

## Microbiological Air Quality and Antibiotic Resistance in Isolated Bioaerosols from Various Activities in Zanzan, Iran

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### ABSTRACT

**Introduction:** The aim of this study is the isolation and primary identification of isolated bioaerosols sampled from different locations, as well as determining their antibiotic resistance profile for the selected bacteria.

**Materials and Methods:** The air samples were collected from 35 stations in 17 buildings (bank, public, healthcare and industrial). An air-sampling pump (Flite3-SKC) with a flow rate of 14.1 l/min was used for five minutes at the respiratory height. The total bacterial count and primary identification were carried out, then the antibiotic susceptibility tests for ten selected antibiotics were conducted by the disk diffusion method.

**Results:** The bacterial population varied at a range of 128-5503 CFU/m<sup>3</sup>, and the fruit and vegetable bazaar of Zanzan showed the highest population of bioaerosols. The bioaerosols population among industrial samples was high. Most isolates were determined as gram-positive Cocci (> 70%) and *Bacillus* spp, respectively. The most antibiotic-resistant bacteria were related to manufacturing activities, showing resistance to Chloramphenicol and Trimethoprim/ Sulfamethoxazole (72%).

**Conclusion:** The type of activity affects the bacterial population and antibiotic resistance in industrial uses. The increase of multi-drug resistant bacteria, especially in medical settings, is known as a community health challenge and needs more attention.

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### Introduction

The particles with biological sources introduced as bioaerosols (bacteria, fungi, viruses, pollen, and biomolecules) are suspended in the air and their sizes vary from 0.02 to 100  $\mu\text{m}$ <sup>1</sup>. The inhaled penetration depth is highly associated with the particle size, wherein the repairable fractions (< 3.3  $\mu\text{m}$ ) play an important role in the epidemiology of diseases<sup>2</sup>. Bioaerosols account for 5-34% of indoor air pollution (IAQ) problems<sup>3</sup>. Most people spend about 80-90% of their time in

indoor environments while inhaling approximately 15000 liters of air per day<sup>1,2</sup>. It is confirmed by studies conducted by the United States Environmental Protection Agency (USEPA) that the health risks from exposure to indoor air pollution could be more severe than the outdoors<sup>2,4</sup>.

The indoor air micro-flora, which is more stable, ranked among the top five risks to public health<sup>3,5</sup>. According to the World Health Organization (WHO) reports, 5000000 people die each year before puberty, because of exposure to airborne

aerosols<sup>4</sup>. Some protocols have been approved in this regard, but no comprehensive standard is determined for all air quality control purposes. The Taiwan Environmental Organization has set the 500 and 100 CFU/m<sup>3</sup> as the standard number of bacteria and fungi, respectively<sup>4</sup>.

Indoor air pollution leads to many health problems affected by the environmental, biological, and personal factors<sup>6</sup>. The environmental factors include the internal sources and their emission rates, air exchange rates (ACHs), the ratio of indoor to outdoor pollution (I/O), and the rate of sedimentation or removal of contaminants on inner surfaces (sinks)<sup>4</sup>. Biological factors can be summarized as the dose of inhaled air pollutants, their biological effect (which could be defined as the number of biological pollutants interacting with a target site), and the way of transmission. The pathogenicity mechanisms and interaction of biological pollutants such as bacteria and fungi are different. The personal exposure levels, individual susceptibilities, and immune responses are highly variable among the population<sup>2</sup>. Over-use and misuse of antibiotics resulting from all the above factors could produce a high population of antibiotic-resistant bacteria with high pathogenicity. The survival of these strains results from a complex interplay of factors, including the physiology and ecology of microorganisms and the whole set of abiotic conditions<sup>2,7</sup>. The transmission of antibiotic resistance genes among pathogenic bacteria and non-pathogen microorganisms has increased<sup>8,2</sup>. This trend of multi-drug resistance in microorganisms is a priority task for medicine and public health<sup>5,8</sup>. More than 0.7 million people die each year because of antibiotic-resistant pathogens, and it is estimated that it will increase to 10 million by 2050. Unfortunately, in Iran, prescription and overuse of antibiotics is a serious problem, leading to an increase in multi-drug resistance among bacteria in hospitals and an increase in nosocomial infections.

