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ORIGINAL ARTICLE

Accessing of Viable Bacteria Captured by Antimicrobial Filters in a Metropolitan Subway of South Korea

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Abstract

In subway stations, where passengers are crowed in enclosed spaces with restricted ventilation, airborne microorganisms have been detected, potentially contributing to the spread of infectious aerosols. In South Korea's metropolitan subways, air purifiers are installed on platforms to reduce particulate matter. Efficiency particulate air (EPA) filters in air purifiers lack disinfection capabilities and can serve as a source of airborne pathogens during filter replacement. In this field study, antimicrobial filters were applied to air purifiers installed on a subway platform to assess their ability to reduce the captured microorganisms. After 1 week of operation, the microbiomes were collected from both the control and test filters, followed by microbial identification. Additionally, the composition of metal elements was analyzed using particulate matter collected by the EPA filters. While 19 types of bacterial species were detected in control filters, the antimicrobial filters showed the presence of 15 bacterial species, with overall 64.71% of antibacterial efficacy. Specifically, the antimicrobial filter exhibited 100% reduction in *Micrococcus* and 93.75% reduction in *Staphylococcus* genus, related to anthropogenic sources.

Keywords Subway station · Antimicrobial filters · Airborne microbiome · 16S rRNA sequencing

Introduction

Airborne pollutants are introduced and generated in subway stations, where passengers are crowded in enclosed spaces with restricted ventilation. Enclosed spaces can become contaminated by suspended particles, bacteria, fungi, and viruses because ventilation affects the dispersion of infectious aerosols in the size range of a few micrometers or less [1, 2]. It has been reported that humans may be a major source of indoor bioaerosols [3], and they can emit approximately 10^6 biological particles per hour, creating a microbial cloud in the surrounding indoor air [4]. Pathogens can be transmitted to other individuals and indoor surfaces through the dispersion of biological particles, such as droplets released during coughing or sneezing. Mainly, airborne transmission occurs by dispersing droplets or droplet nuclei that are a few micrometers or smaller in size and are generated through evaporation. These airborne particles linger in the air for extended periods and can migrate further through indoor airflow. In contrast, droplet transmission generally occurs between persons in close proximity because larger particles are deposited more rapidly. During the severe acute respiratory syndrome (SARS) outbreak in 2003, airborne transmission was shown to occur not only through droplet transmission between individuals in close proximity but also through the dispersion of droplets facilitated by indoor airflows [5–7]. According to Van Doremalen et al., SARS-CoV-2 aerosols can maintain viability for up to 3 h, leading to the prolonged suspension of airborne particles in the air and their ability to travel further through indoor airflow [8]. In a study conducted by Guo et al., air samples were collected in an intensive care unit, and SARS-CoV-2 was detected at 14 out of the 40 sampling locations, indicating that the transmission distance of SARS-CoV-2 particles can go up to 4 m [9].

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In recent years, several studies have reported the microbial distribution in public transportation in urban areas [10]. A study on airborne bacterial concentrations in 25 underground railway platforms in Seoul revealed that airborne bacteria were present on 92% of the tested platforms. Additionally, Gram-negative bacteria were found at two stations (8%), and the total airborne bacterial concentrations at four stations (16%) exceeded 800 CFU/m^3, the maximum level of the Korean indoor bio-aerosol guideline. A study on the distribution of airborne bacteria and fungi in Seoul metropolitan subway stations found that *Staphylococcus*, *Micrococcus*, *Penicillium*, and *Cladosporium* were the predominant genera in both worker and passenger areas [11]. Other investigations on the aerosol microbiome of the Seoul subway stations showed the presence of skin-associated bacterial genera, such as *Cutibacterium*, *Micrococcus*, *Staphylococcus*, *Enhydrobacter*, and *Corynebacterium*, suggesting that microbial composition results from various combinations of environmental and human sources [12]. An investigation into the airborne bacterial environment at an underground subway station in Oslo, Norway, indicated that *Bacillus*, *Micrococcus*, and *Staphylococcus* are the major genera related to anthropogenic sources [13]. In addition to airborne bacteria, microbiome from station and train surfaces in Mexico City was dominated by genera associated with human skin and dust, such as *Cutibacterium*, *Corynebacterium*, *Streptococcus*, and *Staphylococcus* [14]. Source tracking indicated that indoor airborne microbiomes were primarily influenced by outdoor air and the skin of the occupants. For instance, as the concentration of PM2.5 increased, the abundance of *Deinococcus* and *Paracoccus* increased, potentially due to their metabolic capabilities. Furthermore, human-associated microbiomes, such as *Enhydrobacter* and *Micrococcus*, demonstrated correlations with the CO_2 concentration, thereby implying a dense occupant presence and/or insufficient indoor ventilation [15].

