RESEARCH ARTICLE



Contamination of obsterics and gynecology hospital air by bacterial and fungal aerosols associated with nosocomial infections

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Abstract

Bacterial and fungal bioaerosols are a global concern due to nosocomial infections, especially in developing countries. Our study aimed to detect fungal and bacterial bioaerosols in different wards of an obstetrics and gynecology hospital air samples. 240 bioaerosol samples were collected by performing impaction method from different wards of a hospital in the central part of Iran, during two seasons. Fungi genera and bacteria species are recognized by cultivation. Concentrations of bacteria and fungi were ranged from 44 to 75 CFU/m³ and 8 to 22 CFU/m³, respectively. Labor Delivery and Recovery (LDR) and Emergency room had first and second most contaminated air among all the hospital wards. No significant difference between microbial load of wards which used natural ventilation and heating, ventilating, and air conditioning (HVAC) system was observed. The microbial load was not affected significantly by temperature, working shift, and Inpatient Bed Occupancy Rate (IBOR). Fungal load related significantly with relative humidity. *Staphylococcus aureus* (detected in 48.3% of samples) and *Penicillium* (27%) were the most predominant isolated bacteria and fungi, respectively. The results revealed that the level of bacteria and fungi responsible for nosocomial infections in the air of this hospital is very low. Although levels of microbial contamination are relatively low, it is important to investigate the effect of bioaerosols on nosocomial infections, especially in neonates.

Keywords Nosocomial infection · Indoor air pollution · Bioaerosol · Hospital · Bacteria · Fungi

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Introduction

Nosocomial or healthcare-associated infections are introduced as viral, bacterial, and fungal infections which may harm people during their inhabitation in healthcare settings [1]. The World Health Organization (WHO) reported that around 15% of hospitalized individuals get infected by microbial agents in hospital wards [2]. Although person-to-person transmission is the major route for most infections, evidence show infections caused by some microorganisms like staphylococcus and acinetobacter may transmit through the air [3, 4]. In the recent COVID-19 pandemic, medical waste management, health care settings disinfection and air contamination monitoring of hospitals have received more attention [5–8]. Hospitals' indoor air should be healthy for patients to breathe and the level of indoor contaminant or outdoor originated pollutants in the air should be reduced [9]. Based on the present population in hospitals, ventilation system, building and environmental conditions, different types, and concentrations of microorganisms may be existing in the indoor air of



hospitals [10]. Bioaerosols in healthcare settings are originated from outdoor air, respiratory droplets of people, air conditioning stuff, and cleaning activities [11]. Long-term hospitalization and inadequate air exchange rate in hospitals increase the exposure time, and may cause nosocomial infection related to bioaerosols [1, 12]. The most proportion of nosocomial infections causes by bacteria [1]. Also, fungi genera as ubiquitous microorganisms could be found in different types of indoor environments [1, 13, 14]. Fungal spores because of their small sizes are able to easily transmit between different wards and thorough inhalation may cause allergic response, asthma, and infections [15, 16].

Most studies were performed in general or multispecialized hospitals with a wide range of patients and different services and activities [9, 14, 17, 18]; Hence, the determination of influencing variables is difficult. Individuals hospitalized in obstetrics and gynecology hospital are mainly pregnant women and neonates who are known as sensitive groups, and their exposure to high levels of bioaerosols may pose a serious risk of infection in these groups [1]. Some airborne fungi and bacteria which have no impact on healthy people may cause serious problems to vulnerable groups in hospitals [19]. Nosocomial infection causes 4% to 56% of neonates' death around the world, and this rate increases to 75% in the middle east countries [2]. Also, in some studies, it has been reported that bioaerosol contaminations of women and maternity wards were the highest level among different hospital wards [18, 20]. Monitoring of bioaerosols level in hospital air is essential to prevent the spread of nosocomial infections. As mentioned, most deaths from nosocomial infections are related to neonates, therefore it seems monitoring of bacteria and fungi concentration in the maternity and neonatal wards air may probably be effective in preventing infection, disease, and death of neonates. The study aimed to determine the air microbial quality, including bacterial and fungal bioaerosols in different wards of an obstetrics and gynecology hospital in Iran, and its association with ventilation, shifts and environmental factors.

Materials and methods

Description of the sampling sites and strategies

This study was carried out from September 2018 to January 2019 in a maternity hospital, which was located in the center part of Iran. The hospital has eight separate wards including neonates, Neonatal Intensive Care Unit (NICU), maternity, operating theater, surgery, postpartum care, Labor Delivery and Recovery room (LDR), and emergency room. Sampling was performed from three points in all eight wards of the hospital 1 day a month (for 5 months) in two shifts in the morning and afternoon. A total of 240 separate samples (8

wards × 5 months × 2 shifts × 3 sampling point) were taken for fungi and bacteria. The characteristics of sampling sites are presented in Table 1. During the sampling period, patients and staff were present. Sample collection was performed at a height of about 1.2 m for 2.5 min using an Andersen one-stage viable impactor (SKC, USA) at an airflow rate of 28.3 L/min. Impactors were fitted with 100 mm diameter Petri dishes containing Sabouraud Dextrose agar (SDA) supplemented with chloramphenicol and blood agar (BA) for fungal and bacterial sampling, respectively. All samples were transferred to the laboratory in a cold box (4°c) and processed after arrival in the laboratory.

Detection of bacteria and fungi

The fungal plates were kept at room temperature (25 °C) and read after 72–120 h and bacterial plates were incubated at 37 °C for 24–48 h. The total fungal and bacterial concentration was enumerated to colony-forming units per cubic meter (CFU/m^3).

Grown fungal colonies on SDA were recognized based on colony's morphology and microscopically identification under magnifications of 10,40,100. Bacterial colonies grown on BA were isolated based on their morphology and by application of different culture media and biochemical tests, bacteria species were recognized.

Results

The mean concentration of bacterial and fungal bioaerosols in selected 8 wards of the studied hospital is presented in Table 2. the concentration of bacteria and fungi in hospital wards ranged from 44 to 75 CFU/m³ and 8 to 22 CFU/m³, respectively. Table 3 shows the comparison of bioaerosols presence in morning and afternoon shifts. No significant difference between the two shifts was observed. The presence frequency of bacteria species and fungal genera isolated from each ward is presented in Tables 4 and 5, respectively. Among detected bacteria, Staphylococcus aureus was found more frequently than other bacteria and penicillium was the most frequent in comparison with other fungi genera. The Inpatient Bed Occupancy Rate (IBOR) was calculated during the sampling period and was divided into three categories (less than 71%, between 72% to 75% and greater than 76%). The highest frequency of bacteria and fungi was observed in the IBOR less than 71%, but statistical analysis did not show a significant relationship between the IBOR and the frequency of microorganisms. The average temperature recorded during sampling was between 24 and 27 °C and the relative humidity was 30% to 37%. There was no significant relationship between the concentration and abundance of bacteria and fungi with



 Table 1
 Characteristics of the hospital wards

Hospital wards	Area (m2)	No. of Beds	Ventilation system	Sampling locations
Operating Theater	306	4	HVAC* with HEPA** filter	Near toilet, nurse station, between patient bed
Postpartum Care	384	30	Natural ventilation	Near toilet, nurse station, patient room
Surgery	574	31	Natural ventilation	Near toilet, nurse station, between patient bed
NICU	100	12	HVAC	Near toilet, nurse station, between patient bed
Neonatal	70	11	HVAC	Near toilet, nurse station, room
Emergency	147	9	Natural ventilation	Near toilet, nurse station, between patient bed
Maternity	364	11	Natural ventilation	Near toilet, nurse station, between patient bed
LDR	277	7	HVAC	Near toilet, nurse station, room

^{*}Heating, Ventilation, and air conditioning system

temperature, but the relationship between fungi and relative humidity was significant.

Discussion

Indoor air of healthcare settings contains a mixture of bioaerosols such as fungi, bacteria, viruses, and allergens which are originated from different sources include outdoor air, staff and patients (talking, coughing, sneezing), ventilation systems, toilet flushing, and cleaning activities [11, 17]. Among hospital staff, visitors, and patients who are exposed to bioaerosols during their inhabitancy in hospitals, Immunocompromised individuals are at higher risk of probable infections [12, 21]. Therefore, indoor air quality of hospitals is a great concern. In our study, the concentration of airborne bacteria and fungi in hospital wards ranged from 44 to 75 CFU/m³ and 8 to 22 CFU/m³, respectively (Table 2). The average concentrations of detected bacteria and fungi were 57.44 CFU/m³ and 16.13 CFU/m³, respectively, which is in accordance with data found by some authors [9, 22], but lower than that reported by others [17]. Mirhoseini et al. (2015) showed that the mean concentration of airborne

bacteria in hospitals of Isfahan city in Iran was 464 CFU/m³ which is much higher than our results [17]. In the study of Montazeri et al. (2020) concentration of fungal and bacterial aerosols in a burn hospital ranged from 32 to 110 CFU/m³ and 53 to 94 CFU/m³, respectively [23]. Generally, due to the presence of more sources and more favorable environmental conditions for bacteria, the concentration of bacteria in hospitals air is higher than fungi concentration [16]. We found lower concentrations of fungi than bacteria in all samples. Consistent with our results Mousavi et al. (2019) and Bolookat et al. reported the higher level of bacteria in hospitals bioaerosols samples [9, 24].

Table 3 shows the comparison of bioaerosols presence in morning and afternoon shifts. No significant difference between the two shifts was observed. It is indicated that in the studied hospital a constant environmental condition is provided. Other studies reported more bioaerosol presence in afternoon shift, they suggested changes in temperature and relative humidity and presence of visitors as the effective factors on higher microbial load in the afternoon shifts [25, 26].

There was a significant difference between concentrations of airborne fungi and bacteria in different wards, the mean concentration of bioaerosols was highest in LDR and the

 Table 2
 Bacterial and fungal concentration of hospital wards air

	Fungal concentration		Bacterial concentra	ation	Total	Total		
	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value		
Operating Theater	14.28 ± 11.86	0.002	48.57±23.90	< 0.001	62.85 ± 26.42	<0.001		
Postpartum Care	17.62 ± 12.83		56.19 ± 30.22		73.81 ± 34.80			
Surgery	18.75 ± 11.35		57.13 ± 23.43		75.70 ± 28.61			
NICU	8.58 ± 11.64		44.29 ± 28.65		52.87 ± 36.21			
Neonates	14.75 ± 11.55		56.19 ± 30.69		70.94 ± 34.89			
Emergency	14.28 ± 11.86		75.70 ± 30.74		89.99 ± 33.16			
Maternity	18.09 ± 14.97		50.94±26.71		69.03 ± 29.08			
LDR	22.85 ± 14.33		70.47 ± 27.03		93.33 ± 31.33			
Total	16.13 ± 13.03	_	57.44+29.17	_	73.57 ± 33.88			



^{**}high efficiency particulate air

Table 3 Number of isolated bacteria and fungi according to the shift

Sampling time	Fungi		Bacteria		Total		
	P.value	Frequency of positive samples (%)	P.value	Number of positive samples (%)	P.value	Number of positive samples (%)	
Morning shift	0.14	86(71.7)	0.386	114(95)	0.185	116(96.7)	
Afternoon shift		89(74.2)		118(98.3)		119(99.2)	
Total	_	175(72.9)	_	232(96.7)	-	235(97.9)	

lowest in the Neonatal ward (Table 2). Both of these sites used the HAVC system and the concentration differences is probably due to the higher density of people in LDR. However, based on statistical analysis there was no significant differences between natural ventilation and HAVC. Stockwell et al. (2019) reported that the bioaerosols concentration in areas which used mechanical ventilation was significantly less than wards with natural ventilation [12]. The mean concentration of bioaerosols in operating theater was in agreement with some of the other studies [9, 22] and lower from Mirhoseini et al. (2015) [17] and Montazeri et al. (2020) [23] in Iran, which might be related to the recent installation of new filters in the ventilation system and occupant density. The results of previous studies are provided in Table 6.

In the study of Stocks et al. (2010) a relationship between population density and bioaerosols concentration was reported [33]. We used Inpatient Bed Occupancy Rate (IBOR) as the factor of the population of hospitalized patients. Statistical analysis showed no significant relationship between IBOR and bioaerosols concentration. However, it does not mean that the bioaerosols concentration was not affected by the number of patients, because we did not include the number of outpatients in our analysis.

According to our searches, no other study has been performed on a specialized hospital of obstetrics and gynecology, but there are studies that have reported the highest concentration of bioaerosols in the women wards of hospitals [18, 20].

The survival of bioaerosols is influenced by environmental factors include temperature and relative humidity [12, 16]. In our study, airborne bacterial and fungal concentrations in hospital environments were not significantly related to temperature, but fungal concentration was correlated to relative humidity. Similarly, Obbard and Fang (2003) reported no significant relationship between airborne bacteria and temperature in hospital wards in Singapore, but the relationship between humidity and bacterial level was significant [34]. Contrary to our results, Park et al. (2013) Reported that the presence and concentration of bacteria and fungi are correlated to temperature [35]. Some studies found no significant relationship between bioaerosols and environmental parameters in hospital air [9, 28].

Among bacteria, Staphylococcus, Enterococcus and *Pseudomonas aeruginosa* have been reported to play the most important role in causing nosocomial infections [1].

As is presented in Table 4, *staphylococcus aureus* (grampositive) and *Neisseria* (gramnegative) had the most and the

Table 4 Frequency (%) of diagnosed bacteria in different hospital wards

Bacterial Species	Operating Theater	Postpartum Care	Surgery	NICU	Neonates	Emergency	Maternity	LDR	Total	P.value
Staphylococcus aureus	12(40)	18(60)	12(40)	13(43.3)	18(60)	18(60)	12(40)	13(43.3)	116(48.3)	0.332
Staphylococcus epidermidis	11(36.7)	16(53.3)	8(26.7)	5(16.7)	8(26.7)	9(30)	9(30)	14(47.6)	80(33.3)	0.067
Micrococcus luteus	12(40)	8(26.7)	11(36.7)	7(23.3)	9(30)	10(33.3)	10(33.3)	12(40)	79(32.9)	0.84
Micrococcus Roseus	7(23.3)	8(26.7)	10(33.3)	6(20)	9(30)	8(26.7)	6(20)	9(30)	63(26.3)	0.92
Pseudomonas Aeruginase	6(20)	7(23.3)	8(26.7)	11(36.7)	14(46.7)	11(36.7)	7(23.3)	15(50)	79(32.9)	0.094
Pediocock	8(26.7)	4(13.3)	8(26.7)	8(26.7)	7(23.3)	7(23.3)	8(26.7)	9(30)	59(24.6)	0.9
Bacillus	14(46.7)	11(36.7)	9(30)	8(26.7)	10(33.3)	9(30)	8(26.7)	13(43.3)	82(34.2)	0.63
Niseria	1(3.3)	2(6.7)	1(3.3)	1(3.3)	1(3.3)	4(13.3)	4(13.3)	1(3.3)	15(6.3)	0.4
Enterococcus faecalis	2(6.7)	4(13.3)	8(26.7)	3(10)	6(20)	7(23.3)	7(23.3)	8(26.7)	47(19.6)	0.19
Streptococcus pyogenes	7(23.3)	4(13.3)	5(16.7)	2(6.7)	3(10)	4(13.3)	4(13.3)	7(23.3)	37(15.4)	0.58
Other Bacteria	0(0)	1(3.3)	4(13.3)	3(10)	1(3.3)	3(10)	3(10)	1(3.3)	17(7.1)	0.28



Table 5 Frequency (%) of diagnosed fungi in different hospital wards

Fungal Genera	Operating Theater	Postpartum Care	Surgery	NICU	Neonates	Emergency	Maternity	LDR	Total	P.value
Aspergillus Fumigatus	2(6.7)	5(16.7)	4(13.3)	2(6.7)	4(13.3)	5(16.7)	4(13.3)	1(3.3)	27(11.3)	0.62
Aspergillus Niger	3(10)	3(10)	4(13.3)	3(10)	2(6.7)	0(0)	3(10)	3(10)	21(8.8)	0.76
Penicillium	7(23.3)	6(20)	8(26.7)	2(6.7)	7(23.3)	5(16.7)	7(23.3)	8(26.7)	50(20.8)	0.59
Cladosporium	6(20)	3(10)	2(6.7)	3(10)	3(10)	2(6.7)	5(16.7)	6(20)	30(12.5)	0.54
Scopolariopsis	1(3.3)	4(13.3)	2(6.7)	0 (0)	3(10)	4(13.3)	1(3.3)	4(13.3)	19(7.9)	0.32
Alternaria	0(0)	4(13.3)	3(10)	2(6.7)	1(3.3)	3(10)	2(6.7)	4(13.3)	19(7.0)	0.5
Mocur	1(3.3)	2(6.7)	2(6.7)	2(6.7)	0 (0)	1(3.3)	3(10)	1(3.3)	12(5)	0.75
Trichoderma	1(3.3)	4(13.3)	1(3.3)	2(6.7)	1(3.3)	1(3.3)	1(3.3)	2(6.7)	13(5.4)	0.64
Yeast	1(3.3)	0 (0)	1(3.3)	1(3.3)	3(10)	1(3.3)	2(6.7)	3(10)	12(5)	0.58
Psilomycosis	2(6.7)	1(3.3)	3(10)	0(0)	1(3.3)	1(3.3)	1(3.3)	3(10)	12(5)	0.58
Unknown	4(13.3)	5(16.7)	6(20)	1(3.3)	4(13.3)	3(10)	5(16.7)	5(16.7)	33(138)	0.69

least frequency among detected bacteria in hospital wards, respectively. Gram-positive bacteria are more resistant to adverse environmental conditions than gram negatives [12, 21]. Other studies similar to ours reported a higher frequency of gram-positive bacteria and staphylococcus species [12, 36, 37]. Staphylococcus aureus mainly causes blood-borne infections [1]. Staphylococcus spp. are very resistant to dry condition [12] and frequently found in the indoor air of hospitals in Iran which is located in a semiarid area [3, 9, 23]. Some studies suggested staphylococcus sp. as the indoor air pollution bacterial indicators [38]. Still, Gram-negative bacteria are notable for the release of endotoxins. Although endotoxins have not been studied in this study, some authors showed that exposure to endotoxins can have serious outcomes such as septic shock, chest congestion, and even death [39]. As is seen in Table 5, Pseudomonas aeruginosa (gram-negative) had relatively high frequency in LDR and neonatal ward air samples.

Studies reported *Pseudomonas spp.* as the main Gramnegative bacteria isolated from hospital wards [11, 21, 23].

Pseudomonas spp. require moisture for survival and growth, so their presence may be attributed to the existence of wash-room in the vicinity of the sampling area or cleaning and moping activities during sampling [40]. Since pseudomonas species are resistant to many disinfectants, it is difficult to eradicate them from hospitals [21, 40, 41]. Pseudomonas aeruginosa has been observed in infections of all parts of the body, especially infections of the kidneys and urinary tract [1].

Enterococcus is involved in causing surgical-site infections [1]. Although in our study Enterococcus was not more common than other isolated bacteria, its highest frequency was observed in the surgery ward (26%) which can be alarming.

The predominant fungal genera isolated in indoor air of hospital wards were *penicillium* and *aspergillus* species. Inconsistence of our results Montazeri et al. (2020) reported that *penicillium* was the most frequently isolated fungi from hospital wards [23]. *Cladosporium, Penicillium, Aspergillus, Alternaria, Fusarium*, and *Candida* are the most common isolated fungi from hospital air in other studies [11, 36].

 Table 6
 Bioaerosols concentration (bacteria and fungi) in the previous studies

Author (year)	Bacterial aerosols concentration	Dominant bacteria	Fungal aerosols concentration	Dominant Fungi	Country
Ekhaise et al. (2011) [27]	33.5	Staphylococcus	31.5	Aspergillus	Nigeria
Nourmoradi et al. (2011) [28]	254	Staphylococcus	214	Penicillium	Iran
Cabo Verde et al. (2015) [16]	374	Staphylococcus	16.5	Aspergillus	Portugal
Maji et al. (2013) [29]	1641	Bacillus	2830	Aspergillus	India
Hoseinzadeh et al. (2013) [18]	16.05	Staphylococcus	18.8	Aspergillus	Iran
Mirzaei et al. (2014) [30]	56.4	Staphylococcus	_	_	Iran
Mirhoseini et al. (2015) [17]	464	_	_	_	Iran
Sepahvand et al. (2016) [31]	_	_	85.7	Cladosporium	Iran
Demirel et al. (2017) [32]	_	_	47.5	Penicillium	Turkey
Montazeri et al. (2020) [23]	73.5	Staphylococcus	71	Penicillium	Iran



Many studies indicated aspergillus and *penicillium* as the major fungal causative agent of nosocomial infections [11, 12, 23]. These species are resistant to dryness and water scarcity and can survive in different parts of hospitals [11].

According to the WHO guideline 100 CFU/m³ for bacteria and 50 CFU/m³ for fungi are acceptable in the hospital air [42]. In our study, bacterial concentration in wards ranged from 44 to 75 CFU/m³ and fungal concentration ranged from 8 to 22 CFU/m³, which indicates the level of indoor pollution under WHO suggestion.

The most obvious finding of this survey is that the bioaerosols level of the studied hospital is lower than most of the other studies conducted in Iran [14, 17, 23]. Conclusions that can be drawn from bioaerosol detection studies in healthcare settings are highly dependent on study site characteristics and the sampling and detection methodologies used in these studies [11]. In our study, there was only one collection device, so the time of air sampling from all of the selected wards was limited (2.5 min for each sample). Because of the heterogeneous spread of bioaerosols and their different sizes, during the aerosol collection in passive sampling some microorganisms impact on petri dish, while others are still suspended in the air. Therefore, the longer the sampling time, the more detectable microorganisms [12, 43]. On the other hand, as said before bioaerosols concentration in hospital environments may be affected by climate conditions and seasonal changes, activities, population density, and ventilation efficiency [11].

The hospital building is relatively new and disinfection and cleaning activities are managed appropriately. One of the indoor air bioaerosol sources is outdoor air [36]. In this study, the level of bioaerosols in outdoor air was not determined. However, windows during sampling were closed and natural ventilation was not performed at that time. Azimi et al. (2013) reported that opening windows and doors are the most significant route of high concentration of fungi in the hospital air [14]. Low concentration of bacteria and fungi in this study may be related to Viable But Non Culturable (VBNC) state of fungi and bacteria which in this state bacteria and fungi are not culturable but still alive and can cause infection [17]. Molecular techniques like PCR assay could be used to overcome this problem. Furthermore, we used the impaction technique for sampling. Previous studies have reported lower efficiency of impaction and filtration techniques compared to the liquid impingement sampling [44].

An important issue regarding the treatment and control of nosocomial infections is the determination of antibiotic resistance of microorganisms. It is reported that 50 to 60% of nosocomial infections are caused by antibiotic-resistant pathogens.

In this study, although the diversity and concentration of microorganisms were determined, the possibility of transmission of these infections through hospital air was not assessed. Therefore, the possibility or non-possibility of causing risks in these concentrations cannot be assessed. To investigate the possibility of causing infection by microorganisms detected in hospital air, it is recommended that in future studies, quantitative microbial risk assessment be performed using doseresponse models.

Conclusion

In this study, we found different types and frequencies of bioaersols in different parts of an obstetrics and gynecology hospital, and the highest level was found in LDR. However, bacteria and fungi potentially causing nosocomial infections (such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Aspergillus*) were frequently found in this study. Although there was no difference in the concentration of bioaerosols in natural and mechanical ventilation, it seems that ventilation efficiency should be improved to reduce the level of bioaerosols. Given that nosocomial infections often affect neonates; it is necessary to assess the risk of nosocomial infections through exposure to hospital air for neonates.

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Declarations

Conflict of interest The authors declare there is no conflict of interest.

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