The aims of this study is classified into three categories:

a) To assess the bacterial population in the selected buildings with different land uses (banks,

shopping centers, medical centers, industries etc.),

b) To primarily identify bacteria,

c) To determine the antibiotic resistance of bioaerosols bacteria and their multi-drug resistance pattern.

## Materials and Methods

### Sampling location

Zanjan, the capital of Zanjan province, located in the northwest of Iran, was selected for this evaluation. Buildings in different locations and with different uses, and features were selected as sampling sites.

The air samples were collected from 35 stations located in 17 different places and were categorized based on the type of activities into four categories (banks, medical and healthcare, industrial, and public places).

The sampling period was from May to October 2021. All samples were taken on working days and during peak hours (9:00 a.m. to 13:00 p.m.). During the sampling, according to the COVID-19 restrictions, the presence of people in the environment was less than normal.

### Laboratory experiments

Before sampling, the nutrient agar medium (Merck Co., Germany) was prepared in Petri dishes (9 cm). Before placing the Petri dishes inside, the cassette was disinfected and dried using 70% ethyl alcohol to sterilize and remove any initial contamination and biological pollutants<sup>3,4</sup>.

The air samples were collected using SKC, Flite 3 air sample pumps (the SKC Limited Co., USA) and the pump flow was calibrated before sampling, using a Rotameter (Influx, UK). The flow rate of 14.1 l/min ( $2.35 \times 10^{-4}$  m<sup>3</sup>/s) and duration of 5 min was prescribed for this purpose<sup>9</sup>. The sampling probe was placed at a distance of 1.5 meters from the ground to signify the breathing zone, with a suitable distance from the walls and windows<sup>1,4</sup>. The airborne bacteria settle on the surface of the media (9 cm Petri dishes) due to the suction created by the pump for microbial enumerations. The sampling was carried out in triplicates<sup>8</sup>. The location and sampling time were recorded for each sample.

After sampling, the Petri dishes were immediately transferred to the laboratory and incubated at 35 °C for 48 h. Subsequently, the grown colonies were enumerated on nutrient agar medium, and the population was reported as colony-forming units per cubic meter of air (CFU/m<sup>3</sup>). Equation (1) was used for this calculation<sup>8,9</sup>.

#### Equation (1)

$$\text{Microbial Population (CFU/m}^3\text{)} = \frac{\text{Total colony number} \times 1000}{\text{volume of the air sample equal to the flow rate} \times \text{time}}$$

**Microbiological analysis:** Airborne bacteria grown on Petri dishes were initially isolated and then identified by colonial morphological features and microscopic characteristics (bacterial morphology and reaction to Gram staining). Then, the antibiotic resistance pattern was determined for the isolates<sup>2</sup>.

**Antibiotic resistance pattern:** An antimicrobial susceptibility test was performed according to the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol<sup>2</sup>. For standard inoculation, single colonies were transferred to a sterile saline tube by a sterile loop until the turbidity of the solution matched McFarland standard solution corresponding to 3×10<sup>8</sup> CFU/mL. Then, 100 µl of the bacterial inoculum was added and spread over the surface of a Mueller-Hinton agar plate (Merck Company, Germany) using a sterile swab and left to dry off before placing antibiotic discs<sup>2, 10</sup>. Antimicrobial susceptibility testing discs (Padtan Teb Co., Iran) were placed on the inoculated Mueller-Hinton agar plates using sterile forceps while pushing them to ensure complete contact

between the media and the antibiotic disc<sup>3, 10</sup>. In this study, ten antibiotics were selected for testing. The inoculated plates were incubated for 24 h at 35 °C. The diameter of the inhibition zone for each antibiotic was measured on the Petri dish and then compared to the Performance Standards for Antimicrobial Susceptibility Testing from the Clinical and Laboratory Standards Institute (2018)<sup>10</sup>. The inhibition zone size should be divided into three-stage scales:

If the diameter of the growth inhibition zone was less than 15 mm, bacteria would be resistant to the antibiotic (R). A growth inhibition diameter of 16-25 mm indicated that the bacteria had an intermediate level of resistance to the antibiotic (I), while a growth inhibition zone diameter of higher than 25 mm indicated that the bacteria were sensitive to the antibiotic (S)<sup>2, 3</sup>. The multidrug resistance (MDR) bacteria were shown based on the antibiotic resistance pattern.

