

Characteristics of Airborne Particles and Bacteria in Hospital Indoor and Outdoor Air

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ARTICLE INFO

ORIGINAL ARTICLE

Article History:

Received: 23 August 2023

Accepted: 20 October 2023

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Keywords:

Aerosols,
Bacteria,
Particulate Matter,
Hospitals.

ABSTRACT

Introduction: Given that hospital air is one of the important environmental sources for transmission of microorganisms, the importance of airborne transmission in the epidemiology of hospital-acquired infections (HAIs) has gained attention in the past two decades. Therefore, the present study aims to determine the concentration of bacteria in association with airborne particulate matter (PM) in the outdoor and indoor air of two hospital wards.

Materials and methods: The GRIMM 1.109 dust monitor and the Andersen one-stage viable impactor were used for particle counting and bioaerosol sampling, respectively.

Results: The average levels of airborne bacteria sampled from outdoor air were 33 colony-forming units (CFU/m³), and in the air samples of medical and infectious disease wards, they were 76 and 85 CFU/m³, respectively. *Staphylococcus* spp. and *Acinetobacter* spp. were the most prevalent bacteria in the samples. Statistical analysis showed a significant association between PM_{2.5}, and PM₁₀ particle mass concentrations and airborne bacteria concentrations in indoor air samples (P-value < 0.05).

Conclusion: Some bacterial agents of HAIs existed in hospital air and may be problematic for immunocompromised patients. Higher levels of bacteria in indoor air compared to outdoor air may indicate that the bacteria were of indoor origin, such as the presence and activities of people. Moreover, the results showed that particle counting may be a useful tool for airborne bacteria monitoring.

Citation: Atoof F, Gholipour S, Shamsizadeh Z, et al. *Characteristics of Airborne Particles and Bacteria in Hospital Indoor and Outdoor Air*. J Environ Health Sustain Dev. 2023; 8(4): 2125-33.

Introduction

The incidence of nosocomial or hospital-acquired infections (HAIs) is a serious problem in hospitals worldwide^{1,2}. During hospitalization, the patient is exposed to pathogenic or opportunistic

microorganisms from other patients, hospital workers, and numerous environmental sources³. It is estimated that the rate of global HAIs is 0.14⁴.

Hospital air is one of the important environmental sources for transmission of

microorganisms. There is substantial evidence that some airborne microorganisms may cause nosocomial infections^{5,6}. Furthermore, the air conditioning system, respiratory disease, patient respiratory secretions, and infected wounds are also key sources of aerial transmission³. Patients, employees, visitors, ventilation and air conditioning systems, and outdoors all have the potential to introduce pathogenic and non-pathogenic microorganisms into the hospital indoor air⁵. Airborne bacteria caused 10% to 20% of reported endemic nosocomial infections⁵. Hospital air can contain various bacteria, including *Staphylococcus* species (causing Staph infections), *Klebsiella pneumoniae* (causing respiratory and wound infections), *Pseudomonas aeruginosa* (linked to respiratory infections, urinary tract infections, and skin infections), and *Acinetobacter baumannii* (associated with pneumonia, bloodstream infections, and urinary tract infections)^{7,8}. Monitoring and managing these airborne pathogens are critical for infection control and patient safety in healthcare settings^{9,10}. Hospitalized individuals are at a greater risk of infection transmission due to a variety of variables that have been identified. These include actions such as nebulization, suction, and cleaning that create more airborne particles¹⁰. Bioaerosol monitoring at hospitals, in particular, can help with epidemiological investigations of nosocomial infectious illnesses, research on the transmission and control of airborne microorganisms, quality control, and monitoring biohazardous activities¹¹. Furthermore, bioaerosol monitoring in indoor hospital environments might be a significant tool in the prevention of nosocomial illnesses spread by the air. In other words, airborne microorganisms monitoring in hospitals may give information on bioaerosol sources, concentrations, and dispersion, and also serve as a quality control tool⁵.

