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# Characterization of bacterial species and antibiotic resistance observed in Seoul, South Korea's popular Gangnam-gu area

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ABSTRACT

Public transportation facilities, especially road crossings, which raise the pathogenic potential of urban environments, are the most conducive places for the transfer of germs between people and the environment. It is necessary to study the variety of the microbiome and describe its unique characteristics to comprehend these relationships. In this investigation, we used 16 S rRNA gene sample sequencing to examine the biological constituents and inhalable, thoracic, and alveolar particles in aerosol samples collected from busy areas in the Gangnam-gu district of the Seoul metropolitan area using a mobile vehicle. We also conducted a comparison analysis of these findings with the previously published data and tested for antibiotic resistance to determine the distribution of bacteria related to the human microbiome and the environment. *Actinobacteria, Cyanobacteria, Bacteriodetes, Proteobacteria*, and *Firmicutes* were the top five phyla in the bacterial 16 S rRNA libraries, accounting for >90 % of all readings across all examined locations. The most prevalent classes among the 12 found bacterial classes were *Bacilli* (45.812 %), *Gammaproteobacteria* (25.238 %), *Tissierellia* (13.078 %), *Clostridia* (5.697 %), and *Alphaproteobacteria* (5.142 %). The data acquired offer useful information on the variety of bacterial communities and their resistance to antibiotic drugs on the streets of Gangnam-gu, one of the most significant social centers in the Seoul metropolitan area. This work emphasizes the relevance of biological particles and particulate matter in the air, and it suggests more research is needed to perform biological characterization of the ambient particulate matter.

## 1. Introduction

Airborne particulate matter (PM) comprises a sizeable portion (16 %) of biological components [1] and is anticipated to have a substantial impact on both health and the environment. In both natural and urban environments, airborne bacteria represent one of the major components of airborne biological particles [1–4]. Bacteria in the ambient air come from various human and natural sources, including soil, plants, animals, garbage dumps, water bodies, and agricultural practices. Numerous human activities, such as the

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movement and processing of solid waste and sewage, as well as an atmosphere that is favorable to humidity, may increase the quantity of culturable airborne bacteria in an urban area. Bioaerosols can induce or worsen illnesses in humans, animals, and plants. They are vital in the transmission of biological organisms and reproductive materials (pollens, spores, etc.). These have been related to various harmful health effects, including cancer, allergies, asthma, and acute toxic effects.

The present rate of urbanization, which is 55 %, is predicted to increase by 68 % by the year 2050, according to the United Nations Department of Economic and Social Affairs (UN DESA) report "2018 Revision of World Urbanization Prospects". The air load of pollutants, particularly microbiological contaminants, is anticipated to rise dramatically as more rural residents move to metropolitan areas. Most people's daily commutes take up a significant portion of their time. Traveling using public transit saves energy and creates less pollution than driving a private car, which is a major source of air particle emissions in metropolitan areas [5]. Public transportation is regarded as the most significant form of public transportation in significant urban agglomerations. It is a frequently used source because of its great capacity in terms of the number of daily passengers.

Because of urbanization, South Korea has periodic occurrences of Asian dust throughout the winter, which produces massive amounts of particulate matter (PM) and polluting aerosols. We selected the winter season for this study because the ambient air quality of South Korea is influenced by westerlies from China, Mongolia, and Russia [6,7]. It has also been observed that highly concentrated aerosols with complex compositions are particularly harmful to the country's air quality throughout the winter and spring. The South Korea Environment Corporation and the Ministry of Environment continuously assess the quality of the air in high-traffic and multi-use venues. Although dangerous microorganisms like bacteria and fungi are not routinely assessed, the autonomous measuring network that offers this service only detects particulate matter (PM10, PM2.5), carbon dioxide, nitrogen dioxide, and other pollutants. Bioaerosol monitoring is an important task since many bacteria can transmit aerosols and because different particles from surfaces can be moved to a suspended state due to airflow. Most urban microorganism diversity studies focus on a simpler, more coherent protocol of sample collection from various surfaces because there is no universal bioaerosol collection method, whereas bioaerosol diversity in combination with the surface, microbiome assessments has only been conducted for some areas [8,9]. The concentration of outdoor and indoor PM levels in South Korea has been the subject of several studies [7,10–12], but there hasn't been nearly enough study on roadside bioaerosol monitoring.