**Data analysis:** The data were analyzed with SPSS (version 22) and P < 0.05 was considered statistically significant.

#### Ethical Issue

The present study has been approved by the Ethics Committee in University of Zanjan (IR.ZNU.REC.1400.001).

#### Results

The collected samples were divided into four groups based on the type of activity done in each sampling place (Table 1). In the first step, the bacterial counts of each Petri dish were determined and presented in Figure 1.

**Table 1:** Classification of sampling points based on activity type

Group	Activity	Location
Group A	Administrative activity in offices (Banks)	(No.1: Bank Melli, Shohada Rd branch) (No. 2 Bank Melli, Middle Saadi branch) (No. 3 Bank Melli, Khorramshahr branch) (No.4 Bank Melli, Saabz e Midan branch, underground) (No. Bank Melli, Saabz e Midan branch)
Group B	General activities in public places	Zanjan Bazaar (indoor), Sports club, Shopping center and Post office
Group C	Medical activity and health centers	Mousavi Hospital, Valiasr Hospital, Shahid Beheshti Hospital, Health Center No. 8
Group D	Manufacture activities	Zinc Industry, Aviculture

The bacterial counts were divided into four categories based on activity groups:

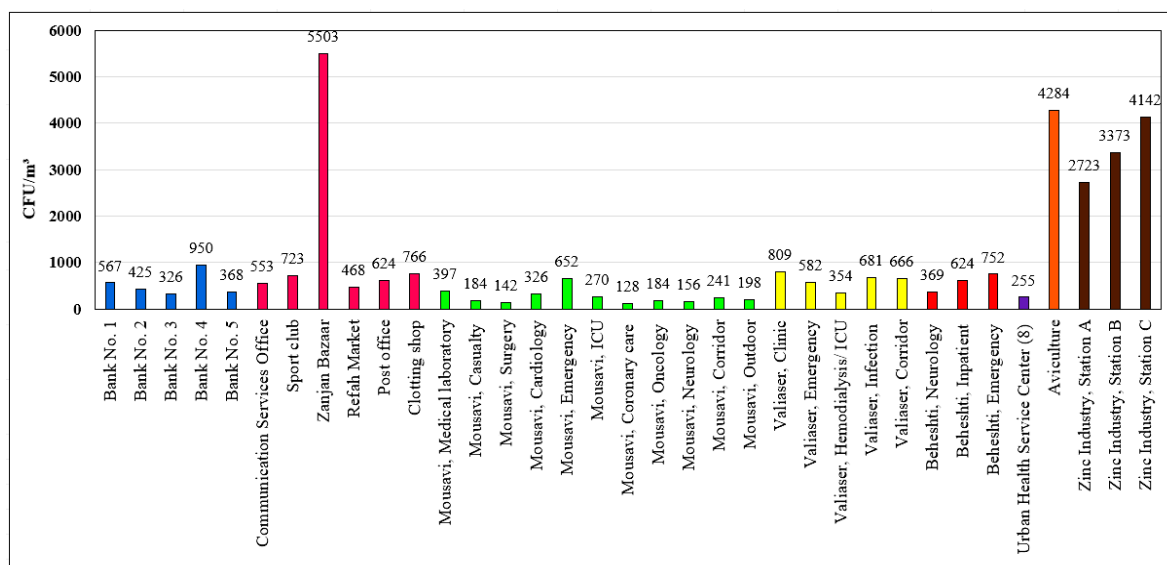
**Group A:** In the first group (five banks), the bacterial population in banks No. 4 and No. 3 was 950 CFU/m<sup>3</sup> (the maximum amount) and 326 CFU/m<sup>3</sup> (the minimum amount), respectively.