Particulate matter (PM) consisting of small solid and liquid particles suspended in the air, can serve as a carrier and reservoir for microorganisms such as bacteria, viruses, and fungi. These microorganisms can attach to or be encapsulated within PM particles, allowing them to remain

suspended in the air for extended periods and potentially facilitating their transport over long distances¹². In outdoor environments, this relationship can have implications for public health, as the inhalation of PM-bound microorganisms can lead to respiratory and other health issues. Additionally, indoor spaces (for example hospital wards) with poor ventilation can accumulate PM-bound microorganisms, contributing to indoor air quality concerns. Understanding and monitoring this relationship is crucial for assessing the potential risks associated with airborne microorganisms and developing effective strategies to mitigate their impact on human health and the environment¹². There are studies which have proposed PM counting as a quicker and more straightforward alternative^{13,14}. In operating rooms, a link has been shown between the number of 5 to 7 micron particles and microbiological infection³. However, little is known about the air quality in hospitals in developing and transitional countries. There has been little research on its usefulness in predicting airborne bacteria in diverse hospital settings⁵. Therefore, monitoring of microbiological air quality can help identify the source of an illness and, as a result, help lower the number of nosocomial infections.

Therefore, this study was carried out to determine the concentrations and diversity of airborne bacteria and to evaluate their association with PM concentrations in different wards of the hospital.

Materials and Methods

The current study was conducted in an educational hospital in Kashan, Iran, during cold seasons, autumn and winter 2018. Cold weather conditions often result in a higher prevalence of respiratory infections, rendering healthcare facilities more susceptible to the transmission of airborne pathogens. Air samples were collected from two sites of the hospital, including the infectious disease ward (60 samples) and medical ward (60 samples). During the research period, outdoor air (60 samples) was also sampled. The

infectious disease ward was chosen due to its high risk of harboring airborne pathogens, given the nature of patients and potential for infectious aerosols. The medical ward, being a more general healthcare setting, provided insight into the overall air quality in areas with diverse patient conditions. Outdoor air sampling served as a reference and control point, helping us understand the levels of bacteria in the external environment. All sampling events were performed in the morning. A total of 60 visits were made to every part of the hospital. As a result, the bacteria and particle concentrations in the air were measured in 180 different samples.

Airborne bacteria

Airborne bacteria were drawn into the Quick Take 30 pump connected with an Andersen one-stage viable impactor with a flow rate of 28 L/min for 15 minutes¹⁵. To simulate the breathing zone, bioaerosols were collected at a height of 1.5 m above ground level. The impactor was loaded with petri dishes containing blood agar¹⁶. All samples were transferred to the laboratory and processed immediately after arrival at the laboratory. For total bacterial analysis, the blood agar plates were incubated at 37°C for 2–3 days. Colonies growing media were enumerated and calculated as colony forming units per cubic meter (CFU/m³). Bacterial colonies were Gram-stained and characterized based on colony, cell morphology, and subsequent tests, including oxidase, catalase, and DNase tests.

Particle counting

The indoor and outdoor PM size distribution during each sampling event was measured using a

GRIMM 1.109 dust monitor (Germany) for an hour.

Quality control

During the data collection and analysis process, several quality control measures were implemented. First, all equipment used for air sampling and particle counting was regularly calibrated to maintain precision and accuracy. Sterile techniques were employed during sample collection to prevent contamination. In the laboratory, rigorous protocols were followed to minimize the risk of cross-contamination during bacterial identification. Positive and negative controls were included in each batch of samples to validate the accuracy of the identification process.

Statistical analysis

SPSS 26.0 was used for statistical analysis. The Kolmogorov-Smirnov normality test revealed that our data distribution is not normal. The Mann–Whitney, a non-parametric test, test was used to compare the results of different groups. The Spearman's rank correlation coefficient was utilized to calculate correlation coefficients between the studied variables (PM and bacterial concentration). Spearman's rank correlation is a non-parametric test that assesses the strength of relationships between variables. P-values smaller than 0.05 were considered statistically significant.

Results

This study focused on culturable bacteria in indoor and outdoor air samples in a hospital over two consecutive seasons, examining their association with PM. Table 1 represents the concentration of airborne bacteria and total suspended particles in different hospital wards.

Table 1: Concentration of airborne bacteria and total suspended particles in different hospital wards

Sampling location	Bacteria (CFU/m ³)			TSP (µg/m ³)		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Outdoor air*	0	119	33.03 ± 28.53	74.6	672.2	231.93 ± 122.68
Indoor air*	0	257	80.98 ± 51.25	21.44	220.9	81.96 ± 43.68
Medical ward	5	188	76.37 ± 45.34	27.36	167.5	76.71 ± 42.08
Infectious disease ward	0	257	85.58 ± 56.94	21.44	220.9	87.22 ± 45.33

* Significant differences were observed between outdoor and indoor air in bacteria (P-value = .000) and TSP (P-value = .000) concentrations.

