Baghdad Science Journal

Volume 23 | Issue 4 Article 27

4-28-2026

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Muheisen, Shahad Emad; Jasim, Israa Mohsin; and Al-Ramahi, Fouad K. Mashee (2026) "Isolation and Identification of Airborne Bacteria within the University of Baghdad Campus, Iraq by using 16S rRNA Gene Technique," *Baghdad Science Journal*: Vol. 23: Iss. 4, Article 27.
DOI: https://doi.org/10.21123/2411-7986.5281

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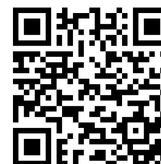
BAGHDAD SCIENCE JOURNAL 2026;23(4):1503–1514



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RESEARCH ARTICLE

Isolation and Identification of Airborne Bacteria within the University of Baghdad Campus, Iraq by using 16S rRNA Gene Technique

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ABSTRACT

This study aimed to identify and classify airborne bacteria in outdoor air within the campus of the University of Baghdad, Iraq, using the passive sampling technique. Air samples were collected from ten sites and analyzed by the 16S rRNA gene sequencing technique. The results revealed significant seasonal differences in both microbial and particulate matter concentrations. Bacterial concentrations were considerably higher during the wet season, with 3238.6 CFU/m^3 compared to the dry season, with 1872.6 CFU/m^3. PM2.5 levels averaged 47.58 $\mu g/m^3$ in the wet season and declined to 11.33 $\mu g/m^3$ in the dry season, while PM10 concentrations reached up to 52.14 $\mu g/m^3$ in the wet season and 10.45 $\mu g/m^3$ in the dry season. The results of the basic local alignment tool (BLAST) showed that the 16S rRNA gene sequences of the isolates were related to (Enterobacter ludwigii, Moraxella osloensis, Peribacillus simplex, Neobacillus drentensis, Priestia aryabhattai, Priestia endophytica, Priestia megaterium, Exiguobacterium mexicanum, Chryseomicrobium amylolyticum, Exiguobacterium sp. and Arthrobacter luteolus). One isolate was only identified at the family level as Enterobacteriaceae. The significant findings were the identification of two pathogenic species, Enterobacter ludwigii and Moraxella osloensis, which are opportunistic bacteria that may negatively affect public health. This study provides a detailed database of airborne bacteria in the study area within the university, which may contribute to the implementation of effective interventions that reduce risks and enhance environmental safety. Nonetheless, studies that examine identification, characteristics and distribution of airborne bacteria in campuses are still rare.

Keywords: Aerosols, Air microbial, Bacteria, Particulate matter, Outdoor air quality, 16S rRNA

Introduction

Air is one of the most basic individual needs, as it contains different particles and microorganisms.[1,2] Particulate matter with a diameter of (PM2.5) includes bioaerosols of size <2.5 μm, which are small enough to arrive and diffuse into lung alveoli. PM10 (<10) μm includes PM2.5 and coarser particles measuring 2.5–10 μm in diameter. Fine particulate matter (PM2.5) can penetrate deep into the respiratory tract, leading to negative long-term effects on human health.[3] Air pollution is one of the most important current environmental issues.[4] A recent study has shown that exceeding the permissible levels of PM in the air, such as in cafes, is associated with changes in human blood parameters, indicating potential effects on public health.[5] Numerous epidemiological studies have demonstrated a strong correlation between elevated hospital admission rates and high concentrations of air pollutants.[6] An estimated 6.7 million

Received 25 March 2025; revised 6 August 2025; accepted 19 August 2025.
Available online 28 April 2026

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https://doi.org/10.21123/2411-7986.5281
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premature deaths are attributed to air pollution each year, with outdoor pollution accounting for 4.2 million of those deaths in 2019.[7]

Aerosols are defined as a mixture of fine solid or liquid particles suspended in air. These particles range in diameter from 10^{-4} to 100μm.[8,9] Bioaerosols constitute a major component of suspended particles in the air.[10] Bioaerosol refers to airborne particles of biological origin, and includes a wide range of microorganisms such as bacteria (pathogenic and non-pathogenic), algae. fungi and viruses.[11] Globally, biological aerosols are estimated to constitute about 25% of the total mass of aerosols in the atmosphere.[12] Numerous studies have shown that between 5% and 34% of indoor air pollution in various environments, such as transportation systems (subways), hospitals, libraries, workplaces, shopping malls and medical clinics, is attributed to biological aerosols, particularly bacterial and fungal components.[11,13] Despite the important increase in research interest in aerosols and bioaerosols in recent years, most studies focus on indoor environments,[10,14,15] while studies on biological aerosols in outdoor air remain limited and constitute a small percentage of the published literature in this field.[16] Several studies have demonstrated the important roles of bioaerosols in influencing climate, ecosystems, human health, and atmospheric dynamic processes.[17] High concentrations of airborne microbes in the air range from 10^3 to 10^7 cells per cubic meter.[18]

