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Evaluation of bacterial bioaerosol diversity and density in the indoor air of elderly care facilities

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Abstract

Background: Exposure to bacteria due to the presence of pathogenic agents in public centers is a significant health issue. This study aimed to identify and quantify different bacterial species present in the respiratory air of 10 elderly care centers in Tehran.

Methods: This cross-sectional study was conducted in public halls and bedrooms across 10 different elderly care centers in Tehran, with three repetitions per location. A total of 60 air samples were collected using three types of media: nutrient agar, MacConkey agar, and mannitol salt agar. Air samples were collected according to the NIOSH 0800 method. The samples were then analyzed using standard microbial diagnostic tests.

Results: The average bacterial bioaerosol concentration in elderly care centers was 180 ± 117.9 CFU/m^3. The highest colony count was observed in the public hall of Elderly Care Center #4, with a concentration of 543 ± 220 CFU/m^3, accommodating 32 elderly residents. A quarter of the sampling points had *Staphylococcus aureus* contamination, with *Staphylococcus aureus* (gram-positive) and *Acinetobacter lwoffii* (gram-negative) as the most common bacteria found.

Conclusion: This study revealed significant variations in bioaerosol concentrations across elderly care centers. The bioaerosol concentrations were higher on average in the bedrooms compared to the common areas. These findings highlight the need for more targeted infection control measures in elderly care facilities. Improved ventilation systems, tailored to the specific conditions of these centers, can help reduce bioaerosol levels. The results also emphasize the necessity of establishing standardized bioaerosol control programs and optimizing building design and environmental conditions to mitigate health risks.

Keywords: Care facilities, Gram-negative bacteria, Gram-positive bacteria, Indoor air quality, Iran

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Introduction

The respiratory system inhales an average of 20 m^3 of air daily (1). Both the outdoor and indoor air can endanger human health if polluted. Indoor air quality is crucial because people spend a significant amount of time inside buildings (s2). Bioaerosols thrive best in environments with high humidity and mild temperatures, which are often found in closed and crowded spaces. Therefore, pollutants can have a greater impact on indoor air quality (IAQ) compared to outdoor air (3-6). Contact with bioaerosols can have many health effects. They can spread diseases, cause poisoning, or trigger allergies. Bioaerosols are biological particles carried by the air and include both dead and live bacteria and viruses, whether disease-causing or not. They also encompass fungi, molds, allergens, endotoxins, and toxins from fungi. Additionally, bioaerosols contain peptides, glycans, pollen, and plant fibers. Their sizes vary from 0.001 to 100 μm (7-9). Scientists refer to this tiny part of bioaerosols as the respirable part, consisting of particles less than 2.5 microns in diameter. These particles are a significant concern because they can penetrate deep into the respiratory system. They are a key component of aerosols, making up to 50% of all aerosol particles. Exposure to bioaerosols is a common aspect of everyone's lives. Germs in closed spaces can exacerbate diseases in already sick individuals, raising the risk of spreading diseases (10-12). Airborne pathogens pose a significant challenge as they are responsible for the spread of airborne diseases. These infections are a global issue, causing millions of deaths annually in low- and middle-income countries (13,14). Bioaerosols are a major cause of air pollution in enclosed or indoor spaces. Studies have indicated that bioaerosols constitute approximately 5%-34% of indoor air pollution, with airborne bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* being commonly detected in elderly care centers (7,8). The World Health Organization (WHO) and other regulatory bodies emphasize the need for maintaining IAQ, particularly in healthcare and care facilities. However, despite these recommendations, existing standards specifically targeting bioaerosol concentrations in elderly care centers are underdeveloped, and current practices often fail to adequately address microbial contamination (15).

In the study conducted by Fouladvand et al, the IAQ in liver transplantation operating rooms (LTOR) demonstrated a higher level of microbial contamination than the guidelines recommended for high-risk environments. To enhance air quality in LTOR, it is advised to implement regular microbial monitoring, ensure the effective functioning of ventilation systems, and prioritize their maintenance and operation (16).