In subway stations, air circulates through ventilation systems, which may be responsible for the spread of infectious aerosols [1]. In the metropolitan subways of South Korea, air purifiers are installed and operated on platforms to enhance the air quality in these areas. Efficiency particulate air (EPA) filters are commonly used in air purifiers to capture submicron-sized airborne particulate matter and bioaerosols. However, EPA filters do not possess disinfection capabilities to inactivate pathogenic microorganisms. Consequently, microorganisms may remain viable and proliferate within the air filtration system. This phenomenon serves as a source for airborne pathogen transmission during filter replacement and disposal. Recently, COVID-19 triggered the active development of antiviral textiles, masks, and filters using various biocidal coating agents, such as metals, metal oxide, natural polysaccharides, alkaloids, inorganic minerals, and organic ionic compounds [16–20]. The standard plate count method (SPC) is commonly used for mesophilic bacteria titration under aerobic conditions. It serves as a suitable and intuitive approach for evaluating the biocidal performance of filter textiles coated with antimicrobial agents. In the case of aerosolized microbiomes, controlled chambers were utilized to assess reduction efficiencies [21, 22]. However, there are only a few studies on the effect of antimicrobial filters on microorganisms captured in occupied spaces. In addition, few case studies have compared the microbiomes collected by regular and antimicrobial filters in the subway. In a field study, we applied antimicrobial EPA filters to air purifiers installed on subway platforms to investigate their reduction effects on captured microorganisms. After 1 week of operation, the microbiomes were collected from both the control and test filters, followed by microbial identification. Additionally, the composition of metal elements, which are abundant in subway station, was analyzed using particulate matter collected by the EPA filters. Overall, this field investigation is significant as it compares the microorganisms captured in regular and antimicrobial filters operated in an occupied space and demonstrates the effectiveness of the antimicrobial filter against identified bacterial genera.

Experimental Details

Preparation of Antimicrobial Filters

The antimicrobial filters were prepared by laminating melt-blown polypropylene (E11 grade) with polyester (PET) coated with biocidal substances. The antimicrobial PET was prepared following previously reported literature [23]. A roll-to-roll method was used to coat a 1.2 m wide and 100 m long PET textile with metal oxide, chitosan and copper nitrate trihydrate. After the roll-to-roll dipping and pressing of the PET in an aqueous dispersion of metal oxide and 10% w/v acrylate copolymer at a speed of 10 m/min, it was subsequently dried at 105 °C. The PET with the coating weight of 7.3 g/m^2 underwent secondary roll-to-roll dipping and pressing process in an aqueous mixture of chitosan (0.625% w/v) and copper nitrate trihydrate (0.625% w/v), followed by drying at 105 °C. Antibacterial and antiviral test of the coated PET were done according to standards KS K 0693 “Test method for the antibacterial activity of textile materials” and ISO 18184 “Determination of the antiviral activity of textile products”. The laminated filter textile was then bent to a thickness of 50 mm and assembled into air filters with dimensions of 350 mm × 450 mm × 50 mm, designed for fitting in a quarter of the air purifiers on the platform.

Measurement of Dust Collection Efficiency

The dust collection efficiency of the control EPA filter and the fabricated antimicrobial filter was measured according to standards KS C 9325: 2011 "air filter element for room air cleaner" which used 2% aqueous solution of potassium chloride as 0.3 μm of test aerosol.

Filter Installation and Sample Collection

For filter installation and sampling, a metropolitan subway station in South Korea served as a testbed platform. Two non-adjacent air purifiers were selected and equipped with control and test filters, respectively, at upline platforms. EPA filters, which are used for routine maintenance, were installed as the control group, whereas antimicrobial EPA filters were designated as the test group. Each air purifier was equipped with four EPA filters. Pre-filters and non-woven filters were used in both the control and test groups to eliminate coarse particles according to the operations manual. Sampling was performed in May 2023 by recovering the control and test EPA filters from the air purifiers that had operated for one week.

