies are mainly vulnerable since they often undergo aggressive procedures. Improvements in newborn attention have led to an increased survival rate, but hospital-acquired infections remain to be a serious concern. Throughout hospitalization, the patient is exposed to pathogenic microorganisms via various environment sources, infected patients and hospital staff (Wan et al. 2011). The spread of these infections must be limited to prevention. The recent previous studies suggest that environmental contamination has an important role in the nosocomial transmission of multi-drug-resistant organisms. The scientific evidences prove that methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa*, *A. baumannii* and *M. tuberculosis* are spread via the aerosol route (Mirhoseini et al. 2016a, b). In hospital environment, the important sources of aerial transmission include air-conditioning system, respiratory secretions of patients, infectious wounds and respiratory illness (Wan et al. 2011). Also, the hospital unit activities, such as those caused by medical staffs and visitors, overcrowding in sensitive wards and ventilation rate are considered the common causes of microbial air contamination (Tang and Wan 2013). Environmental surface contamination can also result from bioaerosols resuspended from hospitalized patients and contaminated devices (Knowlton et al. 2018). The assessment of concentration and types of bioaerosols in the hospital environment can be an efficient indicator of the cleanliness and sanitization level. Consequently, monitoring of microbial air quality in pediatric hospitals will help to detect the source of the infection and, as a result, can assist in reducing health-care-associated infection rates. The purpose of this study was to evaluate levels and diversity of bioaerosols and to assess its relationship with particle mass concentration in different wards in a pediatric hospital in Arak, Iran.

2 Materials and methods

2.1 The sampling sites

The study was conducted from September 2017 to April 2018 in an academic pediatric hospital of Arak University of Medical Sciences, Arak, Iran. Air samples were taken from five sensitive wards, including (1) the cardiac care unit (CCU), (2) the neonatal intensive care unit (NICU), (3) the cancer blood ward (BW), (4) the ENT (ear, nose, throat) operation room (OT1) and (5) the eye operation room (OT2). The characteristics of hospital wards are given in Table 1. Each ward in the hospital was visited eight times. At the time of investigation, all wards of the hospitals were equipped with the central operation, HVAC (heating, ventilating and air-conditioning systems). Furthermore, high-efficiency particulate air (HEPA) filters were within HVAC systems of OT wards. The sampling of air was conducted at a height of about 1.5 m above the ground level. Samples from five hospital wards were collected on one single day of week from 10 a.m. to 1 p.m. after normal cleaning procedures. Simultaneously, ambient concentrations were also measured during the study period. The environmental parameter, temperature and relative humidity were also measured with a humidity and temperature meter (TES-1365, Tiwan) at each site and were about $24\text{C} \pm 2.2\text{C}$ and $35\% \pm 6.7\%$, respectively. During sampling, visitors were limited, but patients, staffs and patient attendants were present.

2.2 Airborne culturable bacteria and fungi

Airborne bacteria and fungi were collected by impaction using the single-stage Andersen sampler (SKC Inc.) with 400 holes. The flow rate of the pump was adjusted to 28 l/min for 5 min. The Andersen impactor was loaded with agar Petri dishes (90 mm diameter) containing tryptic soy agar (TSA) with cycloheximide for bacteria and malt extract agar (MEA) with chloramphenicol for fungi. The sampler surface was disinfected with a 70% ethanol solution prior to each sampling. For total bacteria analysis, the TSA plates were incubated at $37\text{ }^{\circ}\text{C}$ for 2–3 days and the plates of MEA were incubated for 3–7 days at room temperature ($27 \pm 2\text{ }^{\circ}\text{C}$). The quantity of colonies was counted and calculated as colony-forming units per cubic meter (CFU/m³) of air using a positive-hole correction table to adjust colony counts

Table 1 Main characteristics of the hospital wards

Hospital ward	Construction age (year)	Year of construction	No. of beds	Ventilation system	Number of occupants
NICU	20	1999	5	Central operation HVAC	6–8
CCU	18	2001	12	Central operation HVAC	12–14
OT1	58	1961	3	HVAC with a HEPA filter	8–10
OT2	58	1961	3	HVAC with a HEPA filter	10–12
BW	16	2003	12	Central operation HVAC	8

HEPA, high-efficiency particulate air; *HVAC*, heating, ventilating and air-conditioning systems; *NICU*, neonatal intensive care unit; *CCU*, cardiac care unit; *OT1*, ENT (ear, nose, throat) operation room; *OT2*, eye operation room; *BW*, cancer blood ward

from a 400-hole impactor (Macher 1989). Bacterial isolates were identified according to Bergey's Manual and biochemical tests (Faridi et al. 2015). The fungi isolates were identified on basis of macro-morphological and the microscopic characteristics.

2.3 Particle mass (PM_1 , $PM_{2.5}$ and PM_{10}) concentration

The particle mass concentrations (PM_1 , $PM_{2.5}$ and PM_{10}) were measured by a laser photometer (TSI DustTrak 8520). The DustTrak was calibrated by ISO 12103-1 (Arizona Test Dust) and was calibrated to a zero filter during the measurement period.

2.4 Statistical analysis

Statistical analysis was conducted by SPSS 20.0. Kolmogorov–Smirnov test for normality was performed for the use of parametric or nonparametric tests. We obtained min, max, mean and standard deviation values to evaluate the density of airborne bacteria and fungi. The Kruskal–Wallis test was applied to compare the concentration differences of bioaerosol between the sampling locations. The relationships between particle numbers and airborne bacterial concentrations were examined using the nonparametric Spearman's rank correlation method. P values < 0.05 were statistically significant.

3 Results and discussion

3.1 Bioaerosol levels

The mean $\pm$ standard deviation (SD) number of airborne bacteria and fungi is summarized in Table 2.

The mean concentration of indoor airborne bacteria ranged from 19 to 356 CFU/m³. The highest bacteria mean (243 CFU/m³) in indoor air was observed at OT2 ward. Generally, the average number of bacteria in the investigated hospital wards was higher compared to former studies conducted by some authors in ICU and OT wards (Cristina et al. 2012; Dehghani et al. 2018; Hoseinzadeh et al. 2013; Ortiz et al. 2009). A possible description for the peak density of airborne bacteria in the OT wards could be deficient ventilation resulting from building age and design (Table 2). Bacterial air contamination of OTs during surgical actions could be a risk factor for surgical site infection due to sedimentation of airborne bacteria (Mirhoseini et al. 2015). There are variable threshold limits for the airborne bacteria in hospital OTs. Few countries have set airborne bacterial limits for OTs. For example, Switzerland and UK have been limited at 25 CFU/m³ and 35 CFU/m³, respectively, for airborne bacteria in ventilated OTs (Landrin et al. 2005). Iranian standard for hospital indoor air quality (IAQ) has adopted guidelines of the Centers for Disease Control and Prevention (CDC), the Healthcare Infection Control Practices Advisory Committee (HICPAC) and National Institute for Occupational Safety and Health (NIOSH) (Dehghani et al. 2018). The most reports recommend that an acceptable bacterial limit for a working OT is below 180 CFU/m³ (Shaw et al. 2018). However, our findings show higher airborne bacterial levels than the limits. The ventilation systems in this old hospital are not matching the up-to-date engineering standards. The limitations are mostly the site constraints and the financial constraints. The main source of bacteria in the OTs originates from the staff and is often a result of their surgical activities. Nevertheless, it seems that the indoor density of bacteria could be decreased via appropriate

Table 2 Concentration of airborne bioaerosols (CFU/m³) in indoor and outdoor sampling sites

Sampling site	Bacteria			Fungi		
	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD	Max	Min
NICU	152 $\pm$ 95	356	100	26 $\pm$ 12	63	6
CCU	127 $\pm$ 63	206	19	30 $\pm$ 7	44	25
BW	160 $\pm$ 82	281	13	8 $\pm$ 4	19	0
OT1	92 $\pm$ 55	256	31	16 $\pm$ 11	38	0
OT2	243 $\pm$ 77	356	88	17 $\pm$ 5	25	0
Outdoor	1052 $\pm$ 311	1519	344	22 $\pm$ 13	44	6

SD, standard deviation; NICU, neonatal intensive care unit; CCU, cardiac care unit; OT1, ENT (ear, nose, throat) operation room; OT2, eye operation room; BW, cancer blood ward

maintenance of air-conditioning systems and management of human activity, particularly in the hospital OT ward. In this study, the outdoor airborne bacteria level (1052 $\pm$ 311 CFU/m³) was significantly greater than the hospital indoor level (155 $\pm$ 75 CFU/m³) ($P > 0.05$). In the past, some studies have been indicated that the penetration of outside generated particulate matter into indoor environments has been detected to play an important role (Diapouli et al. 2011). Also, the penetration yield of airborne microbial is nearly 100% for a building with natural ventilation, meaning that airborne microbial moving via leaks and openings of building reaches indoors (Prussin and Marr 2015). The other study showed that the outdoor levels of airborne bacteria and virus were about two times higher than in indoor air, proposing that human density might not be the only factor in determining the microbial configuration of indoor air (Prussin et al. 2015). However, there was no significant difference between the outdoor concentrations of airborne fungi (22 $\pm$ 13 CFU/m³) and the indoor concentration (19.5 $\pm$ 6 CFU/m³) ($P > 0.05$). This highlights the inefficiency of hospital ventilation systems for reducing air fungi loads. As presented in Table 2, indoor fungi concentration ranged from 0 to 63 CFU/m³. The highest fungi levels were measured in the NICU ward (26 $\pm$ 12). Our findings also reveal that fungi level ranged between 0 and 38 CFU/m³ in hospital OTs. In this context, Dehghani et al. (2018) showed that the airborne fungi level was ranged from 4.83 to 18.40 CFU/m³ before cleaning actions and after the cleaning actions limited from 1.90 to 8.90 CFU/m³ in nine OTs of an educational hospital. Furthermore, airborne fungi in an OT ward of Nigeria

teaching hospital were obtained between 15 and 24.3 CFU/m³ (Ekhaïse et al. 2010). The airborne fungi concentration depends on several factors, such as ventilation, moisture, temperature, organic matter present in building materials but also outdoor fungal load (Abbasi and Samaei 2018). According to the presented results, the density of total airborne bacteria was higher than the fungal density in indoor and outdoor hospital environments, which is in agreement with some previous studies (Dehghani et al. 2018; Ekhaïse et al. 2010; Faridi et al. 2015). It can be related to the higher airborne concentrations of bacteria than fungi in the soil and the vegetation and the deposition of fungal spores because of the larger aerodynamic diameter (Després et al. 2012).

3.2 Particle mass concentration

Since the microbiological sampling and analysis are time-consuming and labor intensive, it was hoped that the monitoring particulate mass could provide a real-time proxy for bioaerosols. Table 3 shows indoor and outdoor particle mass concentration in the sampling locations. The highest of average PM concentration was observed in OT2 similarity with airborne bacteria level. Results of statistical test showed that the particle mass concentration at different sizes (PM_{1.0}, PM_{2.5} and PM₁₀) was significantly different in sampling wards ($p < 0.05$). Also, the outdoor PM concentration at different sizes was significantly higher than those indoor concentrations ($I/O < 1$), showing a lower number of airborne particles' indoors than outdoors. The relationship between airborne microorganism levels and PM concentrations (PM_{1.0}, PM_{2.5} and

Table 3 Mass concentrations and distribution ratios of suspended particles in indoor and outdoor environments

Sampling site	PM ₁ (μg/m ³) ± SD	PM _{2.5} (μg/m ³) ± SD	PM ₁₀ (μg/m ³) ± SD
NICU	34.6 ± 12.4	39 ± 11.8	49.8 ± 10.2
CCU	40 ± 14.3	44.6 ± 13.4	58.9 ± 15.3
BW	20.8 ± 10.6	23.1 ± 10.9	33.1 ± 12.2
OT1	30.1 ± 9.6	35.2 ± 9.7	39.5 ± 10.3
OT2	48.6 ± 18.5	53.9 ± 19.2	64.3 ± 19.8
Outdoor	65.3 ± 27.3	71.7 ± 27.8	90.3 ± 28.2

SD, standard deviation; NICU, neonatal intensive care unit; CCU, cardiac care unit; OT1, ENT (ear, nose, throat) operation room; OT2, eye operation room; BW, cancer blood ward

PM₁₀) is shown in Table 4. There were significant positive correlations between indoor airborne fungi concentrations with indoor PM_{2.5} and PM₁₀. The previous studies showed that in the normal activities of hospital staffs, the maximum concentration of unicellular (2–5 μm) and multicellular (> 10 μm) fungal spores is usually present in indoor environments and multicellular fungal spores such as *Alternaria* spp. make available a source of larger airborne fungal spore that can be resuspended during indoor activities (Qian et al. 2012). Therefore, it can be concluded that airborne fungi in these indoor environments are mostly existing as unicellular and multicellular fungi. Huang et al. (2018) reported modest and weak positive correlations between indoor airborne fungi with indoor PM_{1–2.5}, PM_{2.5–10} and PM₁₀, respectively. In our study, there was no significant relationship between airborne bacteria and fungi with PM₁ concentration. The past studies revealed that bioaerosols are commonly dispersed on coarse particles with a diameter greater than 1.7 μm, whereas only a proportion is dispersed on fine particles (Huang et al. 2018; Qian et al. 2012). The indoor concentration of PM₁₀ in

the studied NICU was substantially higher than levels measured in NICU (2.9 ± 0.8 μgr/m³) of a private hospital in the USA (Licina et al. 2016). They reported that the average concentrations of PM₁₀ in the single-infant rooms were generally low (< 5 μg/m³), which they attributed to useful building hygiene procedures, efficient particle filtration and relatively low population density.

3.3 Characteristics of bioaerosols

The results showed that airborne Gram-positive cocci (*Staphylococcus* and *Micrococcus*) were the most detected bacterial genera (75%) in all indoor air samples. Gram-negative cocci were also detected and found at higher frequency (6%) in NICU air. The Gram-positive bacilli were higher in OT1 compared to other hospital wards. Gram-negative bacilli were not detected in all air isolates. In the sampling hospital wards, the most predominant bacteria were those belonging to the genus *Staphylococcus* and *Bacillus*, which is consistent with other similar reports (Cabo Verde et al. 2015; León et al. 2016; Mirhoseini et al.

Table 4 Correlation coefficients between bioaerosol concentrations and particle mass concentrations

	Indoor bacteria (CFU/m ³)		Indoor fungi (CFU/m ³)	
	R	<i>p</i> value	R	<i>p</i> value
PM ₁ (μgr/m ³)	0.19	0.9	0.26	0.92
PM _{2.5} (μgr/m ³)	0.145	0.358	0.398**	0.009
PM ₁₀ (μgr/m ³)	0.157	0.32	0.369*	0.016

**p* value < 0.05

***p* value < 0.01

2015). The increase in these bacteria is usually a consequence of the production of a large number of spores of *Bacillus*, while *Staphylococcus* bacteria are related with ward dust and the skin scaling simply dispersed in the indoor air (León et al. 2016). The percentages of fungi and fungi genera in the hospital indoor air are shown in Table 5. The most prevalent genera were *Cladosporium* spp. (19%) followed by *Penicillium* spp. (16%), *Aspergillus* spp. (16%) and *Paecilomyces* spp. (10%). The detected *Aspergillus* fungal colonies included the species *A. flavus* (50%), *A. fumigatus* (31%) and *A. niger* (19%). Many surveys focused on isolation of *Aspergillus* species from the indoor hospital air due to its well-recognized implication on HAI (Cabo Verde et al. 2015; Dehghani et al. 2018; Rostami et al. 2016). Mosayebi et al. (2017) reported that hospital wet cooling system plays the main role in spreading of fungal contamination, particularly *Aspergillus* spp. in hospital indoor environment. Exposure to *Aspergillus* and *Penicillium* fungi can aggravate symptoms of asthma and allergic rhinitis in susceptible individuals (Mirhoseini et al. 2016a). Etienne et al. (Chen et al. 2010) have demonstrated the isolation of *A. fumigatus* from the air, surface and water samples in NICUs environment. For individual fungi, the concentration of *Aspergillus*

above 50 CFU/m³ has also been associated with a higher prevalence of sick building syndrome (Mirhoseini et al. 2016a). Study of microbial air quality in the private hospital in Egypt showed that *Aspergillus* and *Penicillium* were the most predominant fungal genera in the indoors and dust collected on air-conditioning filters. Also, *Aspergillus* was the dominant genera isolated in indoor environments of the OT (100%) and the ICU (90.9%) (Osman et al. 2018). Construction and renovation activities, carpeting, food, ornamental plants and unfiltered air ventilation system are reservoirs in hospitals for *Aspergillus* spp. Many studies have indicated that *Cladosporium* spp. is the main fungus and one of the most common isolates from indoor air of hospitals, which is consist with our results (Chaivisit et al. 2018; Pakshir et al. 2015). Although this genus is responsible for many fungal infections, it is a relatively uncommon cause of nosocomial infections. In this study, the indoor air of CCU (27%) had the highest and OT1 ward (15%) had the lowest fungal pollution. In addition, the main fungal species detected were similar to previous studies in indoor air of different hospitals (Awosika et al. 2012; Chaivisit et al. 2018). The predominant genera can be isolated from the environment that demonstrated they also came from an outdoor source. However, the

Table 5 Isolation rates of fungi (%) in surveyed hospital sites

Fungi	NICU (n = 23)	CCU (n = 29)	BW (n = 18)	OT1 (n = 16)	OT2 (n = 22)	Total (n = 108)
<i>Penicillium</i> spp.	6 (26%)	1 (3%)	4 (22%)	4 (25%)	2 (9%)	17 (16%)
<i>Cladosporium</i> spp.	5 (22%)	9 (31%)	1 (6%)	3 (19%)	2 (9%)	20 (19%)
<i>Aspergillus flavus</i>	3 (13%)	2 (7%)	1 (6%)	ND	2 (9%)	8 (7%)
<i>Aspergillus niger</i>	ND	ND	ND	2 (13%)	1 (5%)	3 (3%)
<i>Aspergillus fumigatus</i>	3 (13%)	1 (3%)	ND	ND	1 (5%)	5 (5%)
<i>Paecilomyces</i> spp.	ND	4 (14%)	2 (11%)	4 (25%)	ND	10 (9%)
<i>Stemphylium</i> spp.	ND	1 (3%)	2 (11%)	1 (6%)	1 (5%)	5 (5%)
<i>Rhizopus</i> spp.	1 (4%)	1 (3%)	ND	ND	ND	2 (2%)
<i>Acremonium</i> spp.	2 (9%)	1 (3%)	1 (6%)	ND	1 (5%)	5 (5%)
<i>Trichoderma</i> spp.	1 (4%)	ND	4 (22%)	ND	ND	5 (5%)
<i>Trichothecium</i> spp.	ND	3 (11%)	ND	ND	ND	3 (3%)
<i>Curvularia</i> spp.	ND	1 (3%)	ND	1 (6%)	ND	2 (2%)
<i>Mucor</i> spp.	ND	ND	1 (6%)	ND	2 (9%)	3 (3%)
<i>Alternaria</i> spp.	ND	ND	1 (6%)	ND	2 (9%)	3 (3%)
<i>Fusarium</i> spp.	ND	ND	ND	ND	1	1 (1%)
Yeast species	ND	ND	ND	1 (6%)	2 (9%)	3 (3%)
<i>Drechslera</i> spp.	ND	ND	ND	ND	1 (5%)	1 (1%)
Unknown	2 (9%)	5 (17%)	ND	ND	ND	7 (6%)

NICU, neonatal intensive care unit; CCU, cardiac care unit; OT1, ENT (ear, nose, throat) operation room; OT2, eye operation room; BW, cancer blood ward, ND not detectable

concentration of fungi indoor levels in CCU and NICU was higher than the outdoor levels, which confirmed the contribution of indoor environment and the increase in fungi with insufficient air-conditioning systems.

4 Conclusion

Microbial concentrations in the pediatric hospital air are related with an extensive range of adverse health effects. Because newborns and babies have undeveloped immune systems, they are recognized immunocompromised patient status. Our result showed that the pediatric hospital units have high levels of microbial contamination and consequently low microbial air quality. The sensitive hospital units, such as OT and NICU, were found to have the highest airborne particle and microbial contamination. The microbial quality of the air in OT wards is a significant factor to control nosocomial infections, and regular monitoring of bioaerosol levels in indoor hospital environments can help decrease the risk of HAIs. This study showed a significant positive association between airborne fungi levels with PM_{2.5} and PM₁₀ concentrations in hospital environment. The modification of ventilation systems and air-conditioning systems is required to decrease the concentrations of bioaerosol that may be introduced into the indoor environment of hospitals, especially in OT units.

Acknowledgements This research was funded by the vice-chancellery for research of Arak University of Medical Sciences (Grant No. 174). The authors wish to extend their thanks to the hospital personnel for their assistance during the study.

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