Bacteria are among the most studied components of biological aerosols, with their concentrations in air showing a wide range from 10^2 to 10^6 cells per cubic meter, and they are characterized by a high taxonomic diversity that reflects the diversity of their environmental sources.[19,20] Airborne bacteria exist either as single cells suspended in the atmosphere or attached to other particles such as soil particles and leaf fragments. This association provides them with relative protection from harsh environmental conditions, enhancing their ability to survive in the air for longer periods.[19] Bacteria grow in moist conditions and are dispersed externally from soil, water, and plants, and are associated with the presence of humans. Water bodies and artificial cooling contribute to their airborne transmission, whereas indoor bacteria are often more diverse than outdoor environment.[21] Decaying organic matter, stagnant water, and wet surfaces are optimal environments for the growth of airborne biological contaminants, while polluted heating and air conditioning systems contribute to the redistribution of these microorganisms into the air, increasing their chances of spread and impacting public health.[22,23]

Bioaerosols cause a variety of health effects and enter the human body through different pathways.[5] vulnerability to the bacterial bioaerosols can prompt a scope of difficulties in the lungs such as allergic reactions, irritation, and inflammation.[4,24] According to the (WHO), about five million persons die each year from exposure to airborne aerosols before they reach puberty.[25,26]

Students, administrative support staff and academics spend approximately 7–9 hours per day on campus during weekdays, the indoor and outdoor air quality in these buildings is a key factor in determining their exposure to air pollution sources and their potential health effects.[27] Thus, the impact of pollutants is associated with increased rates of absenteeism and decreased academic performance.[28]

A study revealed the presence of airborne bacteria and fungi, including species that are human and plant pathogens, such as smut, mildew and rust. Bacteria belonging to the Enterobacteriaceae and Pseudomonadaceae families were also identified.[29] Air samples from a recent hospital study exhibited the highest levels of antibiotic resistance compared to surfaces and food,[30] highlighting the importance of air quality.[31]

Despite the significant increase in aerosol research in recent years, studies focusing on bioaerosols in outdoor environments represent a fewer proportion of research.[16] The present study aims to isolate and diagnose bacterial bioaerosols spread within the University of Baghdad campus in Iraq.

Materials and methods

Description of the study area

Samples were collected from all colleges of the University of Baghdad, Al-Jadriya Campus, in the Rusafa side of Baghdad Governorate, Iraq, with coordinates by time with latitude 33.2723° N, and longitude 44.3792° E. The University has more than 5000 academic staff, including 10,000 postgraduate students and 70,000 undergraduate students, distributed over a number of colleges such as: College of Science for Women (S1), College of Media (S2), University Shopping Center (S3), College of Political Science (S4), College of Engineering Al-Khwarizmi (S5), College of Science (S6), College of Engineering (S7), College of Agricultural Engineering Science (S8), Computer Center (S9) and University Gate (S10), as shown in Fig. 1.

Sample collection

Samples were collected from ten different outdoor sites within the university campus during both the dry and wet seasons, from December to May (2023–2024). Samples were collected monthly from

