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Identification of Bacteria and Fungi in Various Types of Multi-Use Facilities in Bucheon, South Korea

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Abstract: The recent sequential appearance of infectious pathogens has caused ongoing social and economic damage. Despite the very high potential for pathogen transmission within indoor multi-use facilities, there are insufficient measures for the systematic analysis, diagnosis, and reduction in such transmission. Although real-time environmental information is available for pollutants such as particulate matter, carbon dioxide, and nitrogen dioxide in South Korea, an automatic network for the real-time measurements of harmful microorganisms has not yet been established. Therefore, in this study, we analyzed the concentrations of bacteria and fungi in different types of multiple-use facilities in Bucheon, South Korea, using the analytic hierarchy process (AHP) method. All multi-use facilities in the region were classified into six types: facilities for pollution-sensitive groups (e.g., children or the elderly) and transportation-related, public transportation, temperature-controlled, food preparation, and other facilities. Next, the importance of each facility type in terms of bacterial and fungal abundance was evaluated using the AHP method, according to criteria selected using the AHP method. The highest importance was assigned to multi-use facilities for air-pollution-sensitive groups, which were associated with higher user density and more confirmed cases of COVID-19; the second-highest importance was assigned to public-transportation facilities. Bacteria detected at representative multi-use facilities were identified using 16S rRNA sequencing and included the human pathogens *Bacillus anthracis*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Erwinia billingiae*, and *Enterobacter cloacae*. This study is the first to measure monthly and seasonal concentrations of bacteria and fungi at 30 multi-use facilities in Bucheon. The results of this study will be useful for designing systematic measures for the control of infectious bacteria and fungi in various types of multi-use facilities, according to their specific characteristics.

Keywords: 16S rRNA sequencing; analytic hierarchy process (AHP); bacteria; fungi; multi-use facilities

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1. Introduction

The recent sequential appearance of infectious diseases such as Middle East Respiratory Syndrome (MERS) (2015) and Coronavirus disease (COVID-19) (2019) has caused ongoing social and economic damage. Pathogens can spread rapidly in indoor multi-use facilities due to their confined space, high user density, and high user residence times [1–10]. In South Korea, the indoor air quality of high-traffic multi-use facilities (According to the Indoor Air Quality Management Act, this facility is used by unspecified people and includes underground stations, underground shopping malls, and medical institutions) is monitored 24 h per day by the South Korea Environment Corporation and Ministry of Environment. However, the automatic measurement network that provides this service monitors only particle matter (PM_{10}, $PM_{2.5}$), carbon dioxide, nitrogen dioxide, and other pollutants, whereas harmful microorganisms such as bacteria and fungi are not systematically evaluated.

Airborne bacteria comprise live and dead pathogenic and non-pathogenic bacteria. In indoor environments, airborne bacteria can cause infectious diseases, acute toxicity, allergies, and cancer, and are associated with a wide range of adverse health effects [1,3,4,11–15]. Additionally, the transmission of viral respiratory diseases in public facilities is a very important factor [16]. A Swedish study suggested that indoor air quality in school environments could affect morbidity related to asthma symptoms, particularly in terms of airborne bacteria levels [17]. In South Korea, multi-use facilities for pollutant-sensitive groups are required to maintain indoor airborne bacteria levels to less than 800 CFU/m^3, whereas equivalent facilities overseas have recommended maximum levels ranging from 500 to 20,000 CFU/m^3 [1,3].

Concentrations of airborne fungi and bacteria are correlated with the development and exacerbation of asthma [1,2,18,19]. In South Korea, climate change has increased the heat and humidity during the summer rainy seasons and prolonged winters, promoting mold growth [2]. The World Health Organization (WHO) recommends limiting mold concentrations to below 500–1000 CFU/m^3, whereas in South Korea, the recommended standard is below 500 CFU/m^3; however, few countries have regulated mold exposure [1,2,20] and species-centered management of airborne bacterial and fungal exposure remains rare worldwide because such analyses are time-consuming and costly [1,2].

Therefore, systematic analysis, diagnosis, and reduction measures for airborne bacteria and fungi in multi-use facilities are presently insufficient. In this study, we classified multi-use facilities in Bucheon, South Korea, into six categories: facilities for groups sensitive to pollution (e.g., children or the elderly) and transportation-related, public-transportation, temperature-controlled, food-preparation, and other facilities. Next, we analyzed the relative importance of each facility type according to airborne bacterial and fungal concentrations using a combination of 16S rRNA gene sequencing, DNA extraction, and the analytic hierarchy process (AHP). In particular, our study also differed from previous studies in that we used representative multi-use facilities in our analysis. If the previous study only targeted some facilities, this study established a method for surveying and ranking multi-use facilities that may be applied in future studies; this study is also the first to apply 16S rRNA sequencing to identify the main species of bacteria and human pathogens at targeted multi-use facilities. It will be necessary to provide solutions for each facility based on the results of the detection of pathogens harmful to the human body. The results of this study may be used to guide the design of measures to control airborne bacteria and fungi in various types of multi-use facilities to improve public safety.

2. Materials and Methods

2.1. Study Site and Experimental Period

This study included multi-use facilities throughout the Bucheon area, which had the highest rate of traffic congestion in South Korea in 2014 and a population of 15,575 people/km^2 in 2019, representing the highest population density in Gyeonggi Province, South Korea (Figure 1).

Multi-use facilities in Bucheon were surveyed from 15–29 July and then again from 11–18 August 2021. Following these surveys, we selected representative multi-use facilities, in which concentrations of bacteria and fungi were measured from November 2021 to April 2022 using the collision method, which is the main method used to test indoor air quality. The collision method assumes that microorganisms collide with the medium as indoor air is collected. Within each facility, a single site with high concentrations of bacteria and fungi was selected for regular swab analysis using a Pipette Swab Plus kit (3M, St. Paul, MN, USA). In the cotton swab sampling, the surface to be tested is set to 10 cm × 10 cm in the target site where the collision criminal is performed, and sampling is performed three times each. DNA was extracted from the samples using a SPINeasy DNA Kit for Soil (MP Biomedicals Korea, Seoul, Korea), and quantitative analysis and next-generation sequencing (NGS)-based metagenome analysis were performed to identify the bacteria present in the samples.

Figure 1. Study area and sampling locations.

2.2. Experimental Equipment and Mentods

Concentrations of bacteria and fungi were measured using a microbial sampler (MAS-1000 ECO, Taewon Shibata, Seoul, Korea). These measurements were conducted in accordance with the "Measuring Method of Airborne Mold in Indoor Air" (ES 02702.1a) and "Measuring Method of Total Airborne Bacteria in Indoor Air" (ES 02701.1c) indoor-air-quality test standards of the Ministry of the Environment, Korea. The sampling period was 6 months. The single-collection flow rate of the sampler was 100 L/min, once per month. The collection time was 1.5 min, the total collection capacity was 150 L, and the general sampling capacity was 50–200 L. Three samples were taken with a minimum interval of 20 min between samples. Three consecutive samples were collected per sampling location (Figure 2). Bacteria were cultured in TSA medium and Fungi in MEA medium. Following indoor air sampling, bacteria were incubated at 35 ± 1 °C for 48 h, and fungi were cultured at 25 ± 1 °C for 4–5 days. Concentrations are expressed in CFU/m^3.

Figure 2. Suspended microorganism collection and swab analysis.

Following the first survey, we categorized multi-use facilities according to 11 selection criteria, and collected data on 417 people involved with these facilities. Based on the data collected in the first survey, in a second survey, we collected data to evaluate the relative importance of each multi-use facility type in terms of airborne bacterial and fungal concentrations. The selection criteria included the number of confirmed cases of COVID-19, airtightness (i.e., qualitative degree of occlusion), user density, user residence time, fatality rate, overcrowding, airborne population, infection education and control measures, ventilation frequency, and user accessibility. The relative weights of these selection criteria were determined according to the geometric mean for each facility type, with a threshold consistency ratio of ≤ 0.1.

To evaluate the relative importance of each multi-use facility type, we selected a representative multi-use facility for each facility type in Bucheon, and concentrations of bacteria and fungi, as well as temperature and humidity, were measured using the collision method. At each representative facility, three different locations were selected as high-concentration points, and sampling, temperature and humidity were conducted three times in the morning and afternoon once per month. The measurement time in the morning and after was measured at a time when there was a lot of movement of people. A soil kit (SPINeasy DNA Kit for Soil (MP Biomedicals Korea, Seoul, Korea)) was used to extract DNA from swab samples and DNA extraction was conducted as follows. We added 100–500 mg of soil to a lysing matrix E tube, and then added 980 μL of S1 lysis buffer, 120 μL S2 lysis buffer, and 10 μL RNase and vortexed the sample. Next, the sample was homogenized in a fast-prep instrument for 20 s at 6.0 m/s and then centrifuged at 14,000× *g* for 5 min. The supernatant was transferred to a clean 2.0 mL microcentrifuge tube and 250 μL of inhibitor was added. The mixture was shaken 10 times and then centrifuged at 14,000× *g* for 10 min. A supernatant sample (900 μL) was transferred to a clean 2.0 μL microcentrifuge tube and 900 μL of binding solution was added. Next, we transferred 800 μL of the mixture to an S1 column placed on top of a 2.0 μL collection tube, which was centrifuged at 14,000× *g* for 1 min. This process was repeated once and the remaining mixture was discarded. Next, we added 500 μL of Wah buffer to the column and centrifuged it at 14,000× *g* for 1 min. The washing step was repeated, and then the empty column was centrifuged without adding liquid at 14,000× *g* for 2 min for drying. The collection tube was discarded and the column was placed in a 1.5 μL collection tube and air dried for 5 min at room temperature. Next, we heated deep eutectic solvent (DES) buffer to 55 °C using a water bath and then added 100 μL pre-heated DES buffer to the center of the column. The mixture was centrifuged at 14,000× *g* for 1 min to obtain eluted DNA, which was then stored at 20 °C until quantitative analysis.

For NGS-based metagenome analysis, we used polymerase chain reaction (PCR) to target the V3–V4 region of bacteria 16S rRNA. Bacterial concentrations were confirmed using a DNA analysis instrument (Quant-iT PicoGreen, Thermo Fisher Scientific Korea Ltd., Incheon, South Korea). The DNA was amplified by PCR and its presence or absence was verified through electrophoresis. The PCR conditions were as follows: 25 cycles of pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The primers 341F and 805R were used (forward primer: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCGTGG GCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′). Electrophoresis was performed using 10 μL of DNA and 2 μL of loading dye on 0.7% agarose gel. The PCR product was approximately 428 bp in size.

We commissioned 3BIGS (Seoul, South Korea) to perform NSG-based metagenomic analysis using an Illumina MiSeq system (Illumina, San Diego, CA, USA). MiSeq performs cluster generation, sequencing, and data analysis using a single instrument. Each sequence was analyzed from phylum to species using the Ribosomal Database Project pyrosequencing pipeline classifier.

3. Results and Discussion

3.1. Classification of Facilities Based on AHP and Derivation of Selection Criteria

Using the results of the first survey, we classified the multi-use facilities into six categories: facilities for pollution-sensitive groups (i.e., daycare and postpartum centers and facilities for the elderly, infirm, and disabled), transportation-related facilities (i.e., those with large population fluctuations, including underground stations, waiting rooms, passenger terminals, and underground shopping malls), public-transportation facilities (i.e., mobile facilities such as subways, trains, and express buses), temperature-controlled facilities (i.e., low-noise facilities including libraries, museums, art galleries, and exhibition halls), food-preparation facilities (i.e., large bulk stores, restaurants, cafes, funeral halls, movie theaters, and wedding halls), and other facilities (i.e., facilities with high temperature and humidity, including public baths, indoor parking lots, religious facilities, lodging facilities, indoor sports facilities, government offices, swimming pools, entertainment facilities, and call centers).

The 11 selection criteria used in our AHP analysis included the numbers of confirmed cases of COVID-19, airtightness (i.e., qualitative degree of occlusion), user density, user activity, user residence time, fatality rate, overcrowded residences, floating population, infection education and control measures, ventilation frequency, and accessibility [5–10,20–30]. The number of corona confirmed cases was only for the population residing in Bucheon, and it was included because it was necessary to review it as a factor in selecting multi-use facilities from a different perspective from other variables in relation to the corona issue. In addition, as the fatality rate included facilities used by the sensitive class such as medical institutions and nursing facilities, this item was reviewed. The characteristics of each facility are reflected as a whole.

Based on the results of the first survey, in the second survey, we determined the relative importance of each of the six multi-use facility types according to the top six selection criteria: the number of confirmed cases of COVID-19, airtightness, user density, user residence time, fatality rate, and ventilation frequency. The importance of the multi-use facility types was ranked in descending order as follows: facilities for pollution-sensitive groups (0.2094), public-transportation facilities (0.1788), food-preparation facilities (0.1505), other facilities (0.1343), transportation-related facilities (0.1167), and temperature-controlled facilities (0.0994) (Table 1). After ranking the multi-use facility types by importance, we selected representative facilities as target sites for long-term airborne bacterial and fungal concentration measurements, prioritizing those with high user density and numbers of confirmed COVID-19 cases.

Table 1. Multi-use-facility importance rankings based on six selected evaluation criteria.

Facility Type	Weight	Ranking	Evaluation Criteria	Weight	Final Weight	Ranking
Facility for pollutant-sensitive users	0.2094	1	Number of confirmed COVID-19 cases	0.1341	0.028	3
			Airtightness	0.1491	0.017	5
			User density	0.1645	0.029	1
			User residence time	0.1191	0.012	6
			Fatality rate	0.1939	0.029	2
			Ventilation frequency	0.1390	0.019	4
Food-preparation facility	0.1505	3	Number of confirmed COVID-19 cases	0.1373	0.029	2
			Airtightness	0.1639	0.019	5
			User density	0.1728	0.031	1
			User residence time	0.1368	0.014	6
			Fatality rate	0.1512	0.023	3
			Ventilation frequency	0.1469	0.020	4

Table 1. *Cont.*

Facility Type	Weight	Ranking	Evaluation Criteria	Weight	Final Weight	Ranking
Public transportation-related facility	0.1788	2	Number of confirmed COVID-19 cases	0.1293	0.027	2
			Airtightness	0.1586	0.019	5
			User density	0.1776	0.032	1
			User residence time	0.1301	0.013	6
			Fatality rate	0.1658	0.025	3
			Ventilation frequency	0.1498	0.020	4
Temperature-controlled facility	0.0994	6	Number of confirmed COVID-19 cases	0.1276	0.027	2
			Airtightness	0.1668	0.019	4
			User density	0.1722	0.031	1
			User residence time	0.1311	0.013	6
			Fatality rate	0.1639	0.025	3
			Ventilation frequency	0.1430	0.019	5
Transportation-related facility	0.1167	5	Number of confirmed COVID-19 cases	0.1269	0.027	2
			Airtightness	0.1558	0.018	5
			User density	0.1771	0.032	1
			User residence time	0.1263	0.013	6
			Fatality rate	0.1645	0.025	3
			Ventilation frequency	0.1427	0.019	4
Other	0.1343	4	Number of confirmed COVID-19 cases	0.1267	0.027	2
			Airtightness	0.1678	0.020	5
			User density	0.1732	0.031	1
			User residence time	0.1296	0.013	6
			Fatality rate	0.1630	0.025	3
			Ventilation frequency	0.1465	0.020	4

3.2. Verification of Airborne Bacterial and Fungal Concentration Measurements and NGS Analysis

3.2.1. Concentrations of Airborne Bacteria and Fungi

In this study, we calculated the monthly arithmetic mean of total airborne bacteria and fungi in 30 multi-use facilities, and found that their concentration levels rarely exceeded 800 CFU/m^3, which is the maintenance standard for total airborne bacteria, and 500 CFU/m^3, the recommended standard for mold, respectively. These results may reflect that our sampling occurred during winter and spring, when bacterial and fungal concentrations are lowest. Previous studies reported average bacterial and fungal concentrations higher than the recommended and maintenance standards during summer [5–10,20–24]; therefore, continuous monitoring should be performed in future studies.

Total airborne bacterial concentrations were high overall in November 2021, except in food-preparation and temperature-controlled facilities. Total airborne bacterial concentrations were particularly high in “other” facilities such as Bucheon City Hall. In December 2021, total airborne bacterial concentrations were high in facilities for pollution-sensitive groups such as daycare centers, postpartum-care centers, and nursing homes. In January 2022, total airborne bacterial concentrations remained high in nursing homes and daycare centers, and other facilities such as public parking lots and vehicle registration departments had higher total airborne bacterial concentrations than the remaining “other” facilities. In February 2022, total airborne bacterial concentrations were high in daycare centers, public parking lots, vehicle registration departments, and at Bucheon City Hall. In March 2022, the Bucheon Christian Youth Foundation had the highest total concentration of airborne bacteria, and facilities for pollution-sensitive groups such as daycare centers had higher total airborne bacterial concentrations than did other facilities. In April 2022, total airborne bacterial concentrations were high in temperature-controlled facilities such as Wonmi Library and in transportation-related facilities such as Bucheon City Hall Station, Sangdong Station, and Sinjindong Station. Some additional facilities were sampled in March 2022 (Table 2).

In November 2021, total airborne fungal concentrations were high in the 365 Safety Education Center and other facilities such as the Bucheon City Hall parking lot. Airborne fungal concentrations were strongly correlated with humidity. In December 2021, total airborne fungal concentrations remained high in the 365 Safety Education Center and Bucheon City Hall parking lot, as well as public parking lots, postpartum care centers, daycare centers, and other facilities for pollution-sensitive groups. In temperature-controlled facilities such as museums and libraries, and in food-preparation facilities such as CGV Excursion facilities, total airborne fungal concentrations were low. In January 2022, total airborne fungal concentrations were high in facilities such as the 365 Safety Education Center, public parking lots, and facilities for pollution-sensitive groups such as postpartum care centers. Total airborne fungal concentrations were low in cooking facilities such as CGV Excursions and in temperature-controlled facilities such as museums. In February 2022, total airborne fungal concentrations were high in Bucheon City Hall and low in the 365 Education Center, Bucheon Gymnasium, and Bucheon Museum. From March 2022, the sampling area was expanded, and total airborne fungal concentrations were high in facilities such as the Bucheon Civic Center, where the recommended standards were exceeded, and in facilities such as the 365 Safety Education Center, Bucheon City Museum, and Sangdong Library, and temperature-controlled facilities, total airborne fungal concentrations were low. In April 2022, total airborne fungal concentrations were high in facilities such as Bucheon City Hall, Sinjung-dong Station, temperature-controlled facilities such as Wonmi Library, and transportation-related facilities (Table 3).

Based on the measured bacterial and fungal concentrations, we ranked the importance of each multi-use facility type using the AHP technique. Facilities for pollution-sensitive groups were found to have the highest importance in terms of airborne bacterial concentrations, whereas “other” facilities had the highest importance in terms of airborne fungal concentrations (Table 4).

Among samples collected in winter (November 2021 to February 2022), total airborne bacterial concentrations were high in vehicle registration departments, Bucheon City Hall, and daycare centers, postpartum care centers, and other facilities for pollution-sensitive groups; total airborne fungal concentrations were high in “other” facilities such as the 365 Safety Education Center and Bucheon City Hall parking lot. Total airborne bacterial and fungal concentrations were low in temperature-controlled facilities such as libraries and in food-preparation facilities such as CGV Excursion (Figure 3). Among samples collected in spring (March to April 2022), total airborne bacterial concentrations were high in libraries, daycare centers, and subway stations, whereas total airborne fungal concentrations were high in libraries, parking lots, civic centers, subway stations, and various transportation-related facilities. Total airborne bacterial and fungal concentrations were low in museums and the 365 Safety Education Center. Thus, total airborne bacterial and fungal levels showed seasonal variation, with higher concentrations in spring than in winter (Figure 4).

Table 2. Mean and standard deviation (SD) of total airborne bacterial concentrations among various multi-use facilities by type.

Facility Type	Facility	November 2021	December 2021	January 2022	February 2022	March 2022	April 2022	Mean Total Airborne Bacteria (CFU/m^3)	SD
Facility for pollutant-sensitive users	Bucheon City Hall Daycare Center	96.5	64.8	60.2	64.5	177.5	254.3	119.6	72.6
	Bucheon Municipal Specialized Nursing Home for the Elderly	104.0	81.3	119.0	32.5	–	117.8	90.9	32.2
	Labor Welfare Corporation Bucheon Daycare Center	104.5	178.2	92.8	203.8	–	127.0	141.2	42.8
	Loen Postpartum Care Center	106.0	94.0	–	–	–	–	100.0	6.0
	Samsung Future Postpartum Care Center	92.8	46.5	40.3	27.8	37.8	59.0	50.7	21.1
Food-preparation facility	CGV Excursion	26.3	72.0	11.0	14.0	14.3	30.0	27.9	20.9
Temperature-controlled facility	Bucheon City Museum	67.0	22.2	11.8	17.0	14.8	21.3	25.7	18.8
	Sangdong Library	32.5	23.7	18.5	27.5	28.8	52.5	30.6	10.7
	Wonmi Library	71.5	20.8	21.5	31.0	40.3	437.0	103.7	150.0
Transportation related facility	Bucheon City Hall Station	–	–	–	–	54.0	366.0	210.0	156.0
	Sangdong Station	–	–	–	–	58.3	299.8	179.0	120.8
	Sinjung-dong Station	–	–	–	–	69.8	348.8	209.3	139.5
	Sosaeul Station	–	–	–	–	31.3	214.0	122.6	91.4