Metal and Ionic Components Analysis

The metal composition of particulate matter collected in the EPA filters was analyzed using inductively coupled plasma optical emission spectrometry (ICP–OES) (Agilent 5100, USA). To extract metallic components, the filter sample in 2 mL of nitric acid and 6 mL of hydrochloric acid was heated at 200 °C for 16 h. The ionic compounds captured in the EPA filters were analyzed using ion chromatography (882 Compact IC Plus, Metrohm, Switzerland). The water-soluble ionic components were extracted by ultrasonic treatment of the filter sample in 20 mL of ultrapure distilled water for 10 min.

Microbial Identification

The control EPA and test antimicrobial EPA filters, used for one week at the station, were cut at randomly selected regions with a size of 50 mm × 50 mm × 50 mm. Phosphate-buffered saline (PBS; 250 g) was added to three filter specimens per control or test and the mixture was vortexed for 30 min to extract the PBS solutions. Then, the specimen-eluting PBS was filtered out with a 0.45 μM membrane filter. For bacterial cultivation, 5 mL of PBS was added to the membrane filter in a conical tube and the mixture was vortexed to obtain membrane filter-washed PBS. Finally, 1 mL of the PBS solution was evenly smeared onto five tryptic soy broth (TSB) agarose plates. After incubation for 24 h at 37 °C, the bacterial titer on the control and test filters was calculated by counting the colonies on five TSB agarose plates.

Selected colonies with different appearances were inoculated into 5 mL of TSB media, and then incubated for 24 h at 37 °C in a shaking incubator. Each cultured microorganism was streaked to TSB agarose plates. After incubation for 24 h at 37 °C, microorganisms were identified by 16S rRNA sequencing entrusted to Macrogen (South Korea) [24, 25]

Data Analysis

The antimicrobial efficacy was calculated by percentage as per the following equation:

$$\text{Antimicrobial efficacy} = 100 - (\text{colony number of test group} / \text{colony number of control group}) \times 100 \quad (1)$$

All figures were schematized with Prism 8 (GraphPad, US) software.

Results

Filter Installation and Sample Collection

A metropolitan subway station in South Korea was chosen as the testbed platform for the sampling of air filters. The selected station had an average daily passenger count of approximately 25,000 in 2022. At the testbed station, five and six air purifiers were installed and operated 24 h a day on upline and downline platforms, respectively, to reduce particulate matter. For this study, two non-adjacent air purifiers were selected and equipped with control EPA and test antimicrobial filters at the up-line platforms.

The test antimicrobial filters were prepared by laminating melt-blown polypropylene (E11 grade) with the coated PET with metal oxide, chitosan and copper nitrate. The antimicrobial PET prepared by multi-coating using the roll-to-roll process exhibited ≥ 99.999% virus reduction efficiency for SARS-CoV-2 and > 99.9% antibacterial efficiencies against *Escherichia coli* and *Staphylococcus aureus* in 30 min [23]. Metal oxide can generate reactive oxygen species such as hydroxy radicals through catalytic reactions, and copper ions have contact-killing effects on the coated surface. Chitosan has shown the improving the antibacterial efficiency of the coated PET due to intrinsic bactericidal effect attributed amine groups as well as immobilization of copper ions through coordination. Compared to coated PETs with a single agent, the multi-coated PET could reduce the loading amount of coating

materials as well as contact time with bacteria for > 99.9% antibacterial efficiencies.

According to standards KS C 9325: 2011, the control EPA filter and antimicrobial filter showed 96.9% and 95.2% of dust collection efficiency, respectively. The efficiency indicates that both filters belonged to the E11 filter class; this proved to be advantageous to the comparison of microorganisms captured by both the filters. Sampling was performed in May 2023 by recovering the control and test filters from air purifiers that had operated for 1 week.

Metal and Ionic Components Analysis

The metal composition of particulate matter collected by the control EPA filter was analyzed using ICP-OES. As listed in Table 1, iron (Fe) showed the highest concentration ratio of 83.19% among the analyzed metals, which is similar to other studies [26]. Fe in a subway station is usually generated by friction between the train wheels and rail lines. Other major elements were antimony (Sb, 7.20%), silicone (Si, 4.34%), aluminum (Al, 1.46%), barium (Ba, 1.21%), manganese (Mn, 0.71%), zinc (Zn, 0.61%), and copper (Cu, 0.56%). Trace elements, such as chromium, tin, molybdenum, arsenic, zirconium, nickel, strontium, and cobalt (Cr, Sn, Mo, As, Zr, Ni, Sr, Pb, and Co), were also present. Sb, Ba, Mn, Zn, and Mo are typically generated during braking and subsequent steel abrasion. Si and Al are related to soil constituents that originate from outdoor sources. Al is also produced from the abrasion of subway components. Friction between the catenaries and the pantographs is a major source of copper. The ionic compounds captured in the EPA filters were analyzed using ion chromatography. Anions of F^-, Cl^-, Br^-, PO_4^{3-}, SO_4^{2-} and cations of Na^+, NH^{4+}, K^+, Ca^{2+} were detected in the EPA filter samples. These components can originate from tunnel and outdoor sources, such as concrete, soil, and exhaust gas [26, 27].

Table 1 Metallic composition of PM collected in EPA filters

Element	Concentration ratio (%)	Element	Concentration ratio (%)
Fe	83.19	Sn	0.10
Sb	7.20	Mo	0.08
Si	4.34	As	0.07
Al	1.46	Zr	0.07
Ba	1.21	Ti	0.06
Mn	0.71	Ni	0.06
Zn	0.61	Sr	0.03
Cu	0.56	Pb	0.03
Cr	0.21	Co	0.01

Microbial Composition of EPA and Antimicrobial Filters

Figure 1A shows the 20 types of bacteria identified in the test and control groups using 16S rRNA sequencing. Ten detected bacteria belonged to the *Bacillus* genus. While 19 types of bacterial species were detected in the EPA filters, the antimicrobial filters showed the presence of 15 bacterial species. Among these, *Bacillus thuringiensis* was detected only in the antimicrobial filters, whereas *Bacillus sonorensis*, *Micrococcus aloeverae*, *Micrococcus yunnanensis*, *Lysinibacillus macrolides*, and *Bacillus nakamurai* were found solely in the EPA filters (Fig. 1B).

While the EPA filter exhibited a total number of 170 bacterial colonies, the antimicrobial filter had 60 colonies (Fig. 2A), indicating 64.71% of antimicrobial efficacy. The number of colonies for each bacterial genus is shown in Fig. 2B and Table 2. Although *Bacillus* was the most common genus of microorganisms detected, *Staphylococcus* formed the highest number of colonies in the EPA filter (64). In case of the antimicrobial filter, *Bacillus* formed the highest number of colonies (37), which was comparable to the number of *Bacillus* colonies (44) identified in the EPA filter.

In addition, all bacteria, except *Bacillus cereus*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, *Bacillus sanguinis*, and *Pantoea conspicua*, displayed reduced colony counts in the antimicrobial filters compared to the EPA filters. Notably, the colony numbers of *Staphylococcus hominis*, *Staphylococcus epidermidis*, and *Micrococcus aloeverae* reduced more considerably than those of the other microorganisms in the antimicrobial filters (Fig. 2B and Table 2). The efficacy of the antimicrobial filter against each genus was determined to be 15.91% for *Bacillus*, 54.55% for *Lysinibacillus*, 93.75% for *Staphylococcus*, 100% for *Micrococcus*, 46.67% for *Priestia*, and 0% for other (*Pantoea*, *Calidifontibacillus*) (Fig. 3).

Discussion

The effect of antimicrobial filters on the reduction of airborne microbiome was investigated through a field study in a metropolitan subway. Air purifiers equipped with control EPA and test antimicrobial EPA filters were operated in an occupied space on a subway platform. Iron (Fe), with 83.19% concentration ratio, was the most abundant metal in particulate matter collected in the EPA filters for 1 week. Previous studies have shown that the Fe in a subway is usually generated by the wheel–rail friction, and Fe-containing particles (e.g., iