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Assessment of airborne bacteria in the indoor of public-use facilities concentrated on influencing factors and opportunistic pathogenic bacteria

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Abstract

For public-use facilities in urban centers with high occupancy, it is imperative to effectively manage opportunistic pathogenic bacteria due to the diverse range of users, including the immunocompromised population, such as the elderly, children. Therefore, we investigated the concentration of airborne bacteria at several public-use facilities in urban centers in South Korea. The level of total airborne bacteria in the facilities was positively correlated with user density and CO_2 levels. Notably, subway compartments demonstrated particularly high levels of airborne bacteria. Subway compartments and daycare centers contained opportunistic pathogenic bacteria associated with antibiotic resistance. The relative abundance of genera associated with these species showed minor differences by season and facility; the genera *Klebsiella* and *Staphylococcus* showed high relative abundance in subway compartments and daycare centers, respectively. Based on our findings, we recommend enhancing management strategies targeting opportunistic pathogenic bacteria related to antibiotic resistance in the air of subway compartments and daycare centers.

Keywords Urban center · Public-use facility · Indoor air quality · Airborne bacteria · Opportunistic pathogenic bacteria

Introduction

Since the COVID-19 pandemic, there has been a surge of interest in indoor airborne pathogens (Megahed and Ghoneim 2021). In particular, airborne bacteria in indoor air are thought to originate from occupants (Hospodsky et al. 2012). The concentration of bacteria in indoor air falls within the range of 10^4–10^8 cells/m^3, encompassing some bacterial species associated with afflictions such as pneumonia, asthma, and allergic reactions (Bowers et al. 2011; Fujiyoshi et al. 2017).

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The proportion of the global population residing in urban areas was reported as 55% in 2018 and is predicted to increase in the future (UN 2019). Consequently, public-use facilities located in urban centers will witness an increase in user population, thereby lowering indoor air quality (Choi et al. 2022). The swift transmission rate of respiratory infections caused by airborne pathogens, which is enhanced by the dense occupancy in public-use facilities in urban centers, poses a significant challenge (Neiderud 2015; Liu et al. 2016) and therefore remains a major concern. Data on factors that may influence the microbiology of indoor air, such as patterns of human traffic and the surrounding environment in proximity to facilities, remain limited.

Opportunistic pathogens infect individuals with debilitated or compromised immune systems (Berg et al. 2005; Shapira et al. 2020). The escalating dissemination of opportunistic pathogenic bacteria poses a serious risk to public health (Lister et al. 2009). One of the causes of this phenomenon is intractable conditions due to the abuse or overuse of antibiotics, leading to the emergence of antibiotic-resistant bacteria (Wang et al. 2021). Opportunistic pathogenic bacteria include *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K.*

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pneumoniae), *Enterobacter cloacae* (*E. cloacae*), and *Cutibacterium acenes* (*C. acnes*), of which *C. acnes* is an opportunistic pathogenic bacterium in several clinical contexts, whereas others are associated with respiratory ailments (Tada and Hanada 2010; Mayslich et al. 2021). Furthermore, these pathogens are related to antibiotic resistance, thus augmenting their potential harm to immunocompromised populations (Chambers 2001; Fiegel et al. 2006; Gilbert et al. 2010; Chang et al. 2013; Solomon et al. 2017; Platsidaki and Dessinioti 2018; Kozajda et al. 2019; De Canha et al. 2021).

In South Korea, the management of indoor air quality in facilities that cater largely to immunocompromised populations adheres to standards that maintain the quantification levels of total airborne bacterial colony forming units (CFU) per cubic meter (Ministry of Environment 2023). Given the susceptibility of immunocompromised populations to opportunistic infections, facilities that care for them require vigilant management to mitigate the risk of opportunistic pathogenic bacteria. CFU is employed to quantify viable bacteria capable of growth on solid media, hence the utilization of a nucleic acid-based approach that relies on genotypic classification is valuable for microbial identification (Ho and Reddy 2010; Dybwad et al. 2012; Lu and Salzberg 2020; Makrai et al. 2023).

This study investigates the airborne bacteria affecting indoor air quality in public-use facilities located in urban centers. In addition to the conventional CFU method, we employed culture-independent nucleic acid-based analysis to assess the presence of these bacteria. The study encompasses the following aspects: (i) quantification of the total airborne bacteria using CFU and real-time polymerase chain reaction (real-time PCR), and correlation analysis between the bacterial concentration and other environmental factors, such as temperature, humidity, occupancy density, and CO_2 levels; (ii) quantitative assessment of five opportunistic pathogenic bacteria associated with antibiotic resistance in the air using real-time PCR; (iii) validation of genera diversity associated with the five bacterial species using 16S rRNA amplicon sequencing, i.e., nucleic acid-based analysis, and correlation analysis between their relative abundance and the adjacent land-use. Our findings identified facilities that require enhanced management strategies to address airborne bacteria in indoor air.

Materials and methods

Selection of sampling site and sample collection

Table S1 lists the sampling dates and locations for examining the indoor airborne bacteria. Sampling was conducted at facilities governed by the Indoor Air Quality Control Act in South Korea, including daycare centers, libraries, parking garages, subway stations, large retail stores, subway compartments, and train compartments (Ministry of Environment 2014). The selected facilities were located in Seoul, Goyang, Suwon, and Hwaseong, regions categorized as urban centers with population of > 900,000 residents, in accordance with the standards established by the UN Statistical Commission (Eurostat 2021). Sampling was conducted for one year, commencing in August 2021 and terminating in August 2022, especially summer and winter season, differ the most in terms of temperature, humidity, and clothing choices of users, for seasonal comparison. Sampling was conducted during which anti-COVID restriction were being implemented, including patient quarantine, disinfection for routine prevention, and the use of masks indoors.

Dust samples were gathered at a height of 0.8 m, driven by the following reasons: consideration of the height of breathing zone (i) for children due to their higher susceptibility to airborne contaminants in comparison to adults (Chegini et al. 2020; Lee et al. 2021; Yang et al. 2022), (ii) for those who are sitting as they spend longer time in public-use facilities compared with those who are standing. To provide consistency in sampling height across all facilities, a foldable table was implemented as a standardized height of 0.8 m. Nevertheless, the distinct internal characteristics of GCK and KHT made it unfeasible to install the table; therefore, we ensured a consistent sample height by utilizing the intrinsic structural properties.

Closed cassettes (37 mm) equipped with a polyvinyl chloride filter (5 μm pore size, SKL Inc., USA) for collecting dust samples were connected to sampling pumps (Gilian Air plus, Sensidyne, St. Petersburg, USA). The sampling procedure was performed at a flow rate of 4 L/min, which allowed for a flow range of 1,000–3,900 L to traverse the filter. This range was determined based on the users' individual staying characteristics in relation to each facility. Sampling was conducted over a period of 2–3 days. To ensure an adequate amount is captured, two samplers were used, and the resulting filters were combined for genomic DNA (gDNA) extraction. The minimum capture volume was determined based on previous studies (Lee et al. 2021; Yang et al. 2022).