Several factors, such as bioaerosol resistance, meteorological conditions, particle composition, and the nature and location of the emission source, govern the concentration, composition, and survival of bioaerosols. The amount of bioaerosols in congregational places can depend on multiple factors, and their exact quantity may vary. Bioaerosols are airborne particles that originate from biological sources, including bacteria, viruses, fungi, and pollen. Factors influencing the amount of bioaerosols in congregational places include (17-21):

- *Population density*: Crowded places like schools, hospitals, shopping centers, and public spaces have higher levels of bioaerosols.
- *Ventilation*: Proper ventilation systems can reduce the amount of bioaerosols. Conversely, enclosed and poorly ventilated spaces can have higher levels of these particles.
- *Humidity and temperature*: Environmental conditions can affect the proliferation and spread of bioaerosols. High humidity and appropriate temperatures can provide favorable conditions for the growth of microorganisms.
- *Human activities*: Various activities such as talking, coughing, and sneezing can lead to the release of bioaerosols.
- *Cleanliness and hygiene*: Cleaner surfaces and environments have lower levels of bioaerosols. Observing public hygiene can help reduce these particles.

Elderly care centers, characterized by their high population density and limited ventilation, are prime locations for bioaerosol accumulation. The presence of vulnerable individuals, including those with weakened immune systems, makes these environments particularly concerning. Residents in these centers are more susceptible to respiratory infections and other health issues caused by airborne pathogens, including bacteria, viruses, fungi, and pollen (4-6). The interaction between bioaerosols and the aging population increases the likelihood of disease transmission, allergies, and exacerbation of chronic conditions. However, despite this heightened risk, there has been limited research focused on the role and concentration of bioaerosols in such facilities. This gap in research underscores the need for a comprehensive understanding of bioaerosol dynamics in elderly care settings (22-24). In closed spaces, the air may contain a variety of microorganisms, including bacteria, fungi, and viruses. Some of these microorganisms can be harmful to human health (25). Special conditions in spaces like dorms and care centers affect the size and stability of these environments. New people arrive daily from different social backgrounds, with varying health conditions and ages. These conditions, along with factors like smoking, create environments where pathogens can spread. Pathogens are transmitted through airborne particles when people breathe, sneeze, or cough. Air quality in shared spaces, such as elderly care centers, is, therefore crucial. This study aimed to investigate the diversity and concentration of bacterial bioaerosols present in the indoor air of 10 elderly care centers in Tehran in 2023.

Materials and Methods

Study design and sampling location

This cross-sectional descriptive study was conducted over three months, from October to December 2023. The researchers investigated the amount of bacterial bioaerosols in the indoor air of 10 elderly care centers in Tehran, using 60 sampling points. The study examined the presence of bacterial bioaerosols, as well as temperature and relative humidity. The study was divided into two stages: In the first stage, we collected air samples from the accommodation halls (bedrooms and public halls) of each elderly care center. The information collected with each sample included the following parameters: duration of sampling (5 minutes), frequency of sampling (20 samples taken from different indoor locations over three months), type of cultivation environment, time and place of sampling, type of ventilation, number of individuals per hall, temperature, and humidity.

Air sampling

The air sampling device was placed 150 cm above the ground, at the breathing zone of people, and over one meter from the walls. We repeated 20 samples from different indoor locations over three months. The culture medium was prepared in the lab, kept sterile, and refrigerated until use. After sampling, the plate edges were sealed with parafilm to reduce secondary contamination. The plates were then transported to the laboratory while maintaining a cold environment. The sampled plates were incubated at 35-37 °C for 18 hours. In this study, a specialized device for air sampling of bacteria was used according to the NIOSH 0800 standard. Sampling was conducted at a flow rate of 28.3 L/min for 10 minutes on culture media. Due to the high bacterial density on the media, accurate colony counting was not achieved under these conditions, so the sampling time was reduced to 5 minutes for better accuracy. Sampling was conducted in the fall between 9:00 AM and 12:00 PM (2,26).

Culture and identification of bacteria

In the second stage, we counted, identified, and diagnosed the bacterial colonies. To collect all types of bacteria, we used media suitable for both gram-negative and gram-positive bacteria, along with general media. Sampling was performed using three types of media to determine pathogen levels in the air at each station. Nutrient agar was used to measure total contamination, MacConkey agar was used to identify gram-negative bacteria, and Mannitol Salt agar was used to identify gram-positive bacteria. The total number of colonies on Nutrient agar plates was reported as the total contamination at each station. A catalase test was performed on colonies from the Mannitol Salt agar to confirm the presence of *S. aureus*. Following NCCLS guidelines, colonies on MacConkey agar were confirmed as *E. coli* using Eosin Methylene Blue (EMB) Agar, and gram-negative bacteria were identified using a diagnostic gallery test as per NCCLS recommendations (27-30).