Table 1 reveals a higher bacterial level in indoor air compared to outdoor air, while suspended particle concentration is markedly higher in outdoor air. The bacterial counts ranged from 0 to 119 CFU/m³ (mean, 33 CFU/m³) for outdoor air and 0 to 257 CFU/m³ (mean, 80.98 CFU/m³) for indoor air samples. Indoor air bacterial levels were significantly higher than outdoor air (P-value = .000). Notably, the highest bacterial level occurred

in the infectious disease ward, emphasizing the potential indoor origin of bacteria, possibly from human presence.

Table 2 illustrates the mass concentration of particles, indicating higher levels in outdoor air compared to indoor air. PM₁₀ and PM_{2.5} levels in the study were below the World Health Organization (WHO) recommended levels (PM₁₀ = 50 µg/m³, PM_{2.5} = 25 µg/m³).

Table 2: Mass concentration of PMs in the samples

Sampling location	PMs (µg/m ³)	
	PM _{2.5}	PM ₁₀
Outdoor air	9.63	71.94
Indoor air	7.144	38.37
Medical ward	6.48	36.55
Infectious disease ward	7.81	40.2

A positive correlation between PM and bacteria (P-value = .006) concentration in indoor air samples was observed, highlighting the interplay

between PM and bacterial presence (Figure 1).

Table 3 shows the concentrations of PM and airborne bacteria reported in related studies.

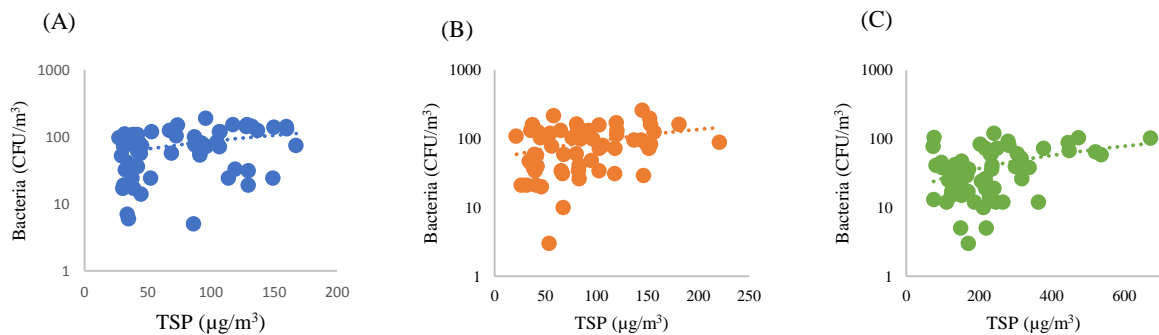


Figure 1: The association between TSP and bacterial concentration. (A) Medical ward, (B) Infectious diseases ward, (C) Outdoor air

Table 3: Concentrations of PM (µg/m³) and airborne bacteria (CFU/m³) reported in studies

First author (year)	Country	PM _{2.5}	PM ₁₀	Airborne bacteria	Reference
		Min-max (average)	Min-max (average)	Min-max (average)	
This study *	Iran	2-51 (7)	11-152 (38)	0-257 (81)	-
Al Rayess et al. (2022)	Lebanon	10-54 (30)	10-65 (33)	20-134 (66)	17
Mirhoseini et al. (2022)	Iran	23-54	33-90	19-356	18
Mousavi et al. (2019)*	Iran	-	16-53	46-126	19
Hwang et al. (2018)	South Korea	-	22-98	40-99	20
Wan et al. (2011)*	Taiwan	-	0.8-55	0-756	3

*Significant correlation has been observed between PM and bacteria levels.

Isolation rates of bacteria in the studied sites of the hospital are presented in Table 4. The most

common bacteria isolated from samples were *Staphylococcus* followed by *Acinetobacter*.