Nevertheless, several authors have emphasized the necessity of researching bioaerosol dispersal to comprehend how airborne contaminants affect human health. The presence of bioaerosols as aggregates connected to suspended PM (such as dirt, dust, skin flakes, sand, etc.) might increase their viability and infectivity [13,14]. This draws attention to the risk posed by the city's exposure to infectious bioaerosols. Due to these circumstances, looking at the bioaerosol load in areas with heavy traffic and risk of human illness is crucial. Human illnesses including pneumonia, TB, brucellosis, anthrax, Q fever, etc. May also be brought on by bacterial bioaerosols [15]. As a result, it is of great importance to characterize bioaerosols in terms of the concentration of viable components [16]. Numerous studies have been done over the years on the characteristics of urban airborne bacteria [3,4,17]. These investigations identified the predominant bacterial species in urban settings and showed that the characteristics and proportions of these species

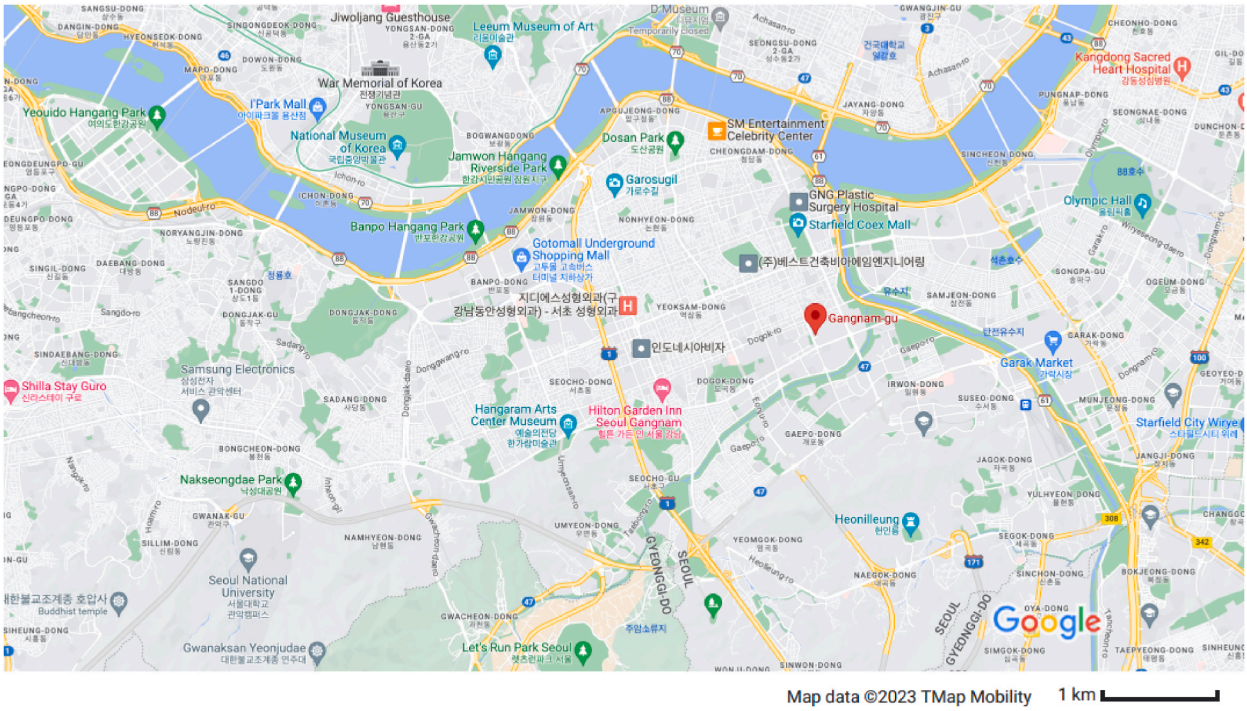


**Fig. 1.** Sampling location map (Gangnam-gu district of Seoul metropolitan area).

differ substantially among urban environments. Traffic intersections are significant outdoor public spaces that offer a dynamic environment for the gathering and movement of people and vehicles. The most vulnerable populations exposed to bioaerosol-related health concerns in these areas include traffic police, residents of neighboring stores, cafes, and restaurants, roadside employees (such as construction and municipal workers), and everyday commuters (such as office workers and students). These considerations make it essential to look into the bioaerosol load in areas with high traffic and risk of human illness. As a result, in this work, we concentrated on tracking the features of the spread of airborne cultivable bioaerosols at a site with high traffic density in the Gangnam-gu region of South Korea. According to our results, airborne bacteria and their byproducts account for a sizable amount of ambient PM. The study highlights the importance of biological particles in the atmosphere and urges more investigation into the biological composition of ambient particulate matter (PM). We attempted to integrate the molecular data on the bacterial population in the air with the characteristics of air fluxes in the main hotspot region of South Korea. This study aims to explore the diversity of airborne bacterial aerosols using 16 S rRNA sequencing and comprehend the size distribution of bioaerosols and their percentage contribution to particulate matter. We also examined the antibiotic drug resistance of the obtained species which can be used to design measures to control airborne bacteria to improve public safety.