Data calculation and statistical analysis

The density of the colonies counted in the air was calculated using equation 1 (31).

$$\mathrm{C}\left(\mathrm{CFU}/\mathrm{m3}\right)=\frac{T\times 1000}{t\times f}\,(1)$$

Where C is the number of colonies per cubic meter of air colony forming unit per cubic meter of air, T is the total number of colonies counted on the nutrient agar medium, and t is time in minutes, and f is flow (L/min).

Descriptive statistics were used to determine the mean, spread, and standard deviation of the data, while analytical statistics were employed to find differences, correlations, and relationships. Data analysis was performed using SPSS software, and the results were compared with the WHO standards.

Results

In this study, we sampled 20 points located in the public halls and restrooms of 10 elderly care centers. We monitored these points and reported the density of bacterial bioaerosols in CFU/m³.

The average bacterial count at the sampling points was 180.8 ± 117.9 CFU/m³. The highest number of colonies was found in the public hall of Elderly Care Center #4, which had a volume of 543 ± 220 CFU/m³ and housed 32 elderly residents. Conversely, the lowest number of colonies was observed in the bedroom of Elderly Care Center #6, which had a volume of 68 CFU/m³ and housed 1 elderly resident.

The analysis revealed that 25% of the sampling points exhibited contamination with *S. aureus*. The results also indicated the presence of the gram- positive bacterium *S. aureus* and the gram- negative bacterium *Acinetobacter lwoffii*, which were the most commonly observed gram-positive and gram- negative bacteria in the sampled areas.

The average concentration of bacterial bioaerosols in the break room and common areas of elderly care centers was 180 ± 117.9 CFU/m³. Table 1 details the colony counts for each care center.

The Kolmogorov-Smirnov test was used to check the normal distribution of the data. Given that the bacterial bioaerosol concentration in the break room and common area followed a normal distribution, a t-test was performed. The results demonstrated that there was no statistically significant difference in the average levels of bacterial bioaerosols between the break room and the common area. Figure 1 illustrates the percentage frequency of gram- negative bacteria. According to the obtained results, the most frequently cultured bacterium was the gram- negative *A. lwoffi*, accounting for 8.4%. *A. lwoffi* is a gram- negative, non-motile bacterium belonging to the family Moraxellaceae. This bacterium is commonly found in various environments, including soil, water, and hospital surfaces. Although *A. lwoffi* is typically not a human pathogen, it can cause infections under certain conditions, particularly in individuals with weakened immune systems. Infections caused by *A. lwoffi* are rare but may include respiratory tract infections, urinary tract infections, and wound infections. Due to its resistance to many antibiotics, this bacterium is of particular concern in the context of hospital-acquired infections. Figure 2 illustrates the percentage distribution of gram- positive bacteria. The results indicate that the most prevalent bacterium in the cultures was *S. aureus*, a gram- positive bacterium, which accounted for 25.6% of the cases. *Staphylococcus aureus* is a gram- positive, coagulase-positive bacterium known for its role as both a commensal organism and a significant pathogen. This bacterium is part of the normal human flora, residing primarily on the skin and mucous membranes, but it can also cause a range of infections when it breaches the body's defenses. In our study, the majority of bacterial bioaerosols were found to be gram-positive, which is consistent with the existing literature reporting a high proportion of gram-positive bacteria in environmental samples (8,14,18-20). These findings align well with previous research. Gram-positive bacteria are commonly

Table 1. The results of bacterial count in elderly care center spaces

Center number	Location	Number of service recipients	Mean temperature (°C)	Bacterial count (CFU/m³)	Range (CFU/m³)
1	Bedroom	3	23	173±46	117-209
	Public hall	12	22	111±60	49-161
2	Bedroom	5	24	249±139	100-404
	Public hall	20	24.8	123±110	111-368
3	Bedroom	4	25	480±250	220-720
	Public hall	24	25	187±63	114-240
4	Bedroom	2	24	100±72	44-158
	Public hall	20	23	543±220	323-784
5	Bedroom	3	24.5	390±245	323-763
	Public hall	18	25	109±76	33-185
6	Bedroom	2	23	68±35	41-99
	Public hall	18	25	125±105	87-340
7	Bedroom	3	22	371±176	187-540
	Public hall	21	24	195±173	75-260
8	Bedroom	2	21	120±75	84-190
	Public hall	15	24	208±115	118-350
9	Bedroom	6	23	320±148	218-448
	Public hall	24	23	290±130	140-370
10	Bedroom	4	24	186±82	111-245
	Public hall	24	25	311±256	105-548

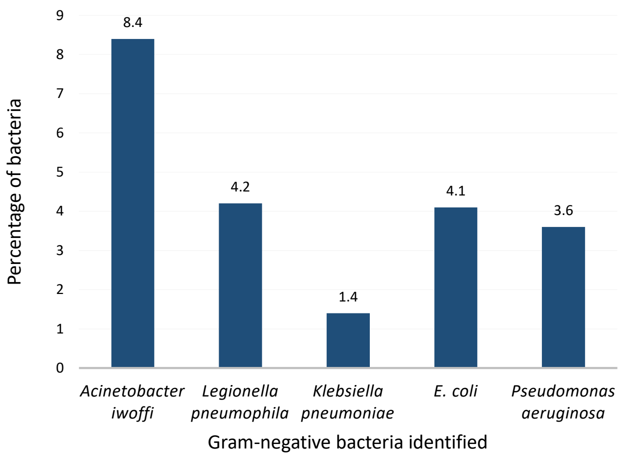

Figure 1. Frequency (%) of gram- negative bacteria in care facilities for the elderly

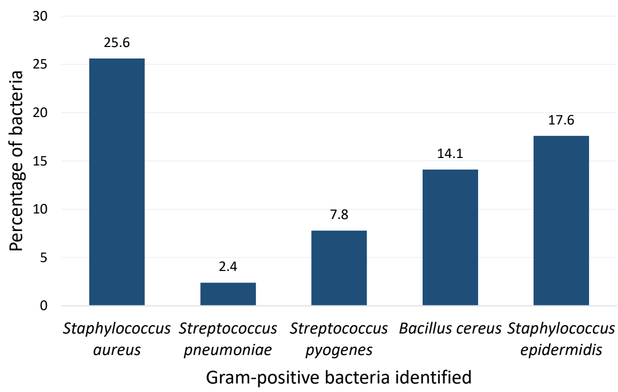

Figure 2. Frequency (%) of gram- Positive Bacteria in Care Facilities for the Elderly

found in a variety of macro- and micro-environments and are a natural component of the flora on human and animal skin, mucous membranes, and hair. Furthermore, gram-positive bacteria exhibit a higher level of resistance compared to gram-negative bacteria, which allows them to survive in unfavorable environmental conditions (32).

Discussion

The air contains many microorganisms that can cause infectious or allergic diseases in humans. Each time air enters the lungs, it can carry bacteria. The number and type of bacteria present determine whether they may cause infections or allergies. Bioaerosols are generally associated with various adverse health effects. Recognizing the importance of this issue, the WHO released a guideline on IAQ in 2009 (21,32). Well-established environmental health studies indicate that exposure to bioaerosols at levels exceeding acceptable limits can cause non-infectious and infectious diseases, including nosocomial infections or healthcare-associated infections, acute toxic effects, cancer, and in severe cases, death, especially for those with weakened immune systems (33,34).

Elderly care centers house many individuals, thereby increasing the risk of exposure to airborne agents, which are significant factors in the spread of diseases among patients. These facilities are very important and require strict standards due to the presence of vulnerable individuals, which heightens the risk of disease transmission. According to WHO guidelines, indoor air should contain 0–500 CFU/m³ of harmless bacteria and zero pathogenic bacteria (35,36).

In a study by Nasir and Colbeck, the density of bacterial bioaerosols in homes was examined, with bacterial colony counts ranging from 1557 to 5036 CFU/m³ (23). Wang et al measured the density of bacterial bioaerosols in a hospital in Taiwan, and found that the lowest and highest bacterial colony counts were 35 and 728 CFU/m³, respectively (24). Gilbert et al investigated the density of bacterial bioaerosols in hospital-breathing air, and found that the lowest and highest bacterial colony counts were 38 and 131 CFU/m³, respectively (25). Pasquarella et al explored bacterial levels in museum air, and reported average, minimum, and maximum bacterial colony counts of 714, 545, and 883 CFU/m³, respectively (26).

The findings of the study reveal that bacterial colonies are highly concentrated, which aligns with previous studies that report elevated levels of bacterial bioaerosols in densely populated and active environments. These bioaerosols can give rise to various health concerns, including inflammation and irritation of the upper and lower respiratory systems, as well as inducing lung allergies in susceptible individuals. The inhalation of these bioaerosols may result in respiratory infections and potentially reduce lung functionality. Additionally, factors such as temperature, humidity, and ventilation have a significant impact on indoor bioaerosol levels, while activities such as coughing, sneezing, and talking, in addition to walking and washing, can release biological particles into the air.

In Figure 2, the amount of bacteria in the indoor air of the monitored halls is depicted. *S. aureus* was identified as the most common bacterium, making up 25% of the bacterial population. This prevalence is attributed to its resistance to temperature fluctuations and environmental conditions, which can lead to respiratory infections in high-risk individuals. The presence of a high number of gram-positive cocci in the air may be due to their lower sensitivity to pressure and heat. Enterococci are resilient bacteria that thrive in harsh conditions and significantly contribute to bacterial pollution. Factors such as the number of occupants, airflow, the condition of the building, and the characteristics of the residents all affect the density of bacterial contamination. In the study by Pereira et al., the average concentrations of bacteria and fungi in the indoor air of nursing homes varied from 121 to 319 CFU/m³ and 63 to 221 CFU/m³ during summer, respectively. In winter, these concentrations ranged from 2.5 to 179 CFU/m³ for bacteria and 21 to 264 CFU/m³ for fungi. The most prevalent bacterial genus identified was *Staphylococcus*, accounting for 39%–58% in summer and 46%–72% in winter. This was followed by *Micrococcus*, which comprised 22%–36% in summer and 10%–26% in winter, and *Bacillus*, which represented 19–28% in summer and 17%–29% in winter. Conversely, *Solibacillus* (3%) and *Kocuria* (1%) were the least frequently identified genera, both of which were only found in summer (37).

This study focused on bacterial bioaerosols in the bedrooms and public halls of elderly care centers, which may not represent other areas, such as kitchens or bathrooms, where different bacterial populations may exist. The sampling size of 20 points may limit the generalizability of the findings, and a longer study duration encompassing different seasons could provide a more comprehensive understanding of variations in bacterial density. While temperature and humidity were monitored, other environmental factors like airflow and building conditions were not thoroughly assessed. Additionally, reliance on culture-based methods may overlook viable but non-culturable bacteria. Lastly, this study did not directly evaluate the health impacts on residents, limiting our ability to draw clear connections between bacterial levels and health outcomes.

Conclusion

Air pollution is widely recognized as a major cause of health problems, impacting both indoor and outdoor environments. Recently, researchers have concentrated on public concerns regarding IAQ. This article summarizes information about the concentration of bacterial bioaerosols in the indoor air of elderly care centers. A thorough understanding of bioaerosol concentrations in the indoor air of healthcare facilities is crucial for evaluating potential health risks to both residents and healthcare staff. The results of this study reveal that the density of bioaerosols varies significantly among different elderly care centers, with gram- positive bacteria being the dominant constituents of the bacterial bioaerosol community. Despite these findings, the study was limited in scope regarding the detailed composition of bioaerosols. Therefore, future research should aim to conduct more extensive analyses of bioaerosol composition in the indoor environments of elderly care centers. Such comprehensive studies are necessary to deepen our understanding of indoor bioaerosols and to evaluate their potential pathogenicity and implications for health.

Overall, the implementation of effective infection control strategies in healthcare facilities is essential to prevent the transmission of hospital-associated infections. The use of appropriate ventilation systems can help reduce the concentration of bioaerosols. Comprehensive bioaerosol control programs in enclosed spaces are necessary. Given the density and age of buildings, it is recommended to further standardize the spaces within elderly care centers, optimally design and install ventilation systems, control temperature and humidity, improve precise disease surveillance, and effectively activate health care units.

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Authors' contributions

Conceptualization: Maryam Meserghani, Pezhman Gheitasian, Anoushiravan Mohseni-Bandpey, Elham Shariatmadari, Farbod Ebadifard Azar.
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Visualization: Maryam Meserghani, Pezhman Gheitasian, Anoushiravan Mohseni-Bandpey.
Writing–original draft: Maryam Meserghani, Pezhman Gheitasian, Anoushiravan Mohseni- Bandpey.
Writing–review & editing: Maryam Meserghani, Pezhman Gheitasian, Anoushiravan Mohseni-Bandpey.

Competing interests

The authors declare that there is no conflict of interests.

Ethical issues

This study was approved by the Ethics Committee of the Iran University of Medical Sciences (Ethical code: IR.IUMS.REC.1402.1164). The authors affirm that all the data gathered during the study are accurately presented in the manuscript, and no information from this study has been or will be published independently elsewhere.

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