Table 4: Frequency of occurrence of airborne bacteria isolated from different sites of the hospital (percent)

Bacteria	Outdoor air	Medical ward	Infectious disease ward
<i>Staphylococcus</i>	5	21	16
<i>Klebsiella</i>	2	0	0
<i>Acinetobacter</i>	3	3	3
<i>Shigella</i>	1	2	3
<i>Salmonella</i>	1	0	0
<i>Bacillus</i>	5	1	1
<i>Hafnia</i>	1	1	1
<i>Diphtheroid</i>	8	0	2
<i>Escherichia coli.</i>	0	2	2
<i>Serratia</i>	1	0	0
<i>Neisseria</i>	2	0	1
<i>Moraxella</i>	1	1	0
<i>Micrococcus</i>	0	0	2
<i>Pseudomonas</i>	0	1	2

Discussion

Hospital environments are complicated environments in which a variety of variables might affect the quantity of bacteria in the air of different sites. In this study, culturable bacteria from indoor and outdoor air samples and their association with PM for two consecutive seasons in an Iranian hospital have been described.

As revealed in Table 1, a higher bacterial level was seen in indoor air than in outdoor air, while the concentration of suspended particles was much higher in the outdoor air.

As mentioned earlier, bacteria level in indoor air samples was significantly higher than in outdoor air (P-value = .000). The infectious disease ward exhibited the highest bacterial level among the indoor air samples, while the lowest level was observed in outdoor air. The elevated levels of bacteria in indoor air compared to outdoor air suggest that the source of these bacteria may be indoor, potentially attributed to the presence of humans. Several studies have reported similar trends, emphasizing the influence of occupants on indoor air quality^{21,22}. The presence of humans in indoor spaces introduces bioaerosols, including bacteria, through activities such as talking, coughing, and shedding skin scales^{21,22}. The infectious disease ward, where the highest bacterial levels were recorded, likely

reflects the increased microbial burden associated with patient care and clinical activities. The WHO recommends a maximum of 100 CFU/m³ for bacteria in hospitals air²³. In the present study, bacterial levels were lower than the WHO recommendation in most of the samples, which reveals the efficient ventilation and/or appropriate disinfection and cleaning activities in the studied wards. According to the observations, natural ventilation such as opening the door and windows was used in addition to the central ventilation (exhaust fan) of the hospital. The low concentrations of bacteria in the samples could be partially attributed to low air temperatures during the sampling period (autumn and winter). There are studies that showed warmer temperatures tend to promote increased bacterial activity and growth, as bacteria thrive in a more favorable environment²⁴. This can lead to higher concentrations of airborne bacteria during periods of elevated air temperature.

Table 2 shows that the concentration of particles was higher in outdoor air than in indoor air. Similar to the present study results, other studies conducted in Iran demonstrated that the indoor concentrations of particles were lower than the outdoor particle concentrations which can be attributed to adequate ventilation systems or lower human activities in the sampling time²⁵⁻²⁷.

According to the WHO guidelines, indoor PM₁₀ and PM_{2.5} concentrations should not exceed 50 µg/m³ and 25 µg/m³, respectively²⁸. In the current study, PM₁₀ and PM_{2.5} levels were lower than the WHO recommended levels, which might be related to effective ventilation. The greater the air exchange rate, the lower the concentration of indoor air particles²⁹. So, it could be said the usage of high-efficiency ventilation systems may have resulted in lower amounts of these particles within the indoor air compared to outdoors in healthcare settings.

Statistical analysis revealed a positive correlation between PM and bacteria (P-value = .006) concentration in the indoor air samples.

In the indoor air, a significant relationship (P-value < 0.05) was observed between the bacteria levels and the concentration of different sizes of particles (including PM_{2.5}, and PM₁₀). Other studies have reported an association between PM_{2.5} and bacteria^{5,30,31}. However, Mousavi et al. reported that the concentration of bioaerosols was only significantly related to PM₁₀, which was due to the passive sampling method and short sampling time, leading to more sedimentation of large particles than smaller ones¹⁹. Due to the time and labor-intensive nature of microbiological samples and analysis, it was thought that monitoring particle mass would provide a real-time estimate for bioaerosols. Monitoring PM alone cannot indicate the concentration of bacteria. Table 3 shows the concentrations of PM and airborne bacteria reported in related studies.

Gram-positive bacteria were observed to be the predominant bacteria and were present in 62% of the samples. Gram-positive bacteria are more resistant to unfavorable condition and disinfection than gram-negatives¹⁶. Messi et al., isolated 107 bacterial strains belonging to different genera in the air-borne bioaerosols in operating rooms of Sassuolo Hospital in Modena, Italy³².