Prior to sampling, all pumps were calibrated using a primary pump calibrator (model 4146, TSI, Shoreview, USA). The digital thermohygrometer (model 605i, Testo, Titisee-Neustadt, Germany) was utilized to measure and record the indoor temperature and relative humidity. Outdoor temperature and precipitation data for the designated sampling period were obtained from the National Climate Data Center of the Korean Meteorological Administration (KMA National Climate Data Center 2023). The number of users in public-use facilities was quantified by direct observation, where the number of people present within the designated sampling area (5 m × 5 m) was periodically recorded. The recorded numbers of users were used as averages for

analysis. The CO_2 levels indoors were measured using an indoor air quality monitoring device (model IMI-1000A, INNO Digital, Republic of Korea). We lacked access to information regarding the building's degree or ventilation capabilities, we were unable to use this data for research. We also lacked comparison of indoor and outdoor samples, because the sampling points were located on different floors of each building, there was a limit to the comparison with the measurable external sample: however, we checked that the difference in floors did not significantly affect the results.

Prior to use, all items and equipment used for sampling were sterilized using UV disinfection (mode HU-4050; Hanshin Medical, Incheon, Republic of Korea) for one hour to eliminate any potential background DNA contamination. After sampling, the cassettes containing the filters were sealed using Parafilm. Sealed filters were carefully placed within sterilized filter containers and thereafter carried with ice packs to ensure a temperature of −4 ℃, minimizing potential changes in microbial communities. All the filter samples were stored at −80 ℃ until analysis.

Extraction of genomic DNA

A total of 300 mg of 212–300 µm sterilized glass beads (Sigma, St. Louis, USA) were placed in a 2 mL microcentrifuge tube with screw cap. After the insertion of two filters divided into smaller pieces within the tube, 600 µL of Cel-Lytic™ B Cell Lysis solution (Sigma, St. Louis, USA) was diluted by a factor of 10 using nuclease-free water (QIAGEN, Hilden, Germany). A homogenizer (Allsheng, Hanzhou, China) operated for five cycles of 30 s at a centrifugal force of $1,484 \times g$ was used for homogenization of the sample. After centrifugation at $8,000 \times g$ for 1 min, the supernatant was carefully collected and transferred to a new tube, which was centrifuged again at $8,000 \times g$ for 1 min, after which the supernatant was aliquoted and transferred to new tubes. Following the pretreatment of the filter, gDNA was extracted following the instructions of the High Pure PCR Template Preference Kit (Roche, Mannheim, Germany). The gDNA was preserved at a temperature of −20 ℃ until further extraction and analysis.

Quantitative analysis of total bacterial DNA and opportunistic pathogenic bacterial DNA

Quantification of total bacterial DNA using quantitative polymerase chain reaction (qPCR) involved the use of primers targeting the 16S rRNA region (Table 1). The mixture of qPCR, with a total volume of 20 µL, was composed of the follows in a 0.2 mL PCR tube (Hyundai Micro, Seoul, Korea): 4 µL of 5 × HOT FIREPol Evagreen qPCR Supermix (Solis BioDyne, Tartu, Estonia), 0.4 µL of forward and revers primer, 1 µL of DNA template, and 14.6 µL of nuclease-free water (QIAGEN, Hilden, Germany). qPCR was performed on a LineGen 9600 instrument (BIOER, Hangzhou, China) with the following conditions: initial denaturation at 95 ℃ for 12 min, thereafter 40 cycles of three-step process, which contained denaturation at 95℃ for 15 s, annealing at 55 ℃ for 20 s, and elongation at 72 ℃ for 30 s. For quantification of total bacteria in air, qPCR analysis was performed based on *Escherichia coli* (*E. coli*). A standard curve was generated by diluting *E. coli*. The concentration of *E. coli* broth used for gDNA extraction was $OD_{600} = 0.7$. Following extraction, the concentration of the DNA solution was measured at 55.0 ng/µL. The solution was diluted by a factor of 10. PCR efficiency was confirmed using a standard curve ($R^2 = 0.999$, PCR efficiency = 100.28%). The concentration of the DNA copy number of total bacteria in the air (copy number/m^3) was determined using linear regression analysis of the standard curve and the Ct values of each sample, as previously reported (Bustin 2000; Lee et al. 2021; Yang et al. 2022):

Table 1 List of primers for analysis of bacterial analysis in this study

Target	Primer	Sequence
Bacteria (qPCR)	Forward	5'-TCCTAC GGGAGG CAGCAG T-3'
	Reverse	5'-GGACTA CCAGGG TATCTAATC CTGTT-3'

$$NC_{E.coli} = \frac{DNAconc. \times NA}{LT_{E.coli} \times WB \times Massconversionfactor} \quad (1)$$

$$f_{E.coli} = \frac{NC_{E.coli}}{10^{n+1}} \quad (2)$$

$$C = f_{E.coli} \times 10^{(Ct_{sample} - b)/m} \times \frac{V_{gDNA}}{V_{air}} \quad (3)$$

The final derived C is the concentration of the bacterial copy number; $NC_{E.\ coli}$ is the copy number of gDNA template (based on *E. coli*, copy number/µL); DNA conc. is the DNA concentration (ng/µL); NA is Avogadro's number; $LT_{E\ coli}$ is the length of template based on *E. coli* (bp); WB is the average weight of base pair (Da/bp); mass conversion factor is the conversion factor to ng; $f_{E.\ coli}$ is the conversion coefficient for quantity of *E. coli*; n is the number of dilutions of the DNA solution to generate the standard curve; C is the concentration of copy number of bacteria in the air (copy number/m^3); Ct_{sample} is the Ct value of each sample; b is the intercept of the standard curve; m is the slope of the

standard curve; V_{gDNA} is the volume of the eluted gDNA (μL); V_{air} is the volume of captured air sample (m^3).

The following five opportunistic pathogenic bacteria associated with antimicrobial resistance were identified in this study: *S. aureus, P. aeruginosa, K. pneumoniae, E. cloacae, C. acnes.* The microbe detection assay (TaqMan™ Microbe Detection Assay, Thermo Fisher Scientific, Vilnius, Lithuania) provided in Table S2 was employed for the quantitative analysis of these bacterial species. The mixture of qPCR, with a total volume of 20 μL, was composed of the following, in a 0.2 mL PCR tube (Hyundai Micro, Seoul, Korea): 10 μL of TaqMan probe (TaqMan™ Fast Advanced Master Mix, Thermo Fisher Scientific, Vilnius, Lithuania), 1 μL of forward and reverse primer, 2 μL of DNA template, and 7 μL of nuclease-free water. The conditions were as follows: initial denaturation at 50 ℃ for 2 min and 95 ℃ for 10 min, thereafter 40 cycles of two-step process, which contained denaturation at 95 ℃ for 15 s, and annealing at 60 ℃ for 60 s. To quantify the aforementioned species in the air, qPCR analysis was performed using the standard curve generated by diluting gDNA samples of each individual bacterial species. The concentration of each bacterial culture used for DNA extraction was measured at $OD_{600} = 0.7$.