## 2. Materials and methods

### 2.1. Study site and experimental period

This study was conducted from 6–8th December 2021 in Gangnam-gu district since winters in Korea have exceptionally high outdoor PM concentrations. Gangnam-gu (Fig. 1) is one of the busiest areas in Korea and is Seoul's wealthy, contemporary core. With a 39.5 $km^2$ size, Gangnam District is Seoul's third biggest district (15.3 sq mi). There were 561,052 people living in Gangnam District as per the 2017 census [18].

### 2.2. Sampling method

A wide-range aerosol spectrometer and optical particle counter (Mini-WRAS, Model 1.371, Grimm Aerosol Technik, Germany) were used to assess the size distributions of all airborne particles over the study period. Particle number concentration (PNC, particles/m3) was assessed at the size range of 0.576–8.001 μm, whereas PM mass concentration ($g/m^3$) was recorded for the aerodynamic diameter less than 1 μm, 2.5 μm, and 10 μm (PM10) (representing 17 size channels). Every time, a jet of fresh compressed air was used to clean the analyzer's input ducts before sampling. Mini WRAS has a time resolution of 1 min and runs at a flow rate of 1.2 L/min. For three days in a row, sampling was done three times at each site from early morning to afternoon and three times from afternoon to night.

Using six-stage viable Andersen Cascade Impactors (Tisch Environmental, Cleves, OH), the airborne cultivable microbiological samples were obtained during the sampling period. Before each sample session, a calibrated dry gas meter was used to maintain the flow rate of 28.3 L/min. Before each sampling event, the samplers were cleaned with 70 % ethanol to prevent any cross-contamination [19]. The sampling strategy chosen was similar to that of Fang et al. [17,20]. The sampling period of 15 min was chosen based on the results of the following investigationsMatolinets et al. [21], provided recommendations for bioaerosol monitoring studies, which state that the time period for bioaerosol sampling should be chosen so that a total of 30–100 microbial colonies may be gathered in a single Petri dish. Both samplers were placed at a minimum distance of 1.0 m between each other and at a breathing height of 1.5 m above ground level (AGL) [22,23]. To optimize the sampling period, a pilot study was carried out at the research site before the sampling campaign. The pilot investigation determined that 30 min was the ideal time to collect the bioaerosol colonies. Colony forming units per cubic meter of air ($CFU/m^3$) were used to represent the bioaerosol concentration after the usual positive-hole correction approach was used to statistically correct the counts [24]. The following equation is used to determine bioaerosol concentration:

$$\text{Bioaerosol concentration CFU/m}^3 = \text{No of colonies/ (flow rate m}^3\text{/min x time (min))}$$

The bacterial colonies were isolated from the cultures and segregated. The 16 S rRNA sequencing process was then performed on the DNA samples obtained from the collected colonies.

### 2.3. DNA isolation and sequencing of 16 S rRNA

The obtained bacterial samples were kept at a temperature of 35 ± 0.5 °C for 24–48 h [25]. To calculate the bacterial abundance, 16 S rRNA gene sequencing was performed. Using the 16 S metagenomic sequencing library preparation methods, a 16 S rRNA sequencing library targeting the V3 and V4 hypervariable regions of the 16 S rRNA gene was produced (Illumina, San Diego, CA, USA). The genomic DNA was extracted using a Soil DNA Isolation kit (SPINeasy DNA Kit for Soil, MP Biomedicals Korea, Seoul, Republic of Korea). The initial PCR used a 12 ng template and region-specific primers that were found to work with Illumina index and sequencing adapters (forward primer: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′).