Similar to the present study results, other studies reported *Staphylococcus* spp. as dominant species isolated from air^{5,16,27,33}. This microorganism is one of the most clinically important pathogens,

causing skin and soft tissue infections, bloodstream infections, and even life-threatening diseases with mortality rates higher than those for acquired immunodeficiency syndrome (AIDS), tuberculosis, and viral hepatitis³⁴. *Staphylococcus* spp. was detected in 21%, 16%, and 5% of the medical ward, infectious diseases ward, and outdoor air samples, respectively. The rise in *Staphylococcus* spp. levels in air samples is a result of ward dust and skin scaling dispersing into the air^{27,35}. Other bacteria are primarily opportunistic pathogens that can be spread by respiratory droplets, causing illness in vulnerable individuals such as immunocompromised patients. It has been reported that methicillin-resistant *S. aureus* (MRSA) account for 30% of nosocomial infections^{8,36}. *Acinetobacter* was one of the most frequent bacteria in detected samples (Table 4). Consistent with the present study, Shamsizadeh et al., (2017) surveyed *A. baumannii* in 11% of air-borne bioaerosol samples of hospitals in Isfahan, Iran and the highest frequency was observed in the intensive care units¹. In other studies, Mirhoseini et al., surveyed β-lactam-resistant bacteria (BLRB) in 4 hospitals in Isfahan, Iran. The most predominant BLRB were *Staphylococcus* spp., *A. baumannii*, and *Acinetobacter* spp.³⁷.

One of the limitations of the current study is the absence of meteorological data and their influence on PM and bacterial concentrations in the hospitals outdoor air. Meteorological factors such as temperature, humidity, wind speed, and precipitation play a significant role in the dispersion and accumulation of airborne particles and microorganisms²⁴. Without detailed meteorological information, this study may not provide a complete picture of how these variables interact with air quality, potentially leaving gaps in the comprehension of their impact on the health risks for patients and healthcare workers.

Overall, understanding the bioaerosol concentrations in indoor hospital air is an important aspect but the data obtained for the bioaerosol composition data is limited. Therefore, a broader analysis of bioaerosol compositions in the indoor hospital air would provide further

knowledge about indoor hospital air bioaerosols and specially to understand their potential pathogenicity. Significant advances in technology and patient management have been made in preventing HAIs, yet transmission persists and is associated with increased costs and increased length of stay during hospital admissions. In this context the use of mechanical ventilation systems is one the important hospital infection-control strategies to prevent HAI transmission³⁸.

Conclusion

Airborne microorganisms and PM are the most serious contaminants of indoor air in hospitals. Therefore, they need to be monitored in hospitals for quality control of the indoor environment. Overall, the concentration of bacteria in air samples of the two studied wards was low, and the presence of higher levels of bacteria in indoor air than in outdoor air showed the indoor origin of bacteria in the air. The concentration of bacteria was below the recommended values by the WHO for indoor hospital wards, which is related to effective ventilation of the wards. The significant positive correlation between airborne bacteria and PM_{2.5}, and PM₁₀ concentrations in indoor air showed that measuring airborne particles might be a simple and rapid way to assess the microbiological quality of the air. Lack of meteorological data and correlation analysis between these data and PM and bacterial abundance is one of the limitations of the present study. Moreover, sampling was performed during autumn and winter. For comprehensive description of microbial quality of hospital air, it was better to conduct the study during a complete year. Moreover, to ensure the safety of patients and healthcare workers, periodically inspecting the ventilation system in the hospital wards is suggested. Further research is required to establish and simulate the effect of various variables on bacterial levels in hospital air.

Acknowledgement

The authors would like to express their gratitude to hospital workers for their support during the study.

Conflict of interests

The authors declare that they have no conflict of interest.

Funding

This research is supported by the Vice Chancellor for Research of Kashan University of Medical Sciences (Grant No: 95117).

Code of Ethics

This study was approved by the Ethics Committee of Kashan University of Medical Sciences (Ethics code: IR.KAUMS.REC.1395.121).

Authors' Contributions

Fatemeh Atoof: Conceptualization, Formal Analysis, Sahar Gholipour: Writing original draft, Zahra Shamsizadeh: Writing original draft, Mohsen Amirimoghaddam: Investigation, Nezam Mirzaei: Conceptualization, Investigation, Supervision, Ali Nazari-Alam: Investigation, Davarkhah Rabbani: Investigation, Mansour Baziar: Investigation, Software, Gholamreza Hoseindoost: Investigation, Gholamreza Mostafaii: Investigation, Abbas Behrami: Investigation

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