Table 2. *Cont.*

Facility Type	Facility	November 2021	December 2021	January 2022	February 2022	March 2022	April 2022	Mean Total Airborne Bacteria (CFU/m^3)	SD
Other	365 Safety Training Center	132.8	11.0	29.3	10.3	12.8	16.0	35.4	44.1
	Bucheon Christian Youth Association Foundation	–	–	–	–	288.0	–	288.0	0.0
	Bucheon City Hall	115.2	57.8	17.0	154.7	41.8	106.5	82.2	47.3
	Bucheon City Hall Parking Lot	88.1	50.1	23.5	139.8	42.3	107.5	75.2	40.3
	Bucheon Civic Center	10.7	11.5	9.2	7.3	39.8	24.5	17.2	11.5
	Bucheon Gymnasium	123.2	14.2	23.3	21.3	58.8	160.3	66.8	55.8
	Cocoming Kids Land	–	–	–	–	–	26.0	26.0	0.0
	Goriul Cave Market Public Parking Lot	38.3	23.2	54.0	42.0	83.5	46.3	47.9	18.5
	Harang Silver Village	–	–	–	–	10.8	–	10.8	0.0
	Mega Plus	–	–	–	–	77.0	–	77.0	0.0
	Starlight Park Public Parking Lot	24.7	27.8	50.2	26.3	15.3	66.5	35.1	17.5
	Middle East Love Market Public Parking Lot	25.0	26.8	56.8	15.8	45.3	110.0	46.6	31.5
	Okgildong Study Capsule Reading Room	–	–	–	–	114.0	–	114.0	0.0
	R2G CrossFit	–	–	–	–	19.0	–	19.0	0.0
	Top Nonsul Korean Language School	–	–	–	–	19.0	–	19.0	0.0
	Vehicle Registration Division	115.8	83.7	68.7	156.8	116.0	177.8	119.8	38.0
	We've the State	–	–	–	–	27.6	–	27.6	0.0

Table 3. Mean and standard deviation (SD) of total airborne fungal concentrations among various multi-use facilities by type.

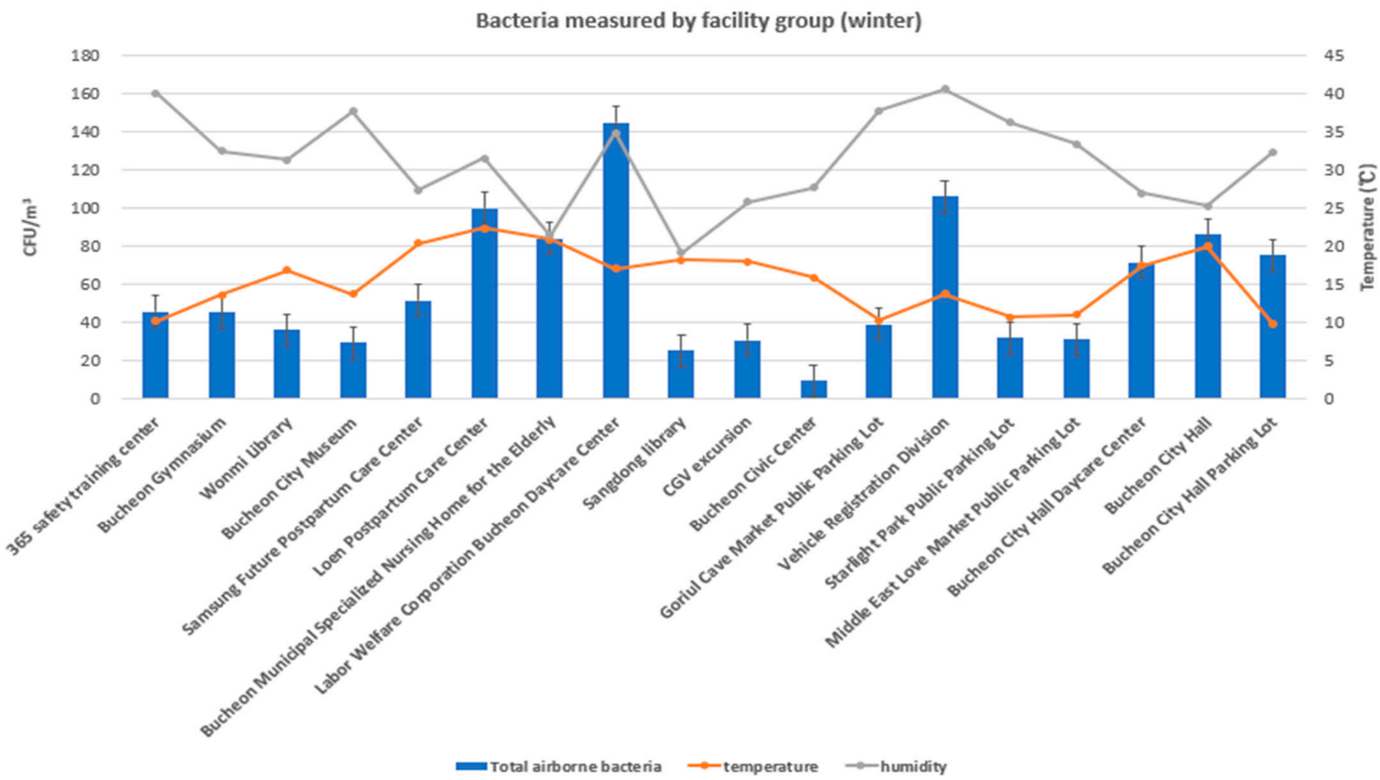
Facility Type	Facility	November 2021	December 2021	January 2022	February 2022	March 2022	April 2022	Mean Fungus Concentration (CFU/m^3)	SD
Facility for pollutant-sensitive users	Bucheon City Hall Daycare Center	110.0	157.3	67.3	69.3	101.8	82.0	97.9	30.8
	Bucheon Municipal Specialized Nursing Home for the Elderly	156.8	71.0	60.5	17.8	–	228.3	106.9	75.6
	Labor Welfare Corporation Bucheon Daycare Center	134.0	151.0	101.8	77.3	–	145.5	121.9	28.1
	Loen Postpartum Care Center	54.0	180.7	–	–	–	–	117.3	63.3
	Samsung Future Postpartum Care Center	167.8	49.2	149.8	43.8	61.8	147.5	103.3	52.4
Food-preparation facility	CGV Excursion	48.3	44.5	23.2	41.8	151.8	115.3	70.8	46.3
Temperature-controlled facility	Bucheon City Museum	126.5	28.7	36.3	14.5	21.8	30.0	43.0	38.0
	Sangdong Library	87.2	123.5	63.2	29.8	30.8	120.5	75.8	38.1
	Wonmi Library	112.5	34.3	56.8	43.5	135.5	466.5	141.5	149.9
Transportation-related facility	Bucheon City Hall Station	–	–	–	–	69.3	374.0	221.6	152.4
	Sangdong Station	–	–	–	–	39.0	322.3	180.6	141.6
	Sinjung-dong Station	–	–	–	–	73.8	490.8	282.3	208.5
	Sosaeul Station	–	–	–	–	107.8	326.0	216.9	109.1

Table 3. *Cont.*

Facility Type	Facility	November 2021	December 2021	January 2022	February 2022	March 2022	April 2022	Mean Fungus Concentration (CFU/m^3)	SD
Other	365 Safety Training Center	348.0	261.8	166.3	16.5	21.5	39.8	142.3	127.9
	Bucheon Christian Youth Association Foundation	–	–	–	–	113.0	–	113.0	0.0
	Bucheon City Hall	118.3	102.5	43.8	155.9	173.2	257.8	141.9	66.3
	Bucheon City Hall Parking Lot	310.6	202.3	67.3	40.5	92.8	264.8	163.0	102.3
	Bucheon Civic Center	66.7	35.0	16.8	20.5	546.5	85.8	128.5	188.5
	Bucheon Gymnasium	129.0	47.0	99.0	11.7	125.0	118.8	88.4	44.0
	Cocoming Kids Land	–	–	–	–	–	57.0	57.0	0.0
	Goriul Cave Market Public Parking Lot	111.2	79.5	96.5	62.0	129.5	328.5	134.5	89.4
	Harang Silver Village	–	–	–	–	35.0	–	35.0	0.0
	Mega Plus	–	–	–	–	123.0	–	123.0	0.0
	Middle East Love Market Public Parking Lot	94.3	171.0	171.0	68.0	45.0	176.3	120.9	53.8
	Okgildong Study Capsule Reading Room	–	–	–	–	202.0	–	202.0	0.0
	R2G CrossFit	–	–	–	–	34.0	–	34.0	0.0
	Starlight Park Public Parking Lot	219.5	109.5	109.0	81.5	91.8	215.3	137.8	57.1
	Top Nonsul Korean Language School	–	–	–	–	26.0	–	26.0	0.0
	Vehicle Registration Division	79.5	80.7	110.5	39.5	93.0	298.3	116.9	83.9
	We've the State	–	–	–	–	103.6	–	103.6	0.0

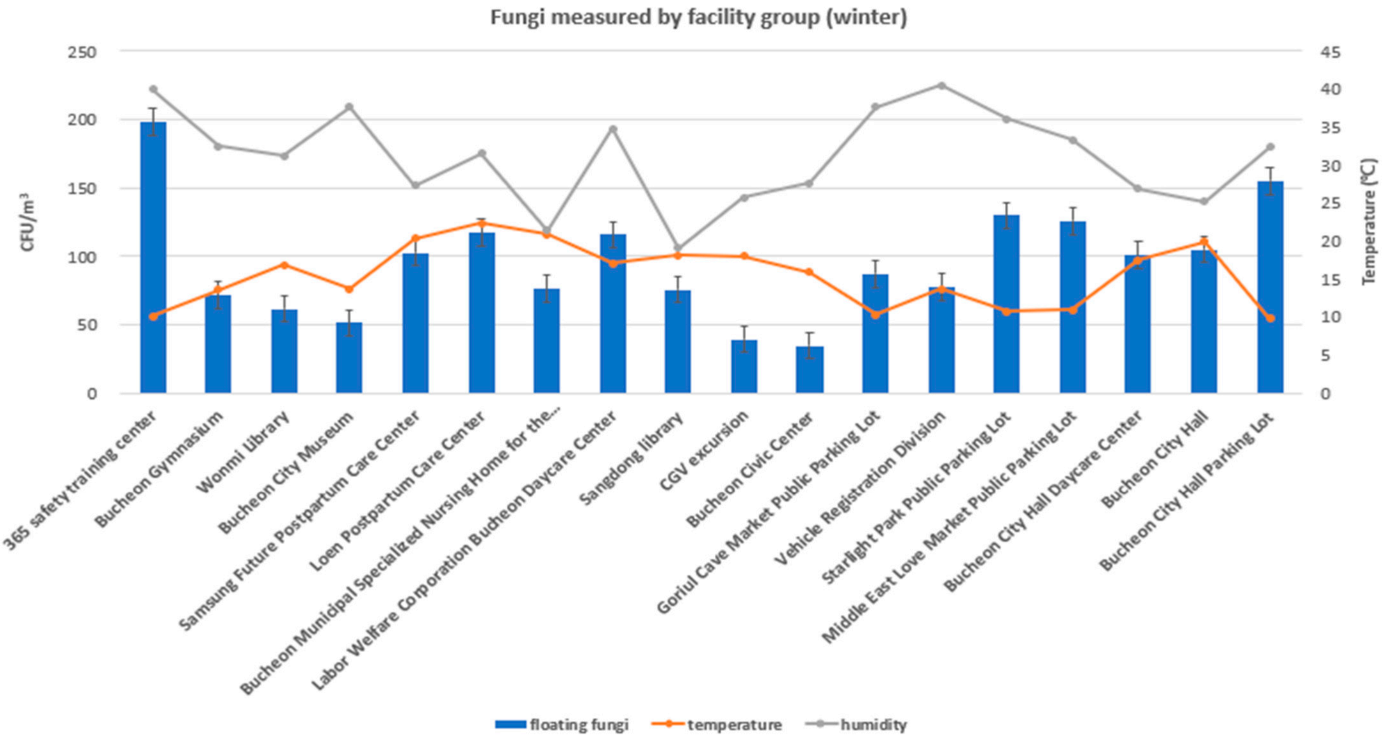
Table 4. Multi-use-facility importance rankings according to bacterial and fungal concentrations.

Facility Type	Mean Bacterial Concentration (CFU/m^3)	Ranking	Mean Fungal Concentration (CFU/m^3)	Ranking
Facilities for pollutant-sensitive users	100.5 ± 34.9	1	109.5 ± 50.1	2
Food-preparation facilities	27.9 ± 20.9	5	70.8 ± 46.3	5
Temperature-controlled facilities	53.3 ± 59.9	4	86.8 ± 75.3	4
Transportation-related facilities	180.2 ± 126.9	3	225.3 ± 152.9	3
Other facilities	67.6 ± 19.0	2	113.2 ± 47.8	1



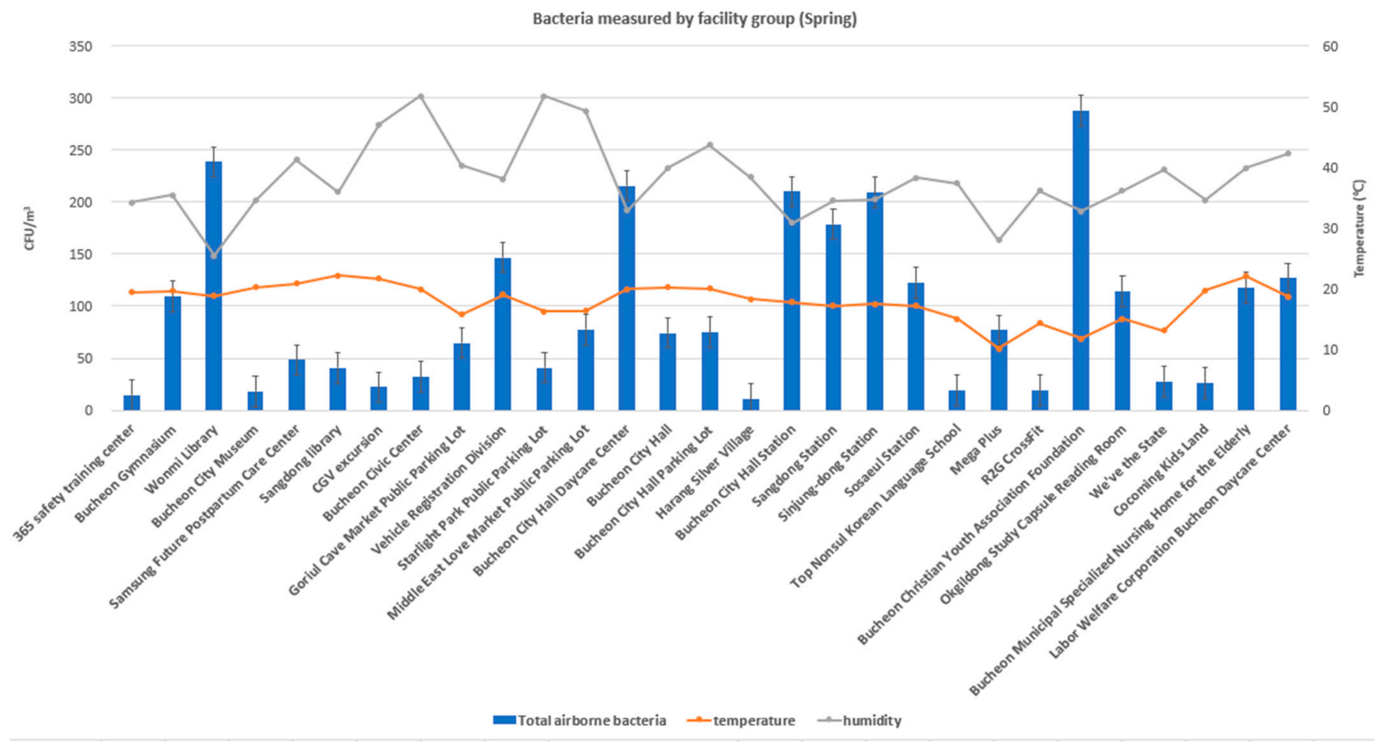
(**a**)

Figure 3. *Cont.*



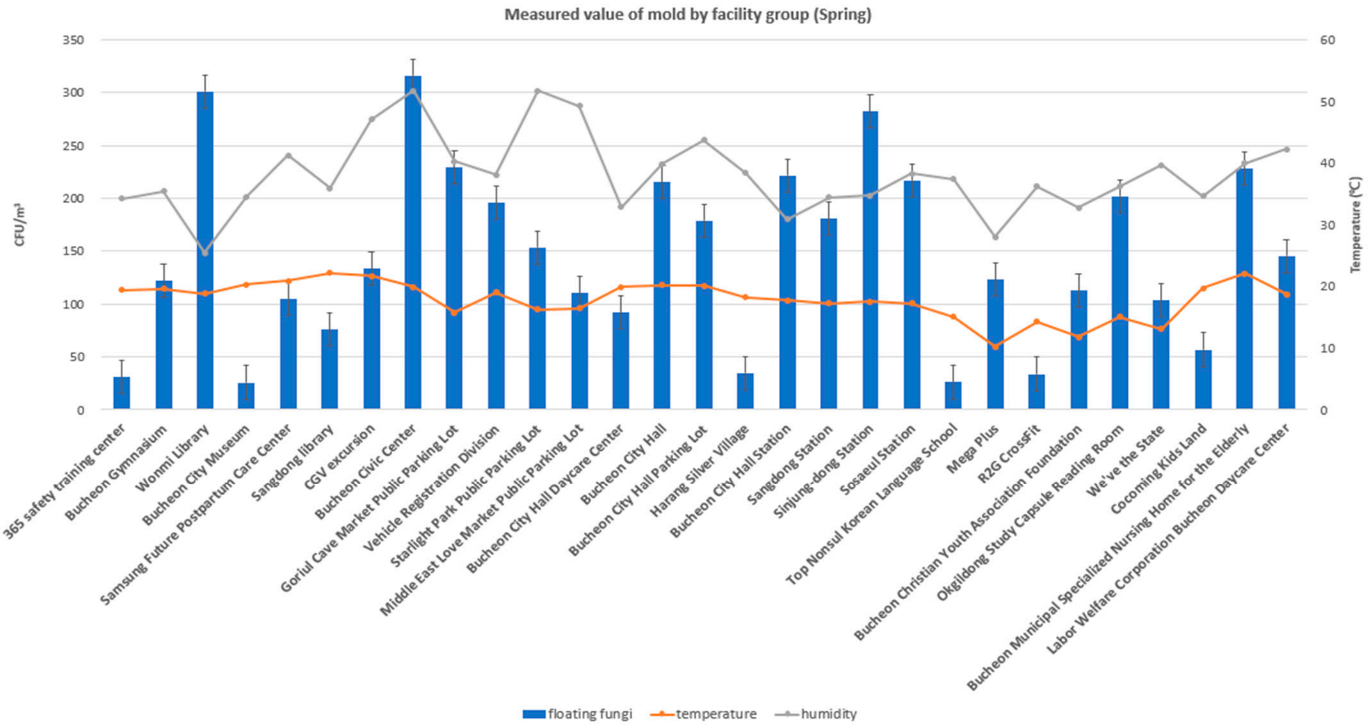
(**b**)

Figure 3. Fluctuation in average winter concentrations of (**a**) bacteria and (**b**) fungi in multi-use facilities with temperature and humidity.



(**a**)

Figure 4. *Cont.*



(**b**)

Figure 4. Fluctuation in average spring concentrations of (**a**) bacteria and (**b**) fungi in multi-use facilities with temperature and humidity.

3.2.2. DNA Extraction and NGS Analysis Results

Based on these results, we selected one or more facilities with airborne bacterial and fungal concentrations within each facility type, and performed cluster analysis for each facility type. Postpartum care centers and daycare centers were selected as representative facilities for pollution-sensitive groups, and libraries and museums were selected as representative temperature-controlled facilities. CGV Excursion was selected as a representative food-preparation facility, the 365 Safety Education Center and Bucheon City Hall vehicle registration department were selected as representative "other" facilities, and Sinjung-dong subway station was selected as a representative transportation facility.

Base-sequence analysis was conducted to identify the most abundant bacteria sampled in representative multi-use facilities to the species level (Figure 5). Among facilities for pollution-sensitive groups, *Acinetobacter lwoffii* (18.32%), *Pseudomonas oryzihabitans* (15.10%), *Sphingomonas jeddahensis* (13.94%), and *Bacillus licheniformis* (10.38%) were detected in daycare centers, as well as low concentrations of the human pathogens *Staphylococcus saprophyticus* (0.06%) and *Bacillus anthracis* (0.01%). A large proportion of the bacteria detected in prenatal care centers consisted of *Acinetobacter pittii* (53.60%), with lower amounts of the human pathogens *Bacillus cereus* (3.71%) and *Bacillus anthracis* (0.99%). In libraries, *Pseudomonas putida* (10.66%) and *Pseudomonas silesiensis* (10.02%) were mainly detected, as well as the human pathogens *Bacillus anthracis* (0.66%), *Bacillus cereus* (0.60%), *Pseudomonas fluorescens* (0.57%), *Erwinia billingiae* (0.39%), and *Enterobacter cloacae* (0.01%). In museums, *Pseudomonas cedrina* (25.23%) and *Lysinibacillus sphaericus* (18.88%) were mainly detected, as well as the human pathogens *Bacillus cereus* (3.99%), *Pseudomonas fluorescens* (2.62%), and *Bacillus anthracis* (1.20%). The food-preparation facility had a high percentage of *Lysinibacillus sphaericus* (36.26%), as well as the human pathogens *Bacillus cereus* (9.13%) and *Bacillus anthracis* (2.89%). In Sinjung-dong subway station, *Tissierella creationophila* (14.00%), *Achromobacter spanius* (11.46%) and *Pseudogracilibacillus auburnensis* (10.41%) were mainly detected, and no human pathogens were found. In the 365 Safety Training Center, the main bacteria detected were *Clostridium subterminale* (15.55%), *Lysinibacillus telephonicus*

(10.53%), and *Erwinia aphidicola* (10.24%), as well as the human pathogens *Bacillus cereus* (7.38%, which was particularly high) and *Bacillus anthracis* (2.22%). In Bucheon City Hall, *Lysinibacillus sphaericus* (17.24%) and *Tissierella praeacuta* (10.21%) were mainly detected, as well as the human pathogens *Bacillus cereus* (7.38%) and *Bacillus anthracis* (2.22%). In particular, as *Bacillus anthracis*, a major cause of bioterrorism in the United States in 2001 and a very dangerous pathogen to the human body, was found in most facilities, it is judged that early management and solution provision are necessary.

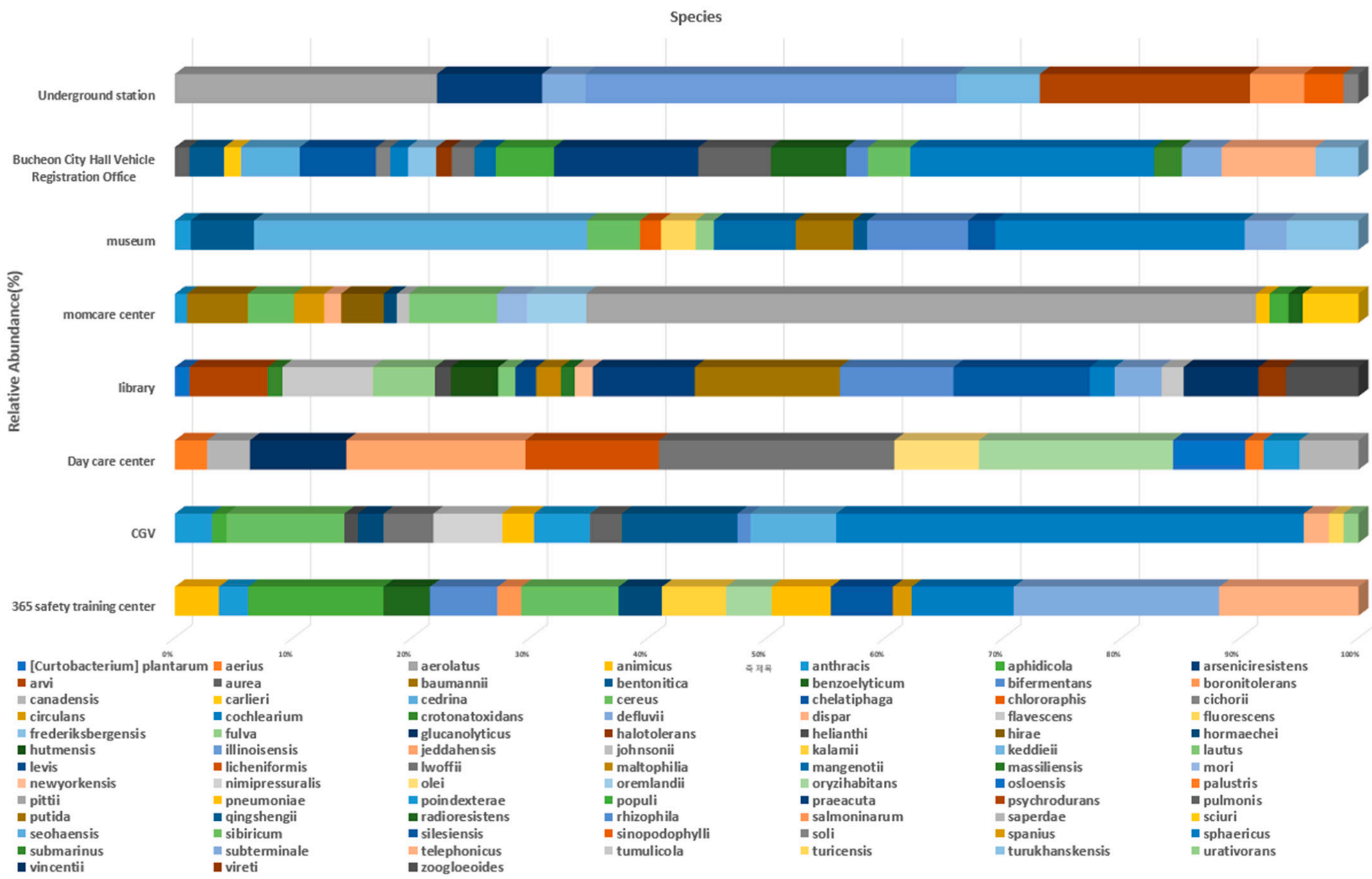


Figure 5. Relative abundances of bacterial species in representative multi-use facilities.

Although other studies [1] did not look at each facility separately, in the case of examining some transportation facilities and facilities used by the sensitive class, *Morganella morganii*, *Acinetovacter guillouiae*, and *Arthrobacter psychrolactophilus* were found to be high in total airborne bacteria at the species level. Additionally, as for the floating mold, *Malassezia restricta*, *Aspergillus penxillioides*, and *Ustilaginidea virens* were mainly found at the species level. As a result of reviewing each facility group in this study, it can be seen that the major types appearing at the species level are different.

The results of this study will contribute to the development of bacterial control measures for each type of multi-use facility. The institution can install and apply a constant monitoring device that measures bacteria and fungi in units of one minute at the point to check the concentration at all times and develop and apply a reduction solution such as air purifier technology, antibacterial film, and hand sanitizer system suitable for the characteristics of each facility.

4. Conclusions

In this study, based on criteria used in the Indoor Air Quality Management Act, we classified multi-use facilities in Bucheon, South Korea into six categories: facilities for pollution-sensitive groups, transportation-related facilities, public transportation facilities, temperature-controlled facilities, food-preparation facilities, and other facilities. We applied the AHP method to the categorized facilities to determine the relative importance of each facility type, as well as the relative importance of the selection criteria used in the former analysis. The results showed that facilities for pollution-sensitive groups were ranked first, followed by public transportation facilities, food-preparation facilities, "other" facilities, transportation-related facilities, and temperature-controlled facilities. The importance of multi-use facilities was found to be influenced mainly by user density and the number of confirmed COVID-19 cases.

Total airborne bacterial and fungal concentrations were measured over a six-month period during winter and spring, when these concentrations are lowest. Previous studies have shown that airborne bacterial and fungal concentrations can exceed recommended limits during summer, whereas we found that recommended limits were not exceeded. Our study also differed from previous studies in that we used representative multi-use facilities in our analysis; therefore, it is difficult to extrapolate our results to wider areas. However, this study established a method for surveying and ranking multi-use facilities that may be applied in future studies; this study is also the first to apply 16S rRNA sequencing to identify the main species of bacteria and human pathogens at target multi-use facilities.

Our AHP results showed that facilities for pollution-sensitive groups were the most important among the six multi-use facility types; based on on-site measurements, these facilities had the highest concentrations of total airborne bacteria and the second highest concentrations of total airborne fungi. The "other" facility type was ranked second and first for total airborne bacteria and fungi, respectively. These results were heavily influenced by user density and the number of confirmed COVID-19 cases; therefore, facilities for pollution-sensitive groups, "other" facilities, and transportation-related facilities require continuous management of harmful microorganisms.

Our 16S rRNA gene-sequencing results identified various bacterial species including human pathogens in each representative multi-use facility. Current indoor air-quality management guidelines recommend limits of 800 CFU/m^3 for bacteria and 500 CFU/m^3 for bacteria and fungi. However, the results of this study indicate that real-time monitoring and library construction of bacterial species is warranted, despite the associated costs. In particular, guidelines for the management of each multi-use facility type should be developed based on the human pathogens detected in this study.

As a limitation of this study, first, there may be differences in species between sampling results by the collision method and 16S RNA analysis. In the culture by collision method, only microorganisms that can be cultured are detected, whereas 16S RNA includes all species. Second, this study has a limitation in that it collects bio-aerosols at specific locations through surface swab analysis. Third, it is necessary to review the funnel route in the species-level analysis derived from this study, and in the future, it will be necessary to review the correlation by constructing continuous data.

In future studies, we plan to expand our sampling strategy to include all multi-use facilities, and to construct a platform that can continuously monitor changes in the concentrations of harmful bacterial species.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Ministry of Environment Republic of Korea. *Research on Advanced Management of Airborne Microorganisms in Multi-Use Facilities*; Ministry of Environment Republic of Korea: Sejong, Korea, 2021.
2. Ministry of Environment Republic of Korea. *Indoor Fungi Identification Analysis and Concentration Research Study*; Ministry of Environment Republic of Korea: Sejong, Korea, 2015.
3. Ministry of Environment Republic of Korea. *Total Airborne Bacteria Standard Rationalization Study*; Ministry of Environment Republic of Korea: Sejong, Korea, 2018.
4. Ministry of Environment Republic of Korea. *Study on Rationalization of Total Suspended Bacteria*; Ministry of Environment Republic of Korea: Sejong, Korea, 2018.
5. Park, S.Y.; Kim, Y.M.; Yi, S.; Lee, S.; Na, B.J.; Kim, C.B.; Kim, J.I.; Kim, H.S.; Kim, Y.B.; Pa, Y.; et al. Coronavirus disease outbreak in call center, South Korea. *Emerg. Infect. Dis.* **2020**, *26*, 1666–1670. [CrossRef] [PubMed]
6. Olsen, J.; Chang, H.L.; Cheung, T.Y.Y.; Tang, A.F.Y.; Fisk, T.; Ooi, S.P.L.; Kuo, H.W.; Jiang, D.D.S.; Chen, K.T.; Lando, J.; et al. Transmission of the severe acute respiratory syndrome on aircraft. *N. Engl. J. Med.* **2003**, *349*, 2416–2422. [CrossRef] [PubMed]
7. COVID-19 National Emergency Response Center, Epidemiology and Case Management Team, Korea Centers for Disease Control and Prevention. Early epidemiological and clinical characteristics of 28 cases of coronavirus disease in South Korea. *Osong Public Health Res. Perspect.* **2020**, *11*, 8–14. [CrossRef] [PubMed]
8. McDonald, L.; Simor, A.E.; Su, I.J.; Maloney, S.; Ofner, M.; Chen, K.T.; Lando, J.F.; McGeer, A.; Lee, M.L.; Jernigan, D.B. SARS in healthcare facilities, Toronto and Taiwan. *Emerg. Infect. Dis.* **2004**, *10*, 777–781. [CrossRef] [PubMed]
9. Song, J.W. Characteristics of Frequent Users in an Emergency Department. Master's Thesis, Sungkyunkwan University, Seoul, Korea, 2010.
10. Hamner, L.; Dubbel, P.; Capron, I.; Ross, A.; Jordan, A.; Lee, J.; Lynn, J.; Ball, A.; Narwal, S.; Russell, S.; et al. High SARS-CoV-2 Attack Rate Following Exposure at a Choir Practice: Skagit County, Washington, March 2020. *Morb. Mortal. Wkly. Rep.* **2020**, *69*, 606–610. [CrossRef] [PubMed]
11. Hwang, S.H.; Seo, S.C.; Yoo, Y.; Kim, K.Y.; Choung, J.T.; Park, W.M. Indoor air quality of daycare centers in Seoul, Korea. *Build. Environ.* **2017**, *124*, 186–193. [CrossRef]
12. Jeon, B.H.; Hwang, I.Y. Concentrations of total culturable microorganisms and their identification in public facilities. *J. Korean Soc. Ind. Acad. Technol.* **2015**, *16*, 868–876.
13. Lee, C.M. Notes on airborne bacteria concentration standards in multi-use facilities. *Air Clean. Technol.* **2019**, *32*, 30–37.
14. Kim, K.Y.; Ryo, Y.M.; Kim, Y.S.; Lee, C.M.; Sim, I.S. Profile of airborne microorganisms distributed in general offices. *J. Korean Soc. Ind. Environ. Hyg.* **2008**, *18*, 11–19.
15. Kim, K.Y.; Park, J.B.; Kim, C.N.; Lee, K.J. Distribution of airborne fungi, particulate matter and carbon dioxide in a Seoul metropolitan subway station. *J. Prev. Med. Public Health* **2006**, *39*, 325–330. [PubMed]
16. Kim, M.K.; Lee, Y.L.; Park, D.S. Analysis of the airflow generated by human activity using a mobile slipstream measuring device. *Environments* **2021**, *8*, 97. [CrossRef]
17. Norbäck, D.; Wålinder, R.; Wieslander, G.; Smedje, G.; Erwal, C.; Venge, P. Indoor air pollutants in schools: Nasal patency and biomarkers in nasal lavage. *Allergy* **2010**, *55*, 163–170. [CrossRef] [PubMed]
18. United States Institute of Medicine Committee on Damp Indoor Spaces and Health. *Damp Indoor Spaces and Health*; National Academy Press: Washington, DC, USA, 2005.
19. American Conference of Governmental Industrial Hygienists. *Bioaerosol Committee Guidelines for the Assessment of Bioaerosols in the Indoor Environment*; American Conference of Governmental Industrial Hygienists: Cincinnati, OH, USA, 1989.
20. World Health Organisation. *Interventions and Actions Against Damp and Mould: Report on a WHO Working Group Meeting*; World Health Organisation: Geneva, Switzerland, 2008.
21. NYU Furman Center. COVID-19 Cases in New York City, a Neighborhood-Level Analysis. New York University Furman Center. 2020. Available online: https://furmancenter.org/thestoop/entry/covid-19-cases-in-new-york-city-a-neighborhood-level-analysis (accessed on 26 August 2022).
22. Chen, Z.; Zhang, W.; Lu, Y.; Guo, C.; Guo, Z.; Liao, C.; Zhang, X.; Zhang, Y.; Han, X.; Li, Q.; et al. From SARS-CoV to Wuhan 2019-nCoV outbreak: Similarity of early epidemic and prediction of future trends. *Cell Host Microbe.* **2020**, *133*, 1112–1114. [CrossRef]
23. American Institute of Architects. *Guidelines for Design and Construction of Health Care Facilities*; American Institute of Architects: Washington, DC, USA, 2006.
24. Dan Koenigshofer, P.E.; Murphy, J.; Walter Grondzik, P.E. *HAVC Design Manual for Hospitals and Clinics*, 2nd ed.; ASHRAE: Atlanta, GA, USA, 2013.
25. Kang, J.H. The effect of infection education on the practice of infection control: Moderating effects of infection control monitoring. *Korean Public Manag. Rev.* **2020**, *34*, 301–321.
26. Wen, Y.; Leng, J.; Shen, X.; Han, G.; Sun, L.; Fei, Y. Environmental and health effects of ventilation in subway stations: A literature review. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1084. [CrossRef] [PubMed]
27. Shashank, G.; Mathis, H.; Jenni, L.; Xuanji, L.; Martin, S.; Jakob, R.; Urvish, T.; Morten, A.; Jakob, S.; Hans, B.; et al. Environmental shaping of the bacterial and fungal community in infact bed dust and correlations with the airway microbiota. *Microbiome* **2020**, *8*, 115. [CrossRef]

28. Mendell, M.J.; Macher, J.M.; Kumagai, K. Measured moisture in buildings and adverse health effects: A review. *Indoor Air* **2018**, *28*, 488–499. [CrossRef] [PubMed]
29. Coleman, K.K.; Nguyen, T.T.; Yadana, S.; Hansen-Estruch, C.; Lindsley, W.G.; Gray, G.C. Bioaerosol Sampling for Respiratory Viruses in Singapore's Mass Rapid Transit Network. *Sci. Rep.* **2018**, *8*, 17476. [CrossRef] [PubMed]
30. Gohli, J.; Bøifot, K.O.; Moen, L.V.; Pastuszek, P.; Skogan, G.; Udekwu, K.I.; Dybwad, M. The Subway Microbiome: Seasonal Dynamics and Direct Comparison of Air and Surface Bacterial Communities. *Microbiome* **2019**, *7*, 160. [CrossRef] [PubMed]