The V3–V4 region of the bacteria's 16 S rRNA was the target of a polymerase chain reaction (PCR) for the NGS-based metagenome study. The use of a DNA analysis tool allowed for the confirmation of bacterial concentrations (Quant-iT PicoGreen, Thermo Fisher

Scientific Korea Ltd., Incheon, South Korea). By using PCR to amplify the DNA, its presence or absence was confirmed using electrophoresis. The PCR conditions were 25 cycles of 3 min of pre-denaturation at 95 °C, 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, 30 s of extension at 72 °C, and 5 min of final extension at 72 °C.

We hired 3BIGS (Seoul, South Korea) to carry out NSG-based metagenomic analysis utilizing an Illumina MiSeq platform (Illumina, San Diego, CA, USA). MiSeq uses a single instrument to carry out cluster creation, sequencing, and data processing. The pyrosequencing pipeline classifier from the Ribosomal Database Project was used to assess each sequence from phylum to species.

### 2.4. Multidrug resistance determination

We combined samples for this study depending on the way they were collected, the location, and the time. This was accomplished by gently combining 300 μL of the original material into 1.5 mL Eppendorf tubes. The following antibiotic concentrations were used to determine the level of bacterial resistance: tetracycline (10 mcg/mL), streptomycin (20 mcg/mL), ampicillin (100 mcg/mL), kanamycin (50 mcg/mL), chloramphenicol (30 mcg/mL), and gentamicin (10 mcg/mL. Before to preparing the nutritional medium, antibiotic solutions were made. After sterilization and cooling to 50 °C, nutritional agar medium was added to, and then chosen antibiotic solutions were added. The resulting liquid was then placed onto Petri plates. As a control, the nutrient agar medium without antibiotic solution was employed. 48 h of cultivation were done at 37 °C. After incubation, each Petri dish's developed colonies were subjected to a quantitative examination. The MALDI-TOF technique was used to prepare samples for species identification after cultures that had been grown in antibiotic-containing media were transplanted into other plates.

### 2.5. Statistical analysis

The taxonomic profiles of bacterial samples were assessed using the *STAMP* software package [26]. Using the Naive Bayes Classifier included in *QIIME 2* and the R programming language, version 3.6.0, the taxonomic makeup of the bacterial species was identified [27, 28]. To evaluate alpha diversity, the Chao1, Shannon diversity matrix was computed using QIIME 2. Phyloseq was used to calculate the incidence-based Jaccard index and the Bray-Curtis dissimilarity to evaluate beta diversity.

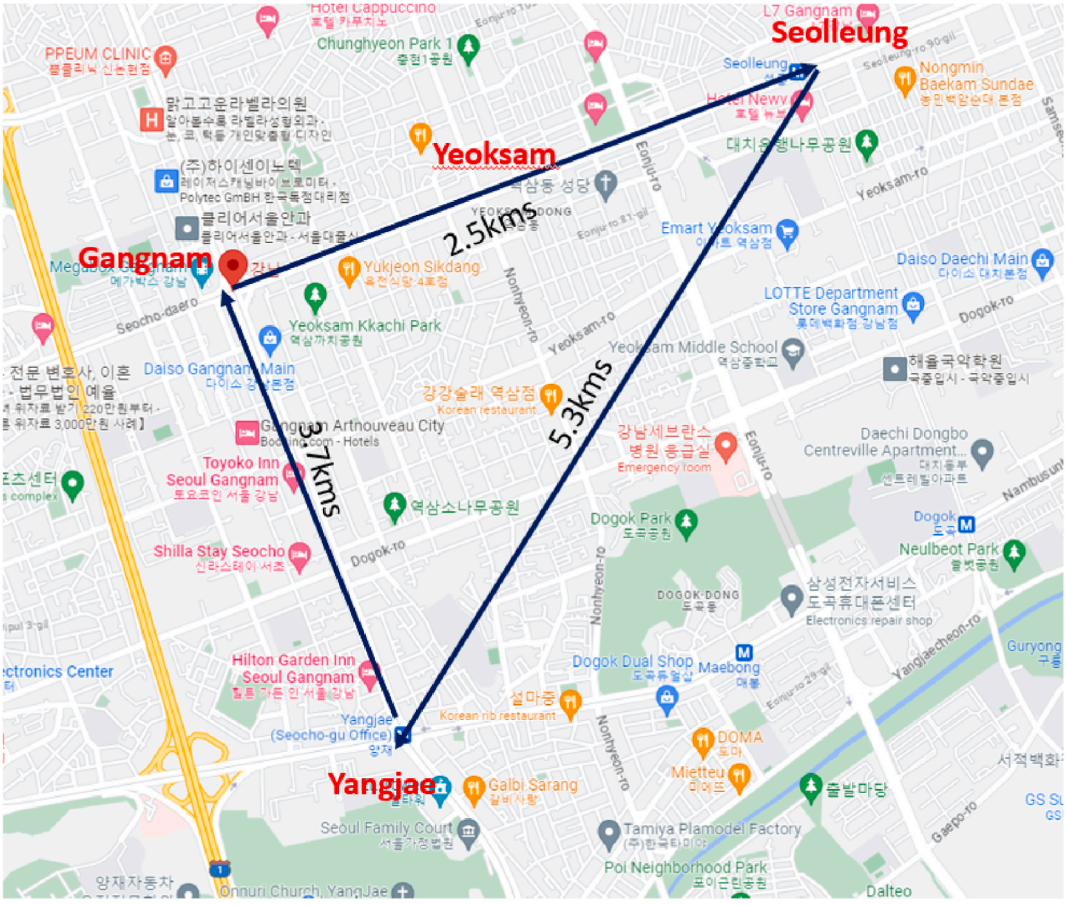


**Fig. 2.** Sampling route for the sampling area with major stops.

Principal Coordinates Analyses (PCoA) 2D plots made with the *ggplot2* software [29] within phyloseq were used to depict the collected distance matrices. Using permutation-based multivariate analysis of variance (PERMANOVA) [30] with 9999 permutations, the comparison categories were used to find statistical differences in bacterial composition among swab samples and filter samples. Within QIIME, statistical significance was determined using $P < 0.05$.

## 3. Results and discussion

### 3.1. PM and bacterial concentration during the sampling period

We collected airborne samples between December 6 and December 8 utilizing a mobile vehicle in campaign mode observation along the route shown in (Fig. 2) to identify any bioaerosol spatial distribution and features. A random range of 67.6 ± 74.3 % relative humidity and −3.6 ± 7.2 °C in temperature were recorded during the sample period. The concentrations of the six size fractions observed throughout the research period were added together to get the total bacterial concentration. The findings of mean total bacterial bioaerosol concentration were found to be 580 CFU/$m^3$. In each instance, bacterial bioaerosol concentrations were below the regulatory limit for public health established by the Ministry of Environment of the Republic of Korea (800 CFU/$m^3$). Throughout the sample period, there was no precipitation. The average $PM_{10}$, $PM_{2.5}$, $PM_4$, and $PM_1$ concentrations for the morning to afternoon sample period (Fig. S1) were determined to be 39.12 μg/$m^3$, 31.38 μg/$m^3$, 35.63 μg/$m^3$, and 26.86 μg/$m^3$, and the same PM concentrations for the afternoon to night period to be 56.95 μg/$m^3$, 43.32 μg/$m^3$, 50.15 μg/$m^3$, and 37.65 μg/$m^3$ (Fig S2).

### 3.2. Microbiome analysis of the bacterial communities

The top five phyla in the bacterial 16 S rRNA libraries were Actinobacteria, Cyanobacteria, Bacteriodetes, Proteobacteria, and Firmicutes, which accounted for >90 % of all readings across all tested sites (Fig. 3) which is similar to results obtained by Cha, Seo et al. [31], for non-Asian dust samples collected in the Daejeon city of South Korea. As shown in Fig. 3, it was observed that Firmicutes and Proteobacteria predominated in the samples gathered from different sites (Fig. 3), with Firmicutes being the most prevalent group. The Bacillus species heavily dominated in the Firmicutes-based dust (Fig. S5). This finding is similar to that of the Jeon et al. research [32], which was carried out in the suburban setting of Seoul's southern district (Republic of Korea). *Bacilli* (45.812 %), *Gammaproteobacteria* (25.238 %), *Tissierellia* (13.078 %), *Clostridia* (5.6975 %), and *Alphaproteobacteria* (5.142 %) were the most abundant classes among the 12 discovered bacterial classes with mean relative abundance >0.1% across all samples (Fig. S3). There were approximately 135 genus groupings with a mean relative abundance (>0.1%) across all the samples, with *Lysinibacillus* with maximum abundance. In this article, the top 15 genera are discussed (Fig. 4). *Paraclostridium, Solibacillus, Pantoea, Psychrobacillus, Peribacillus, Viridibacillus, Crocosphaera, Stenotrophomonas, Rhizobium*, and *Loltiidibacillus* were also discovered, which were not mentioned in (Fig. 4) as the overall mean relative abundance of these genera were lesser than <1 %. In comparison to other types of land types, the phylum Firmicutes predominated in soil on impermeable surfaces. According to studies [33], the presence of proteobacteria is positively correlated with the availability of soil nutrients (such as TC and TN), and they are less common beneath impermeable surfaces than under other forms of land cover. Whereas more Firmicutes were found in the soil around impermeable surfaces, suggesting that Firmicute thrived there. This could be a result of their resilience to drought and drastic environmental changes [34].

### 3.3. Microbial diversity

For bacterial species acquired from a different location, Shannon's Diversity Index was used to determine alpha-diversity along with Simpson and chao1 index were also determined. The Shannon's Diversity index ranged from 5.01 (Yangjae sample) to 6.54

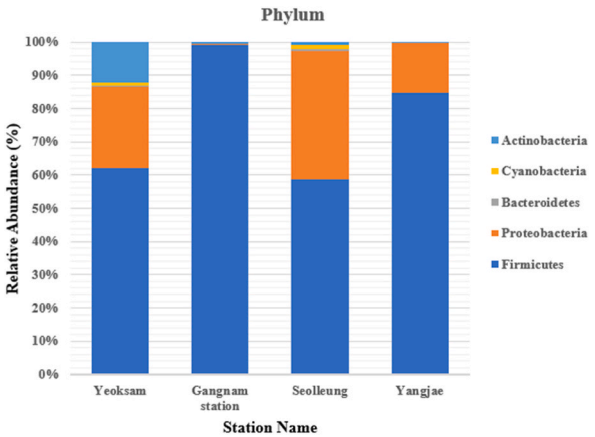


**Fig. 3.** The relative abundance of bacterial OTUs at the phylum level for each sample was taken from various sampling locations.

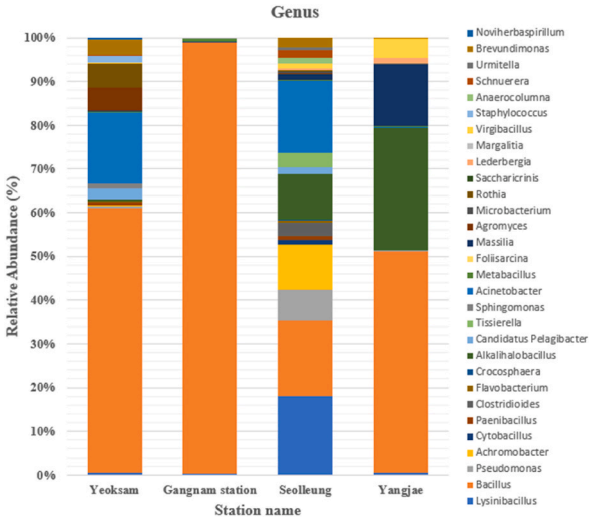


**Fig. 4.** The relative abundance of bacterial OTUs at the genus level for each sample was taken from various sampling locations.

(Seolleung sample). For Yeoksam and Gangnam station, the Shannon's Index was found to be 6.11 and 5.08. The Chao1 index generally displayed higher values for the aerosol samples, providing a more precise evaluation of variety fluctuations [35]. The Yeoksam sample expanded steadily from the start of the study (Chao1 index = 611) and peaked at Chao1 index = 704 during the evening hours. The Chao1 index values for the Seolleung samples increased during the morning rush hour when they hit a plateau and equaled 792–819, and this rise was more pronounced. At the same rush hour, the Chao1 index's highest value was recorded. In general, samples from Gangnam station and Yangjae were distinguished by a lower Chao1 index, which ranged from 320 to 351.

The Bray-Curtis dissimilarity matrix was used in the principal coordinate analysis (PCoA) to assess how samples may be separated (or clustered) based on their geographic location (Fig. 5). This implies that several (biotic and abiotic) causes may be to account for the relationships between bacterial diversity seen in the different sampled settings [8].

### *3.4. Determination of the antibacterial drug resistance of various bacterial species*

One of the biggest concerns to global public health is the increase of antibiotic resistance. Due to the difficulty of treating these bacteria, multidrug resistant staphylococci isolated from healthcare and non-healthcare situations are receiving more and more attention [18,36–38]. Antibiotic resistance of bioaerosol is a critical problem since many bacteria are capable of transmitting aerosols and because different particles from surfaces can be transported to a suspended state as a result of airflow [35]. The collected samples

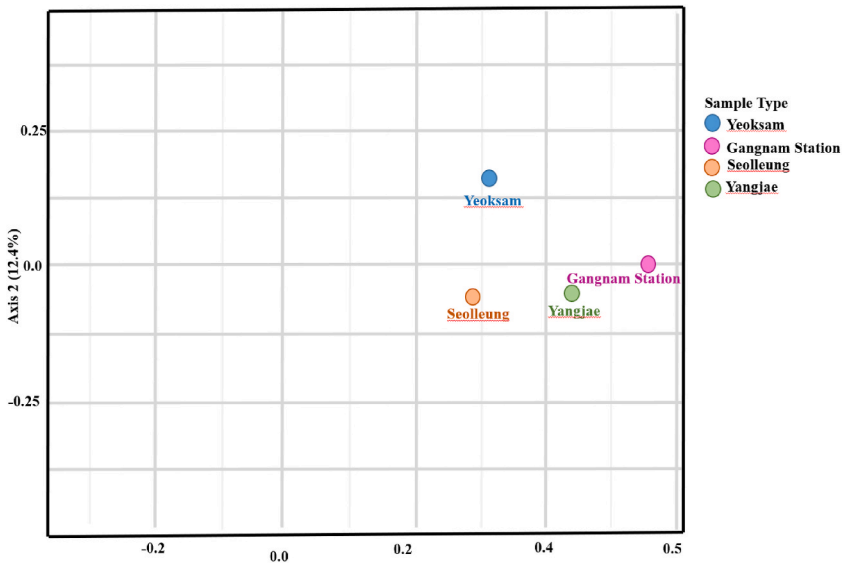


**Fig. 5.** The Bray-Curtis dissimilarity and Jaccard Distance are used to visualize beta diversity in principle coordinate analysis (PCoA).

were grownbu to evaluate the bacterial resistance gene profile. The mechanism of resistance to antibiotic in bacteria has been shown in Fig. 6. During cultivation, twelve morphotypes (MTs) were discovered. 5 colonies among them were recognized based on species (Fig. S1). No. Of the technique of collection or the time of day, all of the species were discovered in the samples taken from the Yeoksam route. Antibiotic resistance was discovered among the identified MTs (Table 1). The *Staphylococcus* species (*S. cohnii, S. saccharolyticus, and S. coreilyticus*) were discovered. The term "opportunistic human pathogen" is also used to describe the staphylococus [36,38]. It was shown that the staphylococcus species were resistant to tetracycline, gentamicin, chloroamplenicol, and kanamycin, which is comparable to the findings made by Refs. [18,37]. Moreover, the Agromyces species (*Agromyces bucanen*) also demonstrated resistance to streptomycin. There was no test for ampicillin resistance for any of the samples.

## 4. Conclusions

1 Bacterial colonies on dust particles are thought to have the potential to affect both human health and the environment [39,40]. Bacterial abundance in the air of our normal non-dust environment is $10^5$ cells/$m^3$, but the abundance of bacteria traveling with dust particles is between $10^4$-$10^5$ cells/$m^3$, and their variety is comparable to that of the soil in the dust source locations. The main source of airborne bacteria in Seoul's ambient environment is human and soil microbial flora. This study demonstrates that a major portion of ambient PM is made up of airborne bacteria and their components. The study emphasizes the significance of biological particles in the atmosphere and calls for more research on the biological makeup of ambient particulate matter (PM). In this work, we examined bacterial composition of the ambient particulate matter, however more research is required to analyse the impact of seasons and fungal composition of the biological component of the PM. The summarized results of this work are as follows-In this work, we tried to combine the molecular information on the bacterial population in the air with the features of air fluxes in the primary hotspot region of South Korea. The airborne bacterial populations from the Gangnam-gu roadside were examined in an effort to determine the characteristics and attributes of air masses.

2 This study provided evidence of the genetic structure, variety, and concentration of the airborne bacterial population.

3 Our findings are consistent with those of the research done by Cha et al., [31]. When compared to the non-Asian dust event in November and December 2014, the fraction of the predominately present phylum Proteobacteria was much lower during the Asian dust event in December 2014. At the same time, dust particles were used to introduce the phyla Actinobacteria and Firmicutes, particularly the genera Bacillus, and Arthrobacter.

4 We also discovered that different microorganism proportions predominate over the typical bacterial phyla reported during Asian dust episodes. Diverse bacteria were also transported during the Asian dust event; however, their proportion was relatively lower than that of the above mentioned species. The modest percentage of newly transported microorganisms cannot be ignored, in fact,

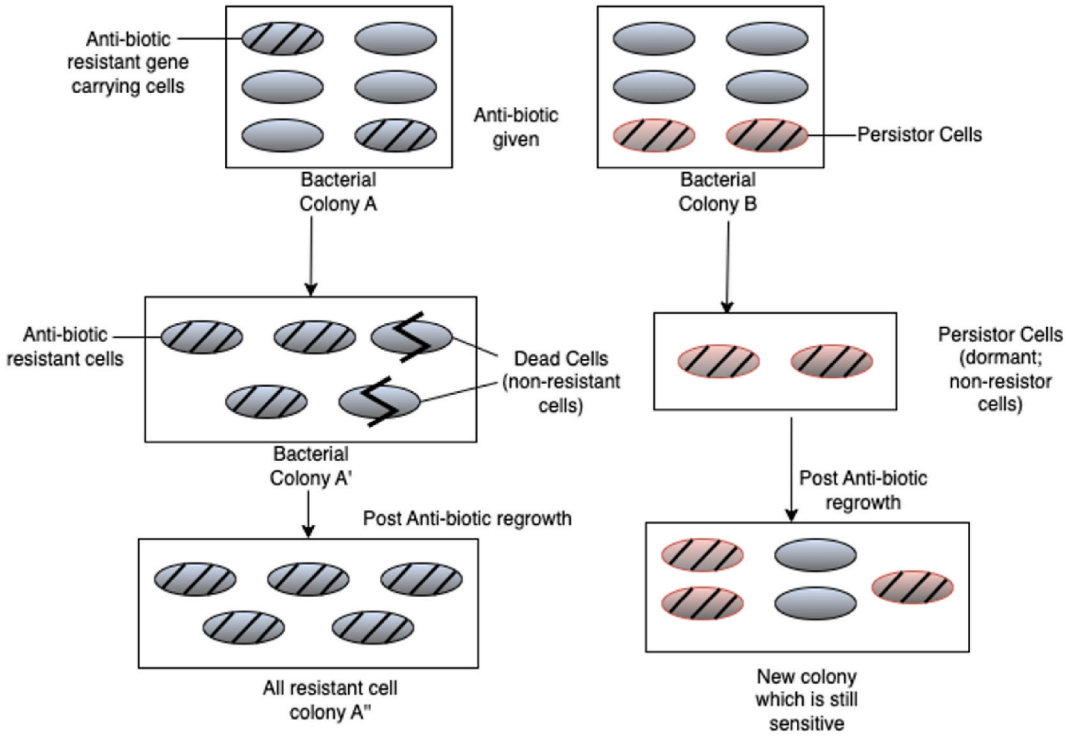


**Fig. 6.** Mechanism of resitance and persistence of bacterial cells against anti-microbial drugs.

**Table 1**
Identification of resistance of various microorganisms to antimicrobial drugs.

| Species | Resistant to antibiotic | Station name |
|---|---|---|
| *Staphylococcus* (*cohnii, saccharolyticus, Coreilyticus*) | Tetracycline (10 mcg/L), Gentamicin (10 mcg/L), Chloroamplenicol (30 mcg/L), Kanamycin (50 mcg/L) | Yeoksam |
| *Agromyces bucanens* | Streptomycin (20 mcg/mL) | Yeoksam |

since the overall number of microorganisms (16 S rRNA gene copy number) has significantly grown (from a few hundred to thousands) in the airborne environment.

The findings of this study will aid in the creation of bacterial management strategies for large hotspots' roadside locations. In addition to developing and implementing a reduction strategy such as air purifier technology, antibacterial film, and hand sanitizer systems appropriate for the location's features, the institution can install and use continuous monitoring equipment that monitors bacteria in units of 1 min at the spot to verify the concentration at all times.

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This study was sponsored by the Ministry of Environment under task code ME23001.

## Data availability statement

Data will be made available on request.
2 of 15.

## CRediT authorship contribution statement

**Shambhavi Sharma:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Ahtesham Bakht:** Data curation, Formal analysis, Methodology. **Muhammad Jahanzaib:** Data curation, Investigation, Methodology. **Minkyeong Kim:** Conceptualization, Investigation, Methodology. **Hyunsoo Lee:** Software. **Choonsoo Park:** Formal analysis, Project administration, Resources, Supervision. **Duckshin Park:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21751.

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