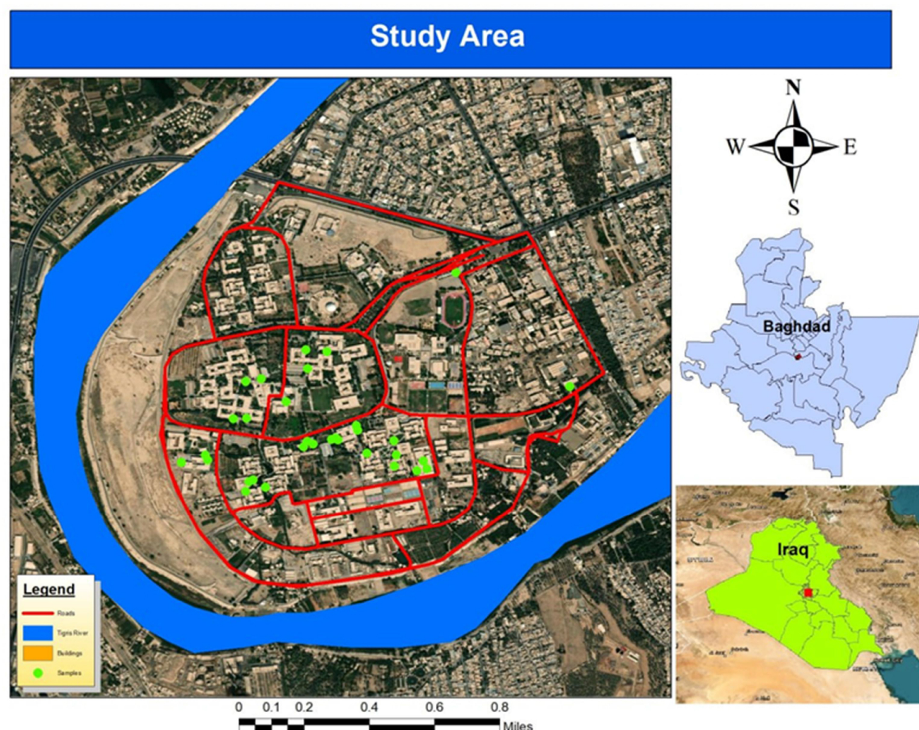

Fig. 1. Map Showing the study area of University of Baghdad in Al –Jadria Campus.

all colleges within the university, to ensure comprehensive coverage of the various academic sites.

The air samples were collected in the morning between 8 a.m. and 12 p.m. Each PM2.5, PM10, humidity, and temperature were sampled using Mini Particle Counter (CEM, China).Passive air sampling technique was used to measure the number of colony-forming units (CFU) of microorganisms. A 9 cm diameter Petri dish, was left open to the air for one hour at a height of one meter above the floor and approximately one meter away from major obstructions and walls.[32]

The collected samples were on the Nutrient agar and incubated at 37 °C for (18–24) hours. Air sampling was processed in the outdoor air conditions of universities to analyze the bacteriological status of the air. Sampling was based on the most significant student gathering spots, which were also close to the student club. During sampling, information of each sample including temperature, relative humidity, place of sampling, and sampling time were noted.

Culturing media

Nutrient Agar (NA) is a solid general-purpose medium used to support the growth of a wide range of non-fastidious organisms and to determine total heterotrophic bacteria counts. To isolate and differentiate specific bacterial groups, several selective and differential media were employed: MacConkey Agar (MCA) was used to determine coliform bacteria, Mannitol Salt Agar (MSA) was used to determine Staphylococcus, and Blood Agar was used to detect the ability to produce hemolysin.[33]

According to the manufacturer's recommendations, all of the media were autoclaved and sterilized for 15 minutes at 121 °C. After being divided among sterile tubes or Petri dishes, they were incubated for twenty-four hours at 37 °C.

Bacterial identification

The bacteria isolated from air samples were identified using morphology (cocci or bacilli). The colony morphology form, margins, surface and gram staining's coloring, were examined under a compound microscope at 100x.[34]

Gram's stain

To examine the morphological characteristics of a single colony from media was captured through a loop and spread on a clean slide, the colony was fixed using heat and stained using crystal violet(primary stain) for 60 seconds, iodine solution for 60 seconds was added onto smear, A few drops of ethyl alcohol

Table 1. Illustrated the conditions for performing a PCR reaction according to the following criteria.

PCR program			
Steps	Tm (°C)	m: s	cycle
Initial Denaturation	94	03: 00	1 cycle
Denaturation-2	94	00: 45	35 cycle
Annealing	56	01: 00	
Extension-1	72	01: 00	
Extension-2	72	07: 00	1 cycle

(as a decolorizer) for 5 seconds, safranin (Secondary stain) for 60 seconds, and then it was examined under a light microscope.[35]

DNA extraction, polymerase chain reaction, and 16s rRNA sequence analysis

Bacterial isolation was performed on nutrient agar. DNA extraction from each airborne bacterial isolate was performed using a G- spin DNA extraction kit (intron biotechnology, (Korea). The DNA products of bacteria were quantified and detected by1% agarose gel and visualized under a UV transmission. The product of DNA was stored at –20°C for further use.

Extracted DNA templates were subjected to polymerase chain reaction (PCR) using a set of (Forward 5’- AGAGTTTGATCCTGGCTCAG- 3’ and Reverse5’- GGTTACCTTGTTACGACTT- 3’) of primers targeting the 16S rRNA gene of isolates using universal primers. The PCR of 16S rRNA amplification was performed as per the manufacturer’s instructions (MultiGeneOptiMax Gradient Thermal Cycler). PCR Mixture of the specific interaction for diagnosis gene were performed in a total volume of 25 μL containing 2 μL of each primer (10 picomols/μl), 5 μL Taq PCR PreMix. Approximately 1.5 μL DNA was added to the reaction as a template prior to completing the volume with free nuclease water 16,5 μL.

The PCR products underwent electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours Table 1. The transilluminator device was used to detect the electrophoresed products, and the images were captured using a digital camera.

The amplified 16S rRNA gene of all isolated bacteria was sent to the laboratories of Macrogen Corporation in Korea, Sanger sequencing was conducted by the ABI-310 Genetic Analyzer System.

Data analysis

The resulting bacterial species were analyzed and aligned using the Basic Local Alignment Search Tool (BLAST) database and compared with the relevant sequences at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) gene database and BioEdit program. The expected value estimates how often the same similarity would occur by chance. Therefore, a low value of E indicates that the degree of similarity between the sequences is high, giving high confidence in the results. Moreover, a value near zero indicates that these sequences are identical. The phylogenetic tree (aligned sequences were conducted and mapped using the MEGA 6 program).[36]

Statistical analysis

Descriptive statistics, including means and standard deviations, were calculated for all studied parameters. Analysis of variance (ANOVA) was then used to determine whether significant differences existed between sites and seasons, with the significance level set at $p < 0.05$.

Results and discussion

Descriptive characteristics of environmental variables and airborne bacterial concentration

The results reflect the seasonal variation in environmental parameters and bacterial concentrations. Clear differences are shown in the statistical tables between the dry and wet seasons. These tables reveal significant variations in the levels of aerobic bacterial concentration, temperature, relative humidity, PM2.5, and PM10.

The result shows descriptive statistics in Table 2, including seasonal differences in environmental and microbial parameters. During the wet season, the mean temperature was significantly lower (17.33 ± 1.97°C) compared to the dry season (32.53 ± 2.90 °C). Similarly, relative humidity levels were higher in the wet season (66.63 ± 4.48%) than in the dry season (47.93 ± 4.74%). For particulate matter, both PM2.5 and PM10 concentrations were substantially higher in the wet season (47.58 ± 18.54 $\mu g/m^3$ and 52.14 ± 33.73 $\mu g/m^3$, respectively) compared to the dry season (11.33 ± 3.68 $\mu g/m^3$ for PM2.5 and 10.45 ± 9.10

Table 2. The descriptive statistical analysis of environmental parameters and airborne bacterial concentrations across seasons.

Parameter	Wet Season			Dry Season		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Temperature (°C)	17.33 ± 1.97	15.30	22.50	32.53 ± 2.90	27.90	38.00
Relative Humidity (RH%)	66.63 ± 4.48	58.20	76.50	47.93 ± 4.74	39.80	56.70
PM2.5 ($\mu g/m^3$)	47.58 ± 18.54	19.00	79.00	11.33 ± 3.68	7.00	23.00
PM10 ($\mu g/m^3$)	52.14 ± 33.73	0.001	99.00	10.45 ± 9.10	0.001	23.00
Bacteria (CFU/m^3)	3238.6 ±1044.3	1126.83	4979.04	1872.6 ± 861.2	458.59	3865.30

Table 3. ANOVA results for seasonal and spatial differences.

Parameter	Wet Mean ± SD	Dry Mean ± SD	F (Season)	p (Season)	F (Sites)	P (Sites)	F (Interaction)	p (Interaction)
Temperature (°C)	17.33 ± 1.97	32.53 ± 2.90	3703.06	< 0.001	24.27	< 0.001	16.29	< 0.001
Relative Humidity (RH%)	66.63 ± 4.48	47.93 ± 4.74	1107.59	< 0.001	15.81	< 0.001	7.99	< 0.001
PM2.5 ($\mu g/m^3$)	47.58 ± 18.54	11.33 ± 3.68	510.98	< 0.001	13.59	< 0.001	14.72	< 0.001
PM10 ($\mu g/m^3$)	52.14 ± 33.73	10.45 ± 9.10	322.70	< 0.001	32.09	< 0.001	22.02	< 0.001
Bacteria (CFU/m^3)	3238.6 ± 1044.3	1872.6 ± 861.2	71.99	< 0.001	7.26	< 0.001	2.50	0.019

$\mu g/m^3$ for PM10). Microbial air quality, measured as bacterial CFU/m^3, also exhibited significant seasonal differences. The bacterial load was markedly higher during the wet season (3238.6 ± 1044.3 CFU/m^3) than during the dry season (1872.6 ± 861.2 CFU/m^3).

The ANOVA results in Table 3 indicate that all measured parameters exhibited highly significant variation with respect to season ($p < 0.001$), with particularly strong effects for temperature ($F = 3703.06$) and relative humidity ($F = 1107.59$). Additionally, site-specific variation was also statistically significant across all variables ($p < 0.001$), indicating spatial heterogeneity in air quality conditions within the study area. Of particular note is the interaction effect (season × site), which was also statistically significant for most variables (e.g., PM10: $F = 22.02$, $p < 0.001$), reflecting the combined influence of temporal and spatial dynamics. The interaction term for bacterial counts was less pronounced but still statistically significant ($F = 2.50$, $p = 0.019$), suggesting that microbial concentrations are influenced by localized microenvironmental factors in conjunction with seasonal conditions.

The results indicated that relative humidity was generally higher during the wet season compared to the dry season. This increase in humidity during the wetter period is considered an important environmental factor, as higher moisture levels can enhance the accumulation of airborne bacteria and promote the deposition of fine particulate matter. These observations align with the findings of.[37] The findings of this study suggest that climatic factors, particularly wind speed and direction, are critical environmental variables influencing the spatial variability of airborne bacterial concentrations across the campus. Wind movement plays a significant role in transporting fine particulate matter (PM2.5 and PM10) from emission sources to surrounding areas, including the dissemination of associated microorganisms.[4,38] The PM2.5 findings are consistent with previous studies, showing higher concentrations during the wet season due to increased humidity and atmospheric stability. However, PM10 levels differed, with literature indicating higher concentrations during the dry season, mainly from dust resuspension and traffic activity.[39] Previous studies confirmed that PM2.5 concentrations are highly influenced by environmental conditions such as temperature and humidity. The seasonal and spatial patterns observed in this study further support that extreme weather conditions significantly impact air pollution levels.[40] Although some research, like that conducted in Sistan, found that there was a decrease in airborne bacterial concentrations with higher relative humidity, the current study found the opposite pattern. Bacterial concentrations rose on the University of Baghdad campus during the wet season, which was marked by higher humidity and lower temperatures.[41]

Composition of airborne bacterial

Molecular identification of microorganisms by the 16S rRNA gene has become a new standard for identifying a wide range of bacteria. The 16S rRNA gene is commonly used for identifying and classifying bacteria because it contains variable and conserved regions, and this gene consists of about 1550 base pairs (bp), making it suitable for accurately distinguishing different types of bacteria.[42]

In this study, the 16S rRNA gene sequence was used to differentiate 12 airborne bacterial isolates. The results showed that one isolate was classified at

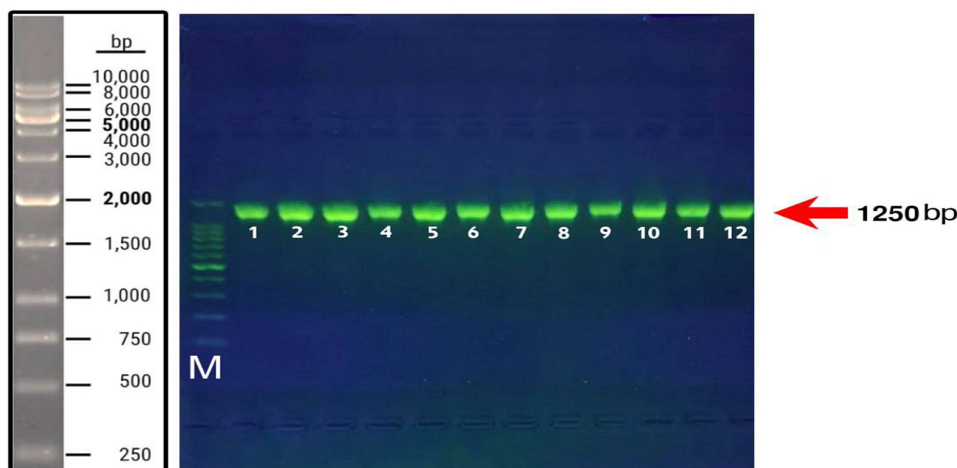

Fig. 2. PCR products of the 16S rRNA gene. Genomic DNA of unknown bacteria identified in PCR was used to amplify the 16S rRNA gene. The reaction products were analyzed on a 1.5% agarose gel and visualized to accurately determine the results. Lane M: DNA ladder (100bp) Lane 1and 2 refer to the samples of bacterial isolates as indicated by S1 and S2. The 16S rRNA gene (about 1250 base pairs) is represented by the red arrow.

Table 4. Polymorphisms of the 16S rRNA gene in isolated bacteria.