Subsequently, the concentration of the extracted gDNA were 2.5 (*S. aureus*), 38.5 (*P. aeruginosa*), 17.6 (*E. cloacae*), 830.5 (*K. pneumoniae*), 226.9 (*C. acnes*) ng/μL. The samples were diluted by a factor of 10. PCR efficiencies were confirmed using standard curves (*S. aureus*: $R^2 = 0.998$, PCR efficiency = 89.34%; *P. aeruginosa*: $R^2 = 0.999$, PCR efficiency = 90.11%; *E. cloacae*: $R^2 = 0.999$, PCR efficiency = 88.90%; *K. pneumoniae*: $R^2 = 0.996$, PCR efficiency = 88.51%; *C. acnes*: $R^2 = 0.999$, PCR efficiency = 93.87%). Determination of the DNA copy number concentration of the five bacterial species in the air was carried out using the same method as for quantification of the total bacteria mentioned above.

16S rRNA amplicon metagenomic sequencing

The sequencing library was prepared according to the Illumina 16S Metagenomic Sequencing Library protocol to amplify the V3-V4 region. DNA was quantified, and DNA purity was assessed using the QuantiFluor dsDNA System (Promega, Madison, USA) and VICTOR Nivo (PerkinElmer, Waltham, USA). Samples with gDNA concentrations below the designated threshold of 10 ng/μL were excluded from sequencing. Amplification of the 16S V3-V4 region of bacterial DNA was conducted using the universal primers listed in Table 1. The amplification process was performed using multiplexing indices and Illumina sequencing adapters. The final products were normalized and pooled using PicoGreen, and the library size was confirmed using a TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). Sequencing was performed using an Illumina MiSeq platform (Illumina, San Diego, CA, USA) and sequence analysis was conducted using Quantitative Insight into Microbial Ecology2 (QIIME2, v.2020.6). In the multiplex phase, each sequence underwent noise sequence filtration and error correction in neighboring sequences using DADA2 (ver.1.1.1). Correction of the amplicon error was achieved by removing the chimeric sequences and singletons. Phylogenetic analysis was conducted using amplicon sequence variants (ASVs).

Determination of CFU

To compare the total bacterial concentrations determined using qPCR and conventional approaches, we employed the impaction method within the framework of the standard method for examining indoor air quality. Capturing microorganisms was accomplished using a single-stage impactor sampler (400 holes, suction flow rate with 20 L/min) (KAS-110, KEMIK Cor., Korea). Each sampling session lasted for 10 min. Prior to sample collection, the sampler was internally sanitized using a 70% ethyl alcohol solution; thereafter, an agar plate was installed.

The collection of samples was carried out at a vertical distance of 0.8 m from the floor while ensuring that the overall flow rate maintained a certain volume and remained below 250 L. To cultivate diverse bacterial species, samples were obtained using tryptic soy agar media (Model SW-M01-050–2, Samwoosnt Itd., Korea). The agar plates utilized were incubated in an incubator (HB-103SI, Hanbaeksci, Korea) at a temperature of 35 ℃ for 48 h. The bacterial concentration was quantified by enumerating and utilizing the number of colonies as a measure of CFU per unit volume of the air sample.

Analysis of the land-use area adjacent to the public-use facilities

The land-use data pertained to spatial data, namely the current land-use status was collected by the Ministry of Land, Infrastructure, and Transport of the Republic of Korea and acquired via the National Spatial Data Infrastructure Portal (National Spatial Data Infrastructure Portal 2019). Spatial data were analyzed using the QGIS Desktop software (ver. 3.28.9). By modifying the method from a previous study that set 500 m as a general pedestrian walking routes (Choi and Park 2017), we delineated the potential walking paths of facility users within a 500 m radius of a facility and visually represented them on a map. The spatial extent within a 500 m radius from the sampling site was converted to a numerical value representing the area in square meters using the field calculator of the QGIS Desktop software. Current land-use status classification categories are derived from the

land-use categorization system developed by the National Geographic Information Institute of Korea (Table S3).

Statistical analysis

Statistical analysis and data visualization were performed using the IBM SPSS Statistics software (ver. 28.0) and R (ver. 4.3.0). Spearman's rank correlation analysis was used to examine the correlation between bacterial DNA concentration and environmental factors. The result was visualized using the 'psych' package in the R software. The Kruskal–Wallis test, a non-parametric statistic, was employed to investigate variations in the concentration of bacterial DNA, user density in each facility, and CO_2 levels across different seasons and facilities. To validate the observed disparities among the groups, the Bonferroni correction was employed to account for multiple hypothesis testing. Prior to conducting the nonparametric test, the data distribution was ascertained to be non-normal using the Shapiro–Wilk test. The concentrations of the five species of bacteria were converted into logarithmic values and visualized using the 'pheatmap' package in the R software. Analysis and visualization of the occupancy of the five bacterial genera were conducted using analysis of similarities (ANOSIM) and nonmetric multidimensional scaling (NMDS) techniques. Both ANOSIM and NMDS were performed using the Bray–Curtis dissimilarity matrix. The Kendall's rank correlation approach was used to examine the relationship between the relative abundance of the five bacterial genera and land-use in the vicinity of the sampling sites within a 500 m radius. To depict the relative abundance of the five bacterial genera among the samples, the data were transformed into z-scores and further visualized using the 'pheatmap' function in the R software.

Results and discussion

Quantitative analysis of total bacteria

To determine the various factors affecting the concentration of total bacteria in the indoor air of public-use facilities, we conducted a correlation analysis between environmental factors and the total bacterial concentration. The average values of the measured environmental factors are listed in Table S4, while the results of the correlation tests are shown in Fig. 1. In addition to the histograms showing each environmental factor on diagonal, the correlation and significance between

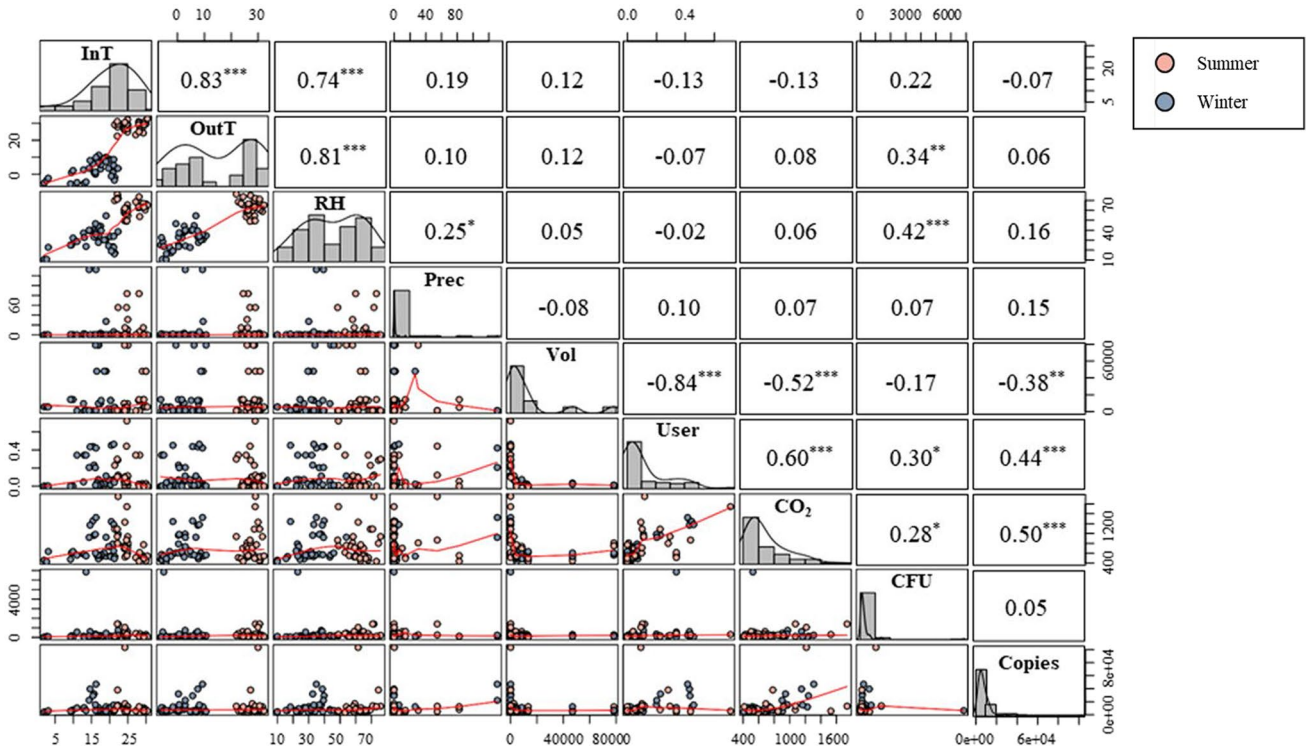

Fig. 1 Correlation between total bacterial concentration and environmental factors. InT, indoor temperature (℃); OutT, outdoor temperature (℃); RH, relative humidity (%); Prec, precipitation (mm); Vol, volume of the indoor of facility (m^3); User, the number of occupants in a public-use facility per unit volume (m^3) of the facility (person/m^3); CO_2, concentration of CO_2 (ppm); CFU, colony-forming unit per unit volume (CFU/m^3); Copies, concentration of copy numbers of bacterial DNA (copy number/$m.^3$). The color of the dots in the scatterplot represents sampling season, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

the factors are displayed. The scatter plots shown below the diagonal illustrate the relationship between several characteristics, with each point distinguished by a hue based on the season in which the samples were obtained. No significant link was observed between the total bacterial concentration determined by copy number and CFU. Unculturable bacteria were considered as one of the reasons, because not all bacteria in the environment grow in laboratory media (Stewart 2012). On the other hand, the nucleic acid-based detection method can obtain DNA sequence information from environmental samples despite the viability of the organism carrying DNA (Amann et al. 1995). The total bacterial concentration based on copy number was significantly correlated with both user density and CO_2 concentration at each facility ($p < 0.001$), with Spearman's rank correlation coefficients of 0.436 and 0.495, respectively. CFU also significantly correlated with both user density and CO_2 concentration ($p < 0.05$ and $p < 0.01$, respectively).

Nevertheless, the correlation coefficients for these variables, 0.26 and 0.31, respectively, were comparatively lower than that of the total bacterial concentration determined by copy number. In summary, our findings suggest a positive correlation between the concentration of total bacteria in the air and both user density and CO_2 levels within each facility. Occupants are one of the primary sources of indoor airborne bacteria; the composition of indoor microbial communities is primarily influenced by human occupation and activity (Fox et al. 2003; Lee et al. 2021; Beasley et al. 2022). In most buildings, the CO_2 level is an indicator of the number of individuals inhabiting the premises, since they are a major producer of indoor CO_2 via exhalation (Rudnick and Milton 2003). In addition, the CO_2 level indoors can fluctuate owing to air circulation and ventilation as well as the volumes of the indoor space (Turanjanin et al. 2014). Therefore, the association between CO_2 levels and the extent of ventilation suggests a slightly stronger correlation between the total bacterial concentration and CO_2 levels in comparison to user density.

Statistical analyses were conducted to investigate the variations in the total bacterial concentration among seasons and types of facilities. The results of the Kruskal–Wallis test showed no significant disparity in the total bacterial concentration between the samples collected during summer and winter ($p > 0.05$) (Fig. 2a). However, a significant disparity was observed across samples collected from each type of facility ($p < 0.01$) (Fig. 2b). The post hoc test revealed significant differences between subway compartments and other types of facilities, including parking garages, subway stations, and large stores ($p < 0.05$). Figure 2b shows that the subway compartments had comparatively elevated levels of total bacterial concentrations in the air.

These results are possibly attributed to the absence of seasonal variation in both user density and CO_2 levels ($p > 0.05$), whereas differences were evident among the different facilities, particularly in the subway compartments (Table S5). Occupants of indoor spaces are prominent contributors to bacteria, hence the user density based on the volume of indoor spaces significantly influences the total bacterial concentration. Moreover, despite the lack of significant differences in CO_2 levels and user density compared