**Group B:** For public activities, the concentration of airborne bacteria in Zanjan Bazaar was 5503 CFU/m<sup>3</sup>, which was higher than other samples. In other places including sports clubs, post offices, and shopping centers, the number of bacteria was in the range of 468-766 CFU/m<sup>3</sup>.

**Group C:** In this group, three important hospitals including Ayatollah Mousavi Hospital (11 stations), Valiasr Hospital (five stations), the Educational Treatment Center of Shahid Beheshti (three stations), and a comprehensive urban health service center (No. 8) were monitored. In Ayatollah Mousavi Hospital, airborne bacteria population varied from 128 to 652 CFU/m<sup>3</sup>, and the lowest and highest amounts were recorded in the CCU and emergency wards, respectively. The

concentration of bioaerosols bacteria in the emergency unit of Beheshti Hospital was 752 CFU/m<sup>3</sup>. Valiasr Hospital yielded the highest concentration of airborne bacteria (809 CFU/m<sup>3</sup>) in clinics and the lowest number of bacteria (354 CFU/m<sup>3</sup>) in the ICU allocated for COVID-19 patients. The population of bacteria in the comprehensive urban health service center (No. 8) was 255 CFU/m<sup>3</sup>.

**Group D:** In the last group, the samples were collected from the outdoor air of an industrial estate (Zinc Functional Estates of Zanjan). Despite the high concentration of acidic components and dust in the air of this region resulting from their type of activities, the number of bacteria in different stations was surprisingly high, ranging from 2723 to 4142 CFU/m<sup>3</sup>. The number of airborne bacteria inside of aviculture was also significant (4284 CFU/m<sup>3</sup>). The results revealed that there was a significant difference in the bacterial population among group D compared to previous ones (Figure 1).



**Figure 1:** Descriptive of sampling places and bacterial density (CFU/m<sup>3</sup>)

The primary identification of samples demonstrated that most of the isolates were gram-positive Coccus, belong to the *Staphylococcus* spp. and different types of gram-positive *Bacillus* spp. were in the second place.

After the primary identification of twenty-seven isolates, the antibiotic resistance profile was determined using Bauer Kirby-Disk Diffusion method<sup>10</sup>. The list of examined antibiotics and the specific doses is presented in Table 2. The

mechanisms of antibacterial action are different based on the type and structure (protein, cell membrane) of bacteria<sup>11</sup>. The negative control was a blank disc, without any inhibition zone around the control discs<sup>10</sup>.

The results of the antibiotic susceptibility test are represented in Table 3, reported as the percentage of the antibiotic-resistant or sensitive bacteria in the population.

**Table 2:** Antibiotics and their doses used in susceptibility test

Group of Antibiotic	Antibiotic	Dose (µg)
Aminocoumarin	Chloramphenicol (C)	30
	Gentamicin (GM)	10
Cephalosporin	Cefotaxime (CTX)	30
Macrolides	Erythromycin (E)	15
Drug against mycobacteria	Rifampicin (RA)	5
Glycopeptides	Vancomycin (V)	30
Penicillins	Ampicillin (AM)	10
	Piperacillin (PIP)	100
Quinolones	Ciprofloxacin (CP)	5
Sulfonamides	Trimethoprim/sulfamethoxazole (SXT)	1.25

**Table 3:** Results of antibiotic susceptibility test based on percentage

Group of Activity Number of tested samples	Administrative 5			Public 3			Medical 12			Manufacture 7		
	R*%	I%	S%	R%	I%	S%	R%	I%	S%	R%	I%	S%
Antibiotic												
Chloramphenicol	20	0	80	67	33	0	25	17	58	72	28	0
Rifampicin	40	0	60	34	33	33	33	8	59	0	0	100
Cefotaxime	40	40	20	67	33	0	25	33	42	0	57	43
Erythromycin	0	60	40	67	0	33	25	42	33	71	29	0
Gentamicin	0	0	100	0	0	100	0	9	91	0	0	100
Vancomycin	40	20	40	67	33	0	42	0	58	0	0	100
Ampicillin	60	20	20	34	33	33	25	25	50	15	28	57
Piperacillin	40	20	40	67	0	33	33	9	58	43	14	43
Ciprofloxacin	40	20	40	0	33	67	8	17	75	71	29	0
Trimethoprim/Sulfamethoxazole	20	40	40	0	33	67	17	8	75	72	0	28

\* R%: Percentage of resistant isolates  
I%: Percentage of intermediate susceptibility  
S%: Percentage of sensitive isolates

Among administrative activities in banks, five bacteria were analyzed and 100% and 80% were sensitive to Gentamicin and Trimethoprim/Sulfamethoxazole, respectively. Moreover, 60% of bacteria were resistant to Ampicillin (Table 3). In the public group, less than 70% of the isolates were resistant to different types of antibiotics such as Piperacillin, Chloramphenicol, Vancomycin, Erythromycin, and Cefotaxime.

Sensitivity to Gentamicin and Ciprofloxacin was observed in 80% and 60% of the bacteria, respectively. In addition, 60% of the isolates were resistant to Piperacillin. In the manufacturing group, the highest antibiotic resistance was observed for Chloramphenicol and Trimethoprim/Sulfamethoxazole (72%).

The pattern of antibiotic susceptibility determined by inhibition zone (mm) is shown in Table 4. The highest MDR was related to six antibiotics, simultaneously. The isolate from bank No. 2 was a gram-positive bacillus, and the isolates from the sports club (public activities), the corridor and laboratory of Mousavi Hospital (medical group) were gram-positive Cocci and resistant to six antibiotics. In addition, the isolates from health center No. 8 (medical group) showed susceptibility to all the tested antibiotics. Based on the data analysis illustrating the administrative activity, 60% of the isolates were resistant to Ampicillin. In

the medical group, the highest population of resistant bacteria was related to Vancomycin, Rifampicin, and Piperacillin. In Beheshti and Valiaser Hospitals, over 50% of strains were resistant to Vancomycin. Gentamicin and Ciprofloxacin susceptibility was in the range of 80-100% of isolates. In the emergency department of Beheshti Hospital, tetra-resistance to Trimethoprim/Sulfamethoxazole, Chloramphenicol, Ampicillin, and Cefotaxime was determined. The isolates from the clinic and emergency of Valiaser Hospital showed tri-antibiotic resistance patterns. The Trimethoprim/Sulfamethoxazole resistance shown in the hemodialysis ward was for COVID-19 patients. No antibiotic resistant bacteria were isolated in the ICU (allocated for COVID-19 patients) and surgery departments. The highest MDR, tetra antibiotic resistance, was determined for Piperacillin, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, and Chloramphenicol in the strain isolated from the Aviculture. All the bacteria were sensitive to Gentamicin, Vancomycin, and Rifampin but resistant to Chloramphenicol. The strain isolated from zinc industrial estate of Zanzan showed a Penta-resistance profile (Erythromycin, Vancomycin, Piperacillin, Chloramphenicol, and Trimethoprim/Sulfamethoxazole), and resistance to Erythromycin was observed in all isolates.

**Table 4:** The profile of multi-antibiotic resistance of isolated bioaerosols

	Sampling place	Isolated bacteria	PIP	E	GM	V	CP	SXT	C	CTX	RA	AM
<b>Banks</b>	Bank No. 1	G <sup>+</sup> /Coccus	I	I	S	R	S	S	S	I	R	R
	Bank No. 2	G <sup>+</sup> /Bacillus	R	I	S	R	I	S	R	R	R	R
	Bank No. 3	G <sup>+</sup> /Bacillus	I	S	S	S	R	I	S	I	S	R
	Bank No. 4	G <sup>+</sup> /Coccus	S	I	S	S	S	R	S	S	S	S
	Bank No. 5	G <sup>+</sup> /Coccus	I	S	S	I	I	I	S	R	S	I
<b>Medical &amp; Healthcare</b>	Mousavi, Medical Laboratory	G <sup>+</sup> /Coccus	R	I	S	R	I	S	R	R	R	R
	Mousavi, Surgery	G <sup>+</sup> /Coccus	S	S	S	S	S	S	S	S	S	S
	Mousavi, Emergency	G <sup>+</sup> /Coccus	R	R	S	S	S	S	S	S	S	I
	Mousavi, ICU	G <sup>+</sup> /Coccus	S	S	I	S	I	S	S	S	S	S
	Mousavi, Corridor	G <sup>+</sup> /Coccus	R	R	S	R	S	S	R	R	R	I
	Valiaser, Clinic	G <sup>+</sup> /Coccus	R	S	S	R	S	S	I	I	I	R
	Valiaser, Emergency	G <sup>+</sup> /Coccus	S	R	S	R	S	R	S	S	S	I
	Valiaser, Hemodialysis	G <sup>+</sup> /Bacillus	S	I	S	S	S	R	S	I	S	S
	Beheshti, Inpatient	G <sup>+</sup> /Bacillus	S	I	S	S	S	S	I	I	R	S
	Beheshti, Neurology	G <sup>+</sup> /Coccus	I	I	S	R	S	S	S	I	R	S
	Beheshti, Emergency	G <sup>+</sup> /Coccus	S	I	S	S	R	I	R	R	S	R
	Health Center No. 8	G <sup>+</sup> /Coccus	S	S	S	S	S	S	S	S	S	S
<b>Public</b>	Communication Services Office	G <sup>+</sup> /Coccus	R	S	S	R	S	S	I	I	I	R
	Sport Club	G <sup>+</sup> /Coccus	R	R	S	R	S	S	R	R	R	I
	Refah Market	G <sup>+</sup> /Coccus	S	R	S	I	I	I	R	R	S	S
<b>Industrial</b>	Aviculture 1	G <sup>+</sup> /Coccus	R	I	S	S	R	R	R	I	S	I
	Aviculture 2	G <sup>+</sup> /Coccus	S	R	S	S	I	R	R	I	S	S
	Aviculture 3	G <sup>+</sup> /Coccus	I	I	S	S	R	S	R	S	S	R
	Zinc Estate 1	G <sup>+</sup> /Bacillus	S	R	S	S	R	R	R	S	S	S
	Zinc Estate 2	G <sup>+</sup> /Coccus	R	R	S	S	I	R	I	I	S	S
	Zinc Estate 3	G <sup>+</sup> /Coccus	R	R	S	S	R	R	R	I	S	I
	Zinc Estate 4	G <sup>+</sup> /Coccus	S	R	S	S	R	S	I	S	S	S

## Discussion

Bioaerosols are a complex mixture in the air, obtained from liquid suspensions that undergo desiccation, whereas those generated as dust or powders are partially rehydrate. The concentration and types of airborne bacteria are important issues in air quality, depending on many environmental and human factors <sup>12</sup>. The increasing incidence of nosocomial and occupational diseases due to bioaerosols exposure indicates the need for thorough knowledge in this respect. In this study, the number and type of airborne bacteria in various places with different activities were evaluated and consequently, the antibiotic resistance profiles for some isolates was monitored. In the administrative activity, the collected samples were from five banks in different places and with various characteristics, and the number of bacteria ranged from 326 to 950 CFU/m<sup>3</sup>. The bacterial count in bank No. 4 was 950 CFU/m<sup>3</sup>, considerably higher than the other samples. The location of this bank

(No. 4) was in a small place, underground, with a large number of clients and adjacent to streets full of traffic. In similar studies, the population of bacteria was 280 CFU/m<sup>3</sup> <sup>13</sup>, 600 CFU/m<sup>3</sup> in Poland and 580 CFU/m<sup>3</sup> in Hong Kong <sup>2</sup>. Similar studies in four office buildings in Poland showed that the concentration of bacteria in the office rooms was in the range of 540-1360 CFU/m<sup>3</sup> <sup>3</sup>, which was in line with the present study.

The study conducted by Sadigh in two universities in Ardebil (2021), showed that the population of bacteria was in the range of 18-132 CFU/m<sup>3</sup> <sup>4</sup>. Two schools similarly reported the highest mean concentration of bacteria in small cafeterias (884 CFU/m<sup>3</sup> in School A and 1906 CFU/m<sup>3</sup> in School B), compared to classrooms and the library <sup>5</sup>. The bacterial count from a primary and high school in an industrial region of Poland was 2205 and 391 CFU/m<sup>3</sup> <sup>14</sup>, suggesting that the population and physical activity affect air pollution <sup>12</sup>. The human responses to exposure is variant and

recovering potentially hazardous microorganisms in routine sampling is difficult; as a consequence, there is no international standard available for maximum levels of bioaerosols in the air <sup>2</sup>.

According to the WHO reports, the total microbial load should not exceed 1000 CFU/m<sup>3</sup> in indoor places, whereas Polish proposals for mesophilic bacteria suggest 5000 CFU/m<sup>3</sup> for public service buildings <sup>2, 15</sup>.

In the present study, the number of bacteria in most public and medical locations was in the acceptable range of population, based on the above standards. However, more attention should be paid to control air pollution in public places. The sampling time (seasons, month or day), ventilation rate, age, construction and building materials could affect the results <sup>14, 16</sup>.

The population of airborne bacteria in the traditional market in Zanjan was significantly high. Zanjan Bazaar is the longest indoor market in Iran, by 200 years of age. The entrance shops are used for selling fresh fruit and vegetable. It is suggested that indoor pollution could be affected by air pollutants from the outdoors, and this bazaar is located adjacent to high-traffic streets in down town. Subsequently, people who are in the bazaar for a long time could be affected by a high concentration of bioaerosols (more than 10<sup>2</sup>-10<sup>3</sup>), especially on the weekend <sup>17, 18</sup>. The count of airborne bacteria in Zanjan Bazaar was far more than the WHO recommended range. In a similar report from a traditional wet market in Taiwan, the bacteria count was reported around 2.7 × 10<sup>4</sup> CFU/m<sup>3</sup> <sup>17</sup>.

In the sports club, placed underground, the airborne bacteria concentration was 723 CFU/m<sup>3</sup>, during quiet hours of activity with only seven people exercising. In the Islamic Republic of Iran, the sports clubs (especially for women) must be located in enclosed places and this point emphasizes more attention to the ventilation systems. Unfortunately, most indoor sport clubs in Iran lack ventilation equipment, and the high population and type of activities in sports clubs increase the bacterial counts. A similar study on sports facilities showed large fluctuations in concentrations of heterotrophic bacteria, ranging

from 38 CFU/m<sup>3</sup> (swimming pool) to 1036 CFU/m<sup>3</sup> (sports hall) <sup>19</sup>. In another report, the number of airborne bacteria in different sports clubs at Ardebil University was between 200 and 728 CFU/m<sup>3</sup> <sup>20</sup>. In fitness centers, resuspension of dust from the ground (because of physical activities and regular contact between the users and surfaces such as exercise instruments, floor mats, and handrails) and high moisture due to intense sweat discharge of the users, promote microbial growth <sup>19</sup>. The ventilation systems, the type of activity and the number of personnel are effective for controlling air pollution bacteria.

Other analyzed samples belonged to medical centers (three hospitals and a health center). The airborne bacteria showed high concentrations in corridors, medical laboratories, and emergency units. In the surgery room, the population of bacteria was 142 CFU/m<sup>3</sup>, at the optimal range (0-250 CFU/m<sup>3</sup>). In general, a population of more than 750 CFU/m<sup>3</sup> was not acceptable in hospital wards <sup>21</sup>. The environmental and public factors (the type of microorganisms and the activity inwards) are in effect <sup>22</sup>. The high bacterial pollution in Valiaser Hospital was related to the hospitalization of infectious diseases, lack of ventilation systems according to the relevant standards, excessive numbers of patients and their families, etc. <sup>22</sup>. According to prior studies in Valiaser Hospital, the population of airborne bacteria rises significantly during the visiting times <sup>22, 23</sup>. The counts of airborne bacteria in some wards (CCUs, surgery rooms, and oncology) were less than other wards. The low population in the oncology ward was due to the disinfection of this area because of its sensitivity, position, unit hygiene standards, and a lack of proximity to other departments with less opening and closing of doors and windows. The low number of bioaerosols bacteria in surgery rooms was due to personnel limitations and fewer open and closed windows and doors <sup>22</sup>. In the emergency department and clinic, the number of personnel, patients and their families could be the most reason for high bioaerosols pollution.

The last type of activities which was aviculture and zinc estate was evaluated in several stations.

The results showed a higher bacterial population compared to the previous activities. In the poultry hall, the low air quality could result in undesirable effects such as respiratory distress, immunosuppression, behavioral changes, and impaired reproductive success<sup>24</sup>. In aviculture, presence of animals, manure and wastes, feed, and bedding materials could explain the high concentration of airborne pollutants. The count of bacteria was 4284 CFU/m<sup>3</sup>, fourth times higher than the recommended WHO guideline. In the report from a poultry house, the bacterial counts were from 40 to  $7.2 \times 10^3$  CFU/m<sup>3</sup><sup>25</sup>. In another research, bacterial population was  $6.6 \times 10^4$  from free-range guinea fowl to  $1.3 \times 10^7$  (conventional guinea fowl)<sup>26</sup>. The results demonstrated that environmental factors such as air temperature, airspeed, relative humidity and ammonium concentration changed the airborne bacterial population in animal farms<sup>27</sup>.

The bacterial counts in the Zinc Functional Estates of Zanjan were between 2723 and 4142 CFU/m<sup>3</sup>, which was similar to another study in an industrial estate in south China, where the population of bioaerosols was 4040 CFU/m<sup>3</sup>. The high level of organic and metal concentrations may increase the microbial viability and their population in industrial estates, confirmed by previous studies<sup>28,29</sup>. The population of air-borne bacteria from a salt mine in Poland was less than the present study<sup>30</sup>. The respirable dust from a coalmine in China containing bioaerosols bacteria was demonstrated as an occupational health risk<sup>31</sup>. The recommended maximum range of air-borne bacteria is 1000 CFU/m<sup>3</sup> for the total number of bioaerosols particles set by the National Institute of Occupational Safety and Health (NIOSH). The American Conference of Governmental Industrial Hygienists (ACGIH) suggested the culturable count for total bacteria not exceed 500 CFU/m<sup>3</sup><sup>30</sup>.

In this study, most isolates were gram-positive bacteria, which are more resistant to environmental factors such as dryness compared to gram-negative bacteria<sup>3,9</sup>. Among the isolates, gram-positive Cocci such as *Staphylococcus* spp. were abundant<sup>3</sup>. The analyzed data showed different profiles of

antibiotic resistance in all tested uses. The increasing population of antibiotic-resistant strains is a public challenge effective for all ecosystems. The multi-drug resistance isolates were monitored frequently in some hospital wards. The data showed that 60% of the isolates in Mousavi Hospital were resistant to Piperacillin. In a similar report, less than 5% of isolates were resistant to Piperacillin although > 50% of the isolates were Gentamicin-resistant<sup>32</sup>. The MDR bacteria are known as a severe challenge in a public health due to the misuse of antibiotics. Antibiotics use in feeding and treatment of animals has increased the number of antibiotic-resistant bacteria. In aviculture, the highest population of resistant isolates were resistant to Chloramphenicol and Sulfamethoxazole, respectively. More than 80% of the air-borne *Staphylococci* isolated from the animal farm were resistant to Methicillin<sup>33</sup>. Chloramphenicol is used for feeding in poultry or livestock farming. Their body analysis has shown that antibiotics remain in different organs<sup>34</sup>, justifying high resistance to Chloramphenicol. In this study the number of bioaerosols and their antibiotic resistance profiles were monitored. The effect of environmental parameters such as temperature, relative humidity (RH), ventilation, wind speed, and human activity on microbial activities needs further investigation.

## Conclusion

In the spread of antibiotic resistance, the activity and location of bioaerosols are two important issues. Airborne pollution and antibiotic resistance profiles in this study were normal for most samples. In order to reduce the population of antibiotic-resistant bacteria, control of the air quality and antibiotic usage is necessary.

## Contributions

The authors confirm their contribution to the paper as follows:

Arezoo Tavakoli conceived the presented idea. All the authors carried out the experiment and samplings. Arezoo Tavakoli developed the theory and performed the computations. Both Arezoo and Azadeh Tavakoli contributed to the final version of

the manuscript.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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