No. of sample	Sampling sites	Type of substitution	Location	Nucleotide	Source	Sequence ID with compare	Identities
1	S6	———	——-	——	*Enterobacter ludwigii*	ID: PP723890.1	100%
2	S1	———	——-	——	*Peribacillus simplex*	ID: MW737655.1	100%
3	S2	Transvertion	103	G\C	*Neobacillus drentensis*	ID: HQ436340.1	99%
		Transvertion	104	G\T			
4	S6	Transvertion	104	G\C	*Arthrobacter luteolus*	ID: MH475928.1	99%
		Transvertion	105	G\C			
		Transvertion	175	A\T			
5	S6	Transvertion	2180825	A\T	*Priestia aryabhattai*	ID: CP041519.1	99%
6	S6	Transition	319	C\T	*Priestia endophytica*	ID: MG651313.1	99%
7	S6	Transition	567	A\G	*Moraxella osloensis*	ID: MT225715.1	99%
8	S6	Transition	71	C\T	*Exiguobacterium sp.*	ID: MG757526.1	99%
9	S4	Transition	181	C\T	*Exiguobacterium mexicanum*	ID: MT509848.1	99%
	S4	Transition	281	A\G			
10		———	——-	——	*Chryseomicrobium amylolyticum*	ID: KP236269.1	100%
11	S7	———	——-	——	*Priestia megaterium*	ID: MN826585.1	100%
12	S5	Transition	190	T\C	*Enterobacteriaceae bacterium*	ID: OK481180.1	99%

the family level, another at the genus level, while the remaining isolates were identified as 10 different species. The polymerase chain reaction (PCR) products were sequenced to 1250 base pairs (bp) for all isolates, and these products were analyzed using agarose gel electrophoresis Fig. 2.

The amplicon was then aligned using the online BLAST tool available in the NCBI database. Analysis of the 16S rRNA gene sequences of *E. ludwigii, P. simplex, C. amylolyticum and P. megaterium showed 100% similarity and N. drentensis, A. luteolus, P. aryabhattai, P. endophytica, M. osloensis, Exiguobacterium sp., E. mexicanum and E. bacterium* 99% similarity with the NCBI sequence database by accession number, substitution type, and nucleotide location, respectively Table 4, Figs. 2 and 3.

The results of BLASTn analysis confirmed that the isolates belonged to the phylum Pseudomonadota, Actinomycetota, while Bacillota exhibited a high relative abundance.

According to the 16S rRNA analysis, twelve bacterial species were added to the bacterial collection of Iraq, and they were registered in NCBI Table 5 with accession numbers as follows:

Analysis of the features of airborne bacterial communities on campus showed a remarkable diversity of bacterial species, including pathogenic species in outdoor environments. Bacterial sources in outdoor air are considered to be primarily from natural elements such as soil, water bodies, and plants, with a limited contribution from human activities.[43] The composition and dispersion of airborne bacteria in

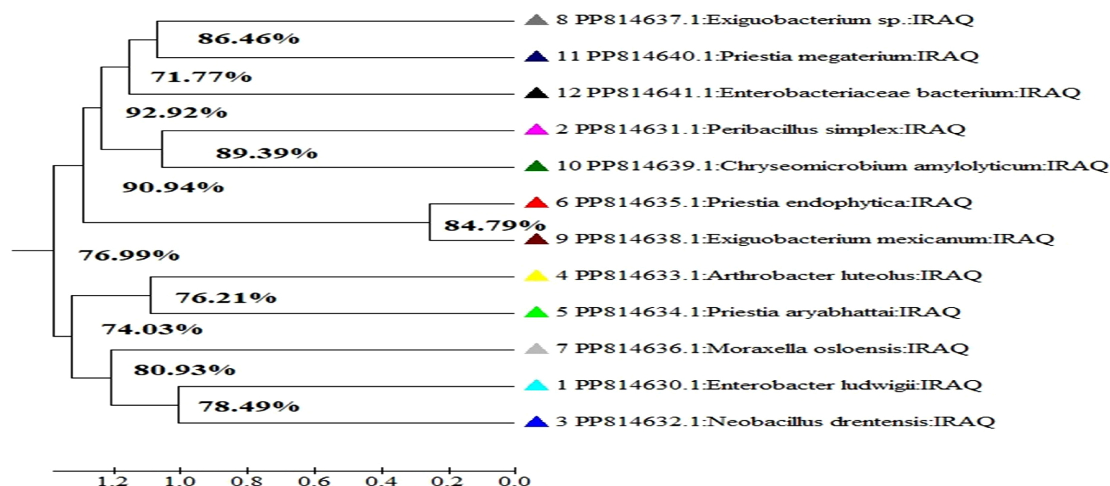

Fig. 3. Phylogenetic tree of bacteria analyzed based on 16S rRNA gene sequences conferred from the GenBank database, and aligned using the BLAST tool from NCBI. The MEGA 6 program was used to construct the phylogenetic trees.

Table 5. Types of airborne bacteria isolated in Iraq and registered in NCBI.

Bacteria	Accession numbers
E. ludwigii	PP814630.1
P. simplex	PP814631.1
N. drentensis	PP814632.1
A. luteolus	PP814633.1
P. aryabhattai	PP814634.1
P. endophytica	PP814635.1
M. osloensis	PP814636.1
Exiguobacterium sp.	PP814637.1
E. mexicanum	PP814638.1
C. amylolyticum	PP814639.1
P. megaterium	PP814640.1
E. bacterium	PP814641.1

the atmosphere is influenced by a number of micro- and macro-factors, including temperature, air humidity, wind speed, emission sources, land use, and particle concentration.[10] Many microbial particles are able to maintain their viability, making them a potential source of infectious diseases and immune reactions. When deposited on surfaces, these particles can remain active for extended periods, increasing the likelihood of their resuspension in the air and impacting the health of exposed individuals.[44] Epidemiological studies have shown a close relationship between deteriorating air quality and negative effects on human health.[45]

In a previous study that examined the effect of vegetation on the microbial composition of ambient air, the abundance of microbial communities, determined by analysis of bacterial 16S rRNA genes, was found to be 2 to 10 times higher in air sampled directly above vegetation than in air sampled from an adjacent non-vegetated area 50 m downwind. The results indicate that approximately 50% of airborne bacteria in samples collected downwind were of plant origin.[46]

Enterobacter ludwigii is an environmental Gram-negative bacterium belonging to the Enterobacter cloacae complex, it exhibits fermentative capacity and is classified as an occasional human pathogen while retaining the overall attributes of the family Enterobacter.[47] *E. ludwigii* is a motile, fermentative, bar formed bacterium, first separated from a clinical example and recognized as another species in 2005.[48] Annual epidemiological reports, such as "Healthcare-associated infections in inpatient units" 2017, indicate that *Enterobacter spp*. were responsible for 8.2% of catheter-associated bloodstream infections (CABSI) in intensive care units in Europe. Further, a study was conducted showing that *E. ludwigii* causes catheter-associated bloodstream infections, which are caused by non- frequent opportunistic pathogens that form massive aggregate on the external surface of central venous catheters (CVC). The strain isolated from these catheters was confirmed to be known for its rapid growth and ability to form biofilms.[49] In addition as a versatile and environmentally oriented species, *E. ludwigii* isolates have been recognized as prominent electrogenic bacteria, adapting to heavy metals and forming biofilms, and are abundant in endophytic bacterial communities.[50,51] Furthermore, *E. ludwigii* isolates have been classified as bioremediation agents, alternating plant defense and capable of performing another important environmental function.[52–54]

In previous studies conducted to detection airborne pathogenic bacteria on campus, the results showed that *Moraxella osloensis* was the most abundant bacterial species in air samples, accounting for 8.66% of

the total pathogenic bacteria. These results are consistent with previous studies, which have shown higher levels of pathogenic bacteria in indoor environments such as laboratories and dormitories compared to outdoor environments.[43] Furthermore, in another study conducted on samples from a hospital entrance, a high diversity of antibiotic resistance genes (ARGs). Moraxella, which carries antibiotic resistance genes, was found in both indoor environments and airborne particles within the hospital.[55] A recent study conducted in a hospital to detect antibiotic resistance showed that the highest level of resistance was observed in air samples, followed by surface samples, and then food samples.[30] Antimicrobial resistance is currently a complex public health issue.[30] Moreover *M. osloensis* is a major cause of invasive infections, especially in immunocompromised individuals. It can cause septic chest, meningitis, bacteremia and other diseases.[56]

In this study conducted on the University of Baghdad campus, two airborne bacteria were identified in the outdoor air *E. ludwigii* and *M.osloensis*. These species were isolated from the College of Science and the central university square, sites known for intensive academic activities, including scientific laboratories, libraries and a student club, resulting in an increasing number of students and the accumulation of bacteria in the air. The current results support the hypothesis that these bacteria can be transferred between indoor and outdoor environments. These findings point to the urgent need for effective preventive measures to limit the spread of these airborne pathogens, especially in closed environments such as universities and hospitals. These measures are essential to protect individuals, especially immunocompromised individuals, who are at higher risk of infection. The airborne transmission of pathogenic bacteria in the atmosphere may pose potential risk to human health.

Airborne microbes in outdoor conditions are significant not only the public heath, but also to agriculture, as they contribute to the dispersal and deposition of phytopathogens on leaf surfaces and stem.[57]

Exiguobacterium mexicanum was isolated and identified as one of the bacterial species in air environments, which is consistent with the findings of Jiangyun Liu *et a*l., who confirmed the presence of this bacterial species in mural and air environments.[58]

In additional, *Priestia megaterium* previously known as (*Bacillus metagerium*)[59] is a Gram-positive, spore -forming, rod-shaped bacterium.[60] Known for its antimicrobial activity against a variety of plant pathogens.[61] *Peribacillus simplex* is a (Gram-positive and spore-forming) bacteria derived from a wide range of environmental sources. Recent studies focusing on these spores have shown multiple beneficial effects, highlighting their importance in environmental and agricultural applications such as biocontrol and bioremediation capabilities.[62,63] In several studies, these bacteria were isolated from soil.[64,65] In the current study, this bacterium was isolated and identified in the outdoor air of the university campus, supporting the hypothesis that its spore-forming ability enables it to transfer from soil to air. Since the environmental nature of the university campus, which contains extensive green spaces and vegetation, the results propose that this bacterium may be transferred from soil to air by environmental conditions.[66]

Conclusion

16S rRNA gene sequencing was used to identify and classify airborne bacteria in the outdoor air of the University of Baghdad. The results revealed a high diversity of bacterial species, with several species identified, some of which are considered pathogenic. Among these species, *Moraxella osloensis* and *Enterobacter ludwigii*, were classified as opportunistic pathogenic bacteria, identified for the first time in this environment. These findings are important for public health, given the potential for airborne transmission of these bacteria. This study contributes to building an information base on the prevalence of microbes in the outdoor environment of the university, which provides a foundation for developing effective strategies to combat these organisms. A deeper understanding of their dissemination mechanisms and potential health effects is also required, especially under changing environmental conditions. Furthermore, the study recommends conducting additional research to assess the prevalence of these species in different environments, with a focus on enhancing prevention and control strategies. These efforts should include field and experimental studies to determine the risks associated with the presence of these bacteria in public places, which contributes to ensuring public health safety.

Authors' declaration

- Conflict of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.

- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' contributions statement

S.E.M and I.M.J conceived of the presented idea. S.E.M developed the theory and performed the computations. I.M.J and F.K.M.A verified the analytical methods. I.M.J encouraged S.E.M to investigate and supervise the findings of this work. All authors discussed the results and contributed to the final manuscript.

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عزل وتشخيص البكتيريا المحمولة جواً داخل حرم جامعة بغداد، العراق باستخدام تقنية جين 16S rRNA

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الخلاصة

الهدف من البحث عزل وتشخيص البكتيريا المحمولة جوًا في الهواء الخارجي داخل حرم جامعة بغداد، العراق، باستخدام تقنية الترسيب السلبي. تم جمع عينات الهواء من عشر مواقع وتحليلها باستخدام تقنية تسلسل جينات rRNA S16. أظهرت النتائج وجود فروق موسمية واضحة في تراكيز البكتيريا والجسيمات الدقيقة، حيث كانت التراكيز البكتيرية أعلى بشكل ملحوظ خلال الموسم الرطب 3238.6 CFU/m³ مقارنة بالموسم الجاف 1872.6 CFU/m³. كما سجلت مستويات PM2.5 متوسطًا قدره 47.58 ميكروغرام/م³ في الموسم الرطب، وانخفضت إلى 11.33 ميكروغرام/م³ في الموسم الجاف. أما تراكيز PM10 فقد بلغت 52.14 ميكروغرام/م³ في الموسم الرطب، و10.45 ميكروغرام/م³ في الموسم الجاف. أظهرت نتائج أداة المحاذاة المحلية الأساسية (BLAST) ان تسلسلات rRNA S16 للعزلات كانت تشير الى (Enterobacter ludwigii, Moraxella osloensis, Peribacillus simplex, Neobacillus drentensis, Priestia aryabhattai, Priestia endophytica, Priestia megaterium, Exiguobacterium mexicanum, Chryseomicrobium amylolyticum, Exiguobacterium sp., Arthrobacter luteolus). وتم تشخيص عزلة واحدة فقط عند مستوى العائلة Enterobacteriaceae. وكانت اهم النتائج التي تم الحصول عليها هو التشخيص والتعرف الى نوعين من البكتريا الممرضة Enterobacter ludwigii و Moraxella osloensis وهما بكتيريا انتهازية قد تؤثر سلبًا على الصحة العامة. توفر هذه الدراسة قاعدة بيانات مفصلة عن البكتيريا المحمولة جوًا في منطقة الدراسة داخل الجامعة، مما قد يساهم في تنفيذ تدخلات فعالة تقلل من المخاطر وتعزز السلامة البيئية. ومع ذلك، لا تزال الدراسات المتعلقة بتحديد وخصائص وتوزيع البكتيريا المحمولة جوًا في الحرم الجامعي نادرة.

الكلمات المفتاحية: الهباء الجوي، الميكروبات المحمولة جواً، البكتيريا، الجسيمات الدقيقة، جودة الهواء المحيط، rRNA S16.