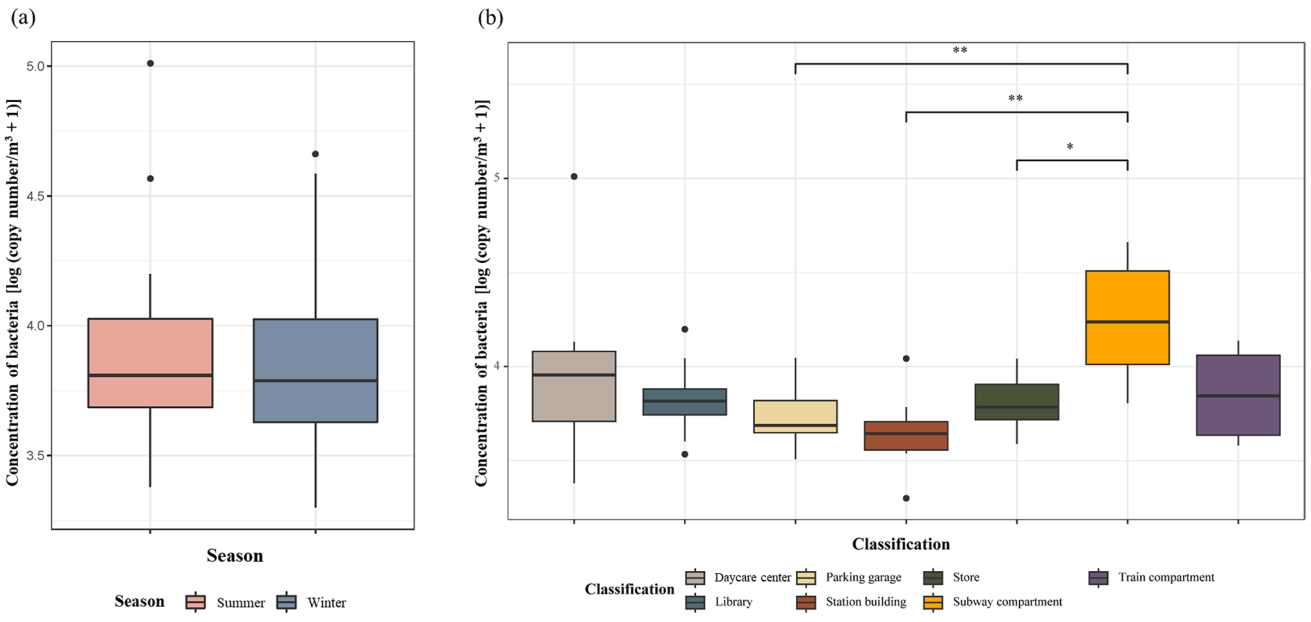

Fig. 2 Analysis of total bacterial concentration by (**a**) season and (**b**) facility classification. Boxplots demonstrate minimum, Q1, median, Q3, and maximum values from bottom, $^{**}p < 0.01$, $^{*}p < 0.05$

with the train compartment, the subway compartments demonstrated an elevated total bacterial concentration. Previous research has indicated that the movement of passengers and the opening and closing of doors can lead to the suspension of particles and an increase in the concentration of particulate matter indoors (Qiao et al. 2015; Cha et al. 2018). During sampling, subway compartments traversed 66 stations with doors opening and closing during the round trip, for a total running time of 2 h. Meanwhile, the train compartments traversed 20 stations with door opening and closing during the round trip over a running time of 6 h. In contrast to train compartments, re-suspension of particulate matter occurred frequently in subway compartments, increasing the airborne bacterial concentration.

Quantitative analysis of the five species of opportunistic pathogenic bacteria

Quantitative analysis of opportunistic pathogenic bacteria, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *E. cloacae*, *C. acnes*, associated with antibiotic resistance and detected in air, was conducted for public-use facilities located in urban centers and visualized by seasons (Fig. 3a and b). *S. aureus* was detected in subway compartments in both summer and winter samples. Moreover, high levels of *S. aureus* were observed in daycare centers, specifically during summer. *P. aeruginosa* was identified in the subway compartments during winter. *K. pneumoniae* was not detected in any sample. During the summer season, *E. cloacae* was detected in daycare centers. Except for one sample collected in summer and seven in winter, *C. acnes* was detected in all the remaining samples. *S. aureus* is not only known as a symbiotic bacterium, as it establishes colonization within the nasal cavity of 20–40% of the human population, but is also an opportunistic pathogenic bacterium capable of inducing various illnesses, including skin and wound infections, pneumonia, and sepsis (Kozajda et al. 2019). Furthermore, *S. aureus* has a propensity for acquiring resistance genes against a multitude of antibiotics, thus necessitating heightened attention to its effective management (Kozajda et al. 2019), and can persist for extended periods, ranging from weeks to months, on dry, inert surfaces (Kramer et al. 2006; Hübner et al. 2011).

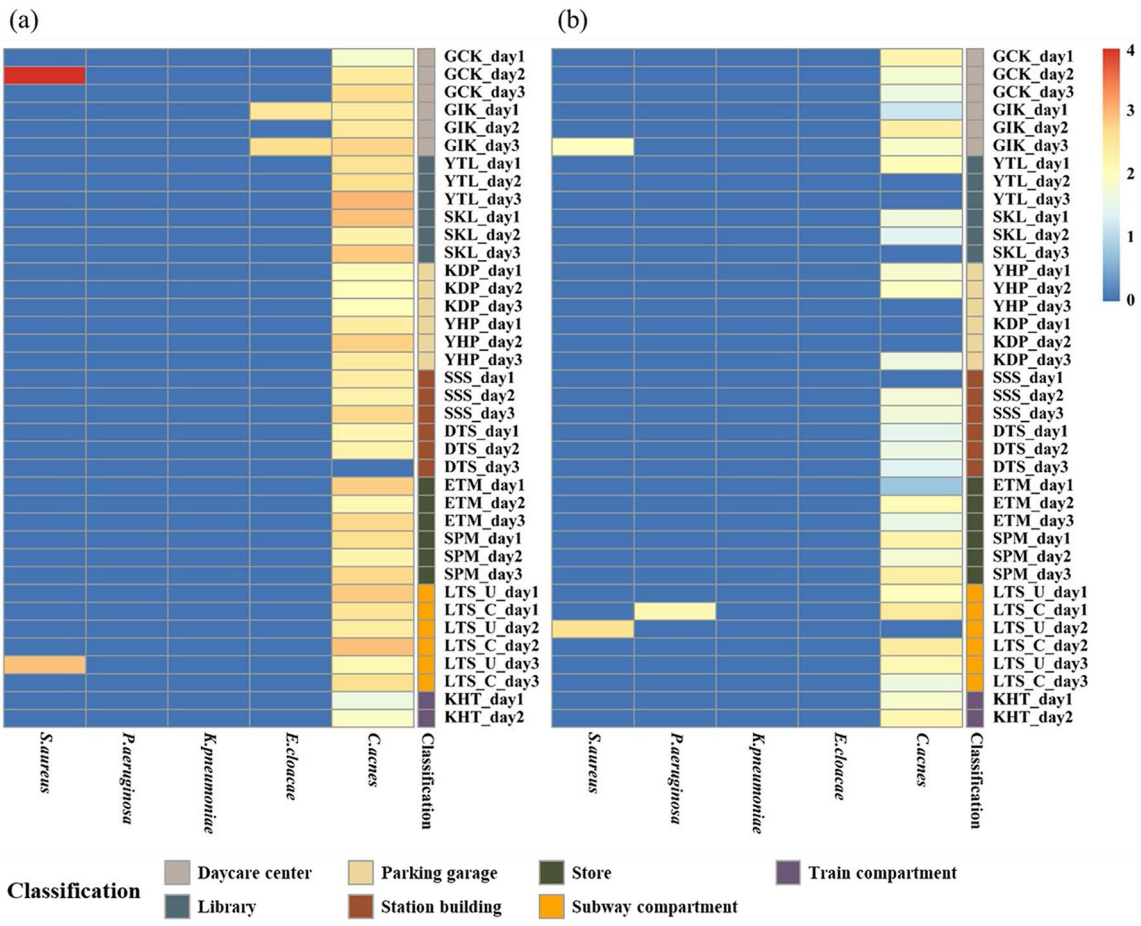

Fig. 3 Heatmaps demonstrating detection concentration of each bacterial species [log (copy number/m^3 + 1)] in samples collected in (**a**) summer and (**b**) winter

The presence of bacteria in surface dust is linked to airborne microorganisms through processes, such as deposition and resuspension. Hence, it is imperative to conduct routine surface cleaning in establishments such as subways and daycare centers, where elevated amounts of opportunistic pathogenic airborne bacteria have been identified. For *S. aureus*, it is advisable to employ alternative disinfectants due to its inherent resistance to quaternary ammonium-based formulations (Martinez et al. 2022). However, indiscriminate and intense disinfection should be avoided due to the investigation that disinfectant by-products can continuously promote antibiotic-resistant bacteria (Chen et al. 2021; Mantilla-Calderon et al. 2019). *P. aeruginosa* is also known for its resistance to numerous types of antibiotics, its ability to persist on desiccated inert surfaces, and, infrequently, its association with community-acquired pneumonia (Kramer et al. 2006; Solomon et al. 2017; Bassetti et al. 2018). *E. cloacae* is also an opportunistic pathogenic bacterium linked to urinary tract, respiratory tract, and other infections (Ramirez and Giron 2023). Hence, given the identification of these two species in the airborne bacterial composition of both subway compartments and daycare centers, it is crucial to prioritize disinfection protocols and strict hygiene standards to reduce the likelihood of infection. *K. pneumoniae* is commonly present on the mucosal surfaces of both humans and animals and in contaminated water and soil. In humans, *K. pneumoniae* has been identified as a symbiotic organism found in the respiratory and digestive systems, with a detection rate of 1–6% in the nasopharynx (Podschun and Ullmann 1998; Grimont and Grimont 2015).

Indeed, the absence of a confirmed presence of *K. pneumoniae* was indicated by the detection rate of *K. pneumoniae* in nasopharyngeal samples and implementation of required mask-wearing in indoor settings of public-use facilities as a response to COVID-19 during the sampling period. The presence of *K. pneumoniae* in indoor air is concerning due to its ability to cause pneumonia through airborne transmission, which is associated with high death rate (Fiegel et al. 2006). *C. acnes*, a gram-positive bacterium commonly found on the human skin, exhibits both environmental and airborne growth capabilities (Dekio et al. 2021). In contrast to other bacterial species, *C. acnes* was present in most samples, possibly because it is a symbiotic microorganism typically found on human skin, hence its presence in the air of public-use facilities was a result of its release from the skin of users visiting the facilities (Rozas et al. 2021). There is evidence that human skin cells, which naturally shed from the epidermis, can be emitted into the indoor air or settle on the floor and are subsequently re-suspended, strongly influencing bacterial communities in the air (Hospodsky et al. 2012). The genus *Cutibacterium*, influenced by these mechanisms, is found in regions of the human skin with sebum secretion, including the forehead, nasolabial folds, external ear canal, nostrils, and areas behind the ears where wrinkles are prevalent (Grice and Segre 2011). The variations in clothing among users visiting public-use facilities in Korea are attributed to the contrasting climatic conditions during hot summers and frigid winters. The samples collected during winter had a comparatively low, or even not detected, concentration of *C. acnes* in comparison to summer samples. This could be attributed to the fact that users visiting public-use facilities tend to have limited skin exposure during the winter. Nevertheless, owing to the inherent exposure of the skin normally inhabited by *Cutibacterium* spp., irrespective of seasonal variations, *C. acnes* was detected in most samples.

Occupying characteristics of five genera associated with opportunistic pathogenic bacteria

Prior to analyzing the relative abundance of bacterial genera linked to opportunistic pathogenic bacteria associated with antibiotic resistance, specifically *Staphylococcus, Pseudomonas, Klebsiella, Enterobacter, Cutibacterium*, the sequencing depth of each individual sample for 16S rRNA amplicon sequencing for metagenomics was verified using rarefaction curves (Fig. S1a and b). In the rarefaction curves, the average ASVs of samples collected over a period of three consecutive days is expressed. Amplification of the samples was successful, as indicated by the convergence of the slope. To gain insight into the occupancy patterns of the five specified genera in public-use facilities, the relative abundance of each genus was assessed using the ANOSIM test and NMDS, with a classification of seasons and facility types. The ANOSIM test yielded statistically significant differences between the seasonal and facility groups, albeit with only a slight dissimilarity: $R = 0.21$, $p < 0.001$ in the seasonal group (Fig. 4a); $R = 0.19$, $p < 0.001$ in the group classified by facility type (Fig. 4b); the number of permutations was 9,999 in both groups. This was visualized in the NMDS plots by seasonal (Fig. 5a) and facility groups (Fig. 5b). A previous study conducted in libraries indicated that the dissimilarities observed between buildings in terms of bacterial communities were more significant than seasonal fluctuations (Rintala et al. 2008), while another study conducted in daycare centers revealed no discernible seasonal patterns in bacterial communities was not discovered (Prussin et al. 2016). These observations suggest that the distribution of the aforementioned five genera do not demonstrate distinct seasonal or facility category variations within indoor environments, where there is a reduced degree of temperature fluctuation compared with outdoor environment. Accordingly, the relationship between indoor and occupant bacterial communities suggests that factors such as the number of occupants and their clothing can indirectly influence these clusters beyond the direct influence of seasons or facility

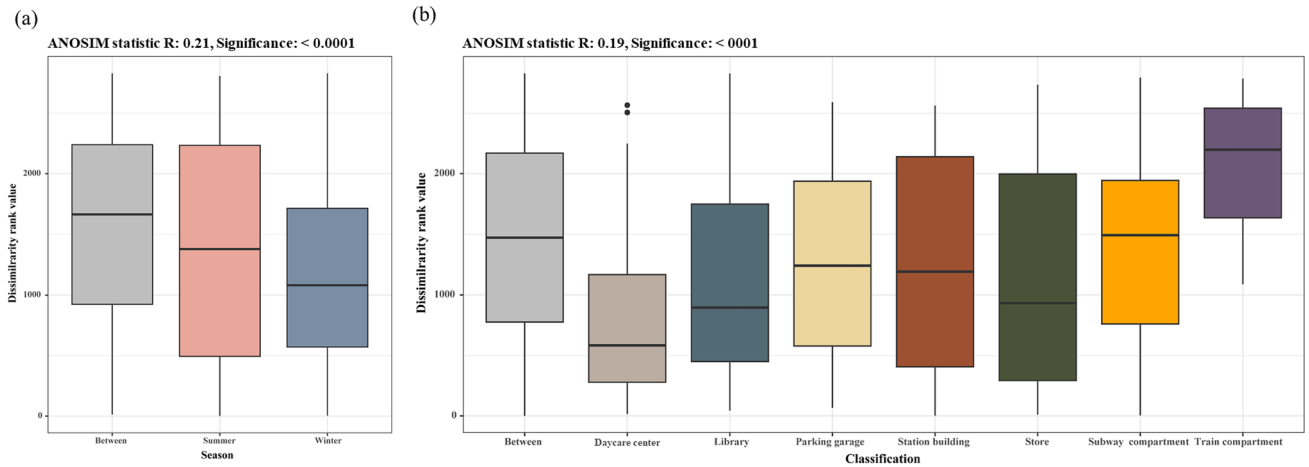

Fig. 4 Analysis of similarities (ANOSIM) of the occupancy by five bacterial genera grouped by (**a**) season and (**b**) facility: *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Cutibacterium*. The number of permutations for both groups was 9,999

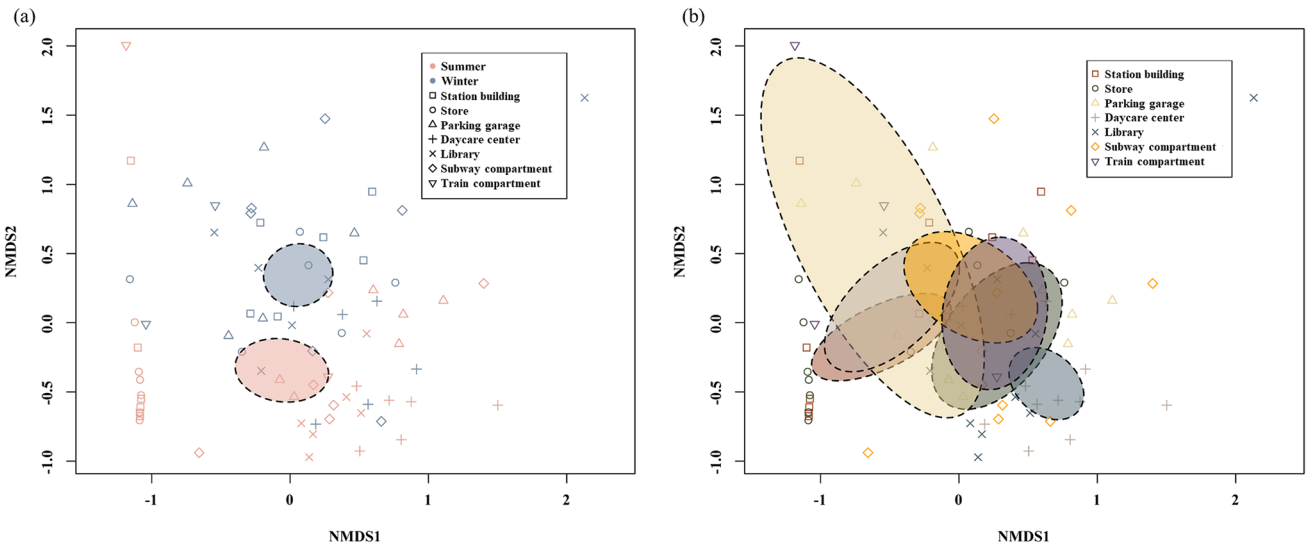

Fig. 5 Nonmetric multidimentional scaling (NMDS) map of the occupancy by five bacterial genera grouped by (**a**) season and (**b**) facility: *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Cutibacterium*. Colored ellipse: 95% confidence interval using standard error

classification. Moreover, the combination of additional factors might have influenced the outcomes.

To examine the occupancy patterns of *Staphylococcus, Pseudomonas, Klebsiella, Enterobacter,* and *Cutibacterium* genera in public-use facilities by seasonal variations, we conducted a comparative analysis of the relative abundance of these genera, which involved the transformation of metagenomic data into z-scores and visualization (Fig. 6a and b). In summer samples, *Klebsiella* had a notably high prevalence in the subway compartments (LTS-C), whereas *Staphylococcus* exhibited a high prevalence in the daycare center (GIK). Additionally, *Pseudomonas* and *Enterobacter* displayed significant occupancy rates in another daycare center (GCK). Conversely, in winter samples, *Klebsiella* was predominant in subway trains (LTS-U), whereas *Staphylococcus* was prominent in childcare facilities (GIK). *Pseudomonas* showed high occupancy in another daycare center (GCK). The findings pertaining to daycare centers were in line with those of prior research conducted on this subject. Deng et al. (2016) showed that *Staphylococcus* exhibited a propensity for uniform dispersion within the indoor air of childcare facilities. Lee et al. (2007) conducted a study in which the presence of *Staphylococcus* and *Pseudomonas* on various surfaces, including toys and countertops, were consistently observed across four facilities. These results are evidence of the

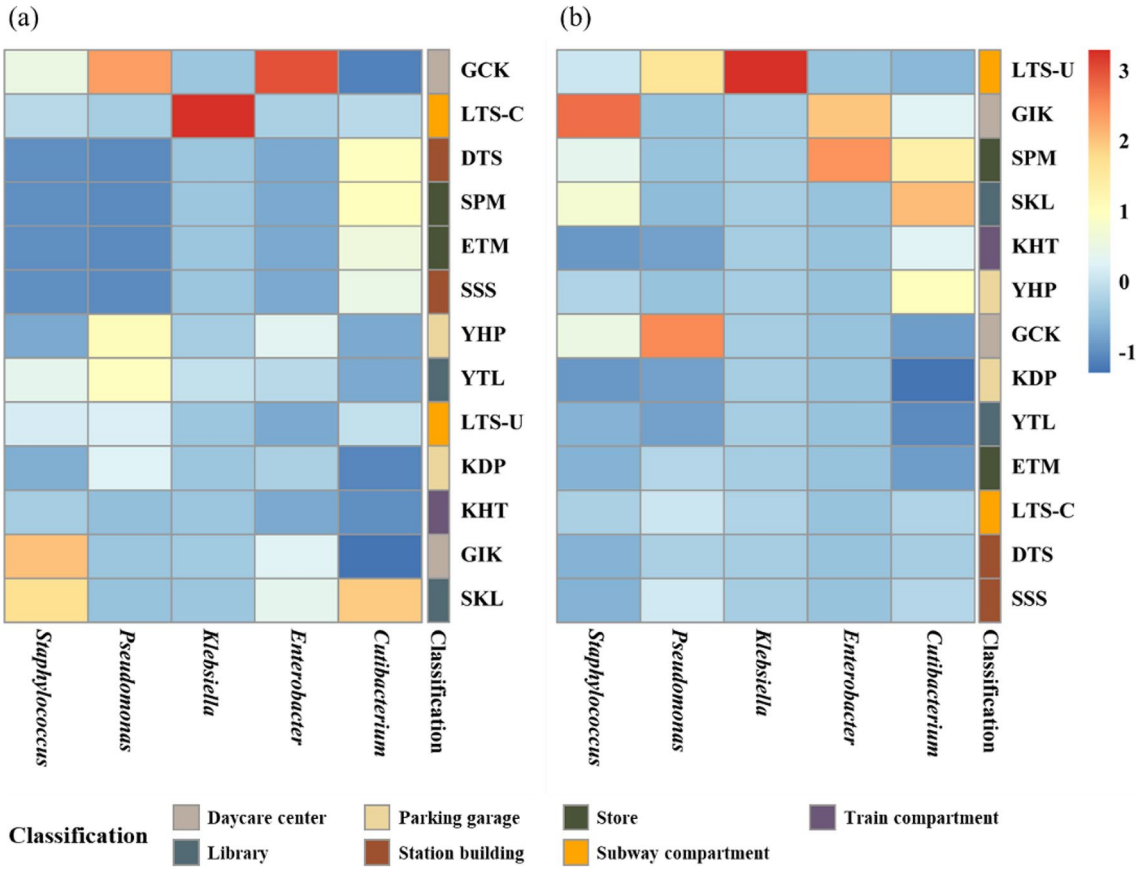

Fig. 6 Heatmaps demonstrating the relative abundance of five of bacterial genera expressed in z-score among the samples collected in the (**a**) summer and (**b**) winter

widespread presence of *Staphylococcus* and *Pseudomonas* genera within daycare centers.

To ascertain the other factors affecting the bacterial community, we analyzed the correlation between the five bacterial genera linked to opportunistic pathogenic bacteria associated with antibiotic resistance and land-use patterns within a 500 m radius from the sampling site (Table 2). The composition of airborne bacterial communities can be influenced by several local surface factors, including vegetation cover, land management practices, and the amount of bare soil; consequently, the composition of airborne bacterial communities may exhibit variations across several land-use categories (Shaffer and Lighthart 1997; Bowers et al. 2011). Moreover, there

Table 2 Correlation between occupancy of bacterial genus and land-use type

Season	Genus	Land-use type	Kendall's rank correlation coefficient ($p < 0.05$)
Summer	*Staphylococcus*	Mixed forest land	0.804
		Extensive park	0.704
		Stream	-0.640
	Pseudomonas	Educational and military installation	0.656
		Stream	-0.640
	Enterobacter	Extensive park	0.704
		Stream	-0.640
Winter	*Staphylococcus*	Barren or artificially created grassland	-0.643
	Pseudomonas	Mixed forest land	-0.624

are instances wherein particles adhered to garments can be liberated into the atmosphere due to bodily motion, thereby constituting a potential route for outdoor bioaerosols to enter the indoor environment (McDonagh and Byrne 2014). Bioaerosols are deposited on the surface of garments through contact with the skin of the wearer and the surrounding environment (Licina and Nazaroff 2018). We focused on introducing diverse bacterial clusters, depending on the land-use of the surrounding area, into indoor public-use facilities, with pedestrians serving as carriers. Analysis of correlations conducted on summer samples indicated a positive link between *Staphylococcus* and the extent and abundance of mixed forestland and extensive parks. The abundance of soil microorganisms in mixed forests and extensive parks with significant population movement can be attributed not only to their ubiquitous distribution in nature but also their propensity to aggregate on human skin, sweat glands, and mucous membranes (Götz et al. 2006; Antelmann 2015). *Pseudomonas* is positively associated with the spatial extent and abundance of educational and military installations, and is a pervasive genus that is extensively distributed across natural environments and encompasses several species that engage in symbiotic relationships with plants, as well as affiliated with insects, animals, and pathogens affecting humans (Mulet et al. 2009; Peix et al. 2018). *Enterobacter* positively correlated with nearby extensive parks, which is possibly attributed to its capacity to thrive in diverse habitats, including soil, plants, human epidermis, and animal waste (Davin-Regli et al. 2019). All five genera were negatively correlated with streams, although due to the limited presence of a single facility, including a stream in the vicinity of 500 m, it was not possible to generalize this correlation. However, according to prior research, elevated relative humidity enhances the surface adherence of particles and lead to the condensation of smaller particles into larger ones, consequently augmenting the rate of particle re-suspension (Zheng et al. 2019). Hence, those who traverse routes near rivers may have lower bacterial transportation rates owing to reduced bacterial re-suspension. In samples collected in winter, *Staphylococcus* was negatively correlated with barren or artificially created grasslands, while *Pseudomonas* was negatively correlated with mixed forest land.

The organization of soil bacterial communities is influenced by diverse environmental conditions, including seasonal variations in plant physiology (Chemidlin Prevost-Boure et al. 2011). This suggests that the reduction in plant biomass, particularly the vegetation of the specific soil classification system, during winter altered the bacterial population, ultimately affecting the transport and introduction of bacteria into indoor environments.

Conclusion

This study examined the presence of airborne bacteria in indoor public-use facilities located in four urban centers in South Korea and observed positive correlations between the aggregate concentration of bacteria in the air, user density, and CO_2 levels. The concentration of airborne bacteria in subway compartments was greater than that in the other facility categories. Except for *K. pneumoniae*, opportunistic pathogenic bacteria associated with antibiotic resistance were identified in the samples obtained from daycare centers and subway compartments. In the context of the related bacterial genera, *Klebsiella* exhibited a greater relative abundance in samples collected from subway compartments, whereas *Staphylococcus* demonstrated a higher relative abundance in samples obtained from daycare centers compared with other sampling sites. While research on the quantification of opportunistic pathogen species in the air remains limited, our findings showed that subway compartments and daycare centers exhibit elevated concentrations of such bacteria compared to other facilities. These bacteria pose a risk to immunocompromised populations.

Hence, it is imperative to prioritize the treatment of airborne bacteria, particularly in such facilities, to minimize the potential harm of opportunistic pathogenic bacteria. In subway compartments, characterized by high passenger density and frequent population movement, it is imperative to implement strategies, such as regular disinfection protocols, to effectively manage and control airborne bacteria in indoor air. Furthermore, as day care centers are frequented by vulnerable populations such as children, it is necessary to implement routine disinfection protocols and other management strategies to maintain an environment that is both safe and conducive to optimal health. During the sampling period, the Korean government also recommended quarantine management and the use of personal masks to prevent COVID-19. We recognize that this may have led to an underestimation of the results of this study and recommend further investigation of the changes resulting from the relaxation of quarantine regulations. We investigated the correlation between surrounding land-use facilities and several bacterial genera. Although this study was able to find a partial correlation between land-use and opportunistic pathogenic bacteria due to limited sampling sites, the addendum of research pertaining to land utilization and meticulous monitoring of passenger routes may yield more definitive outcomes. Further research will contribute to the development of indoor environments in cities that prioritize public health, such as strategies for rearranging specific land-uses that may affect the bacterial community in close proximity to facilities frequented by immunocompromised populations.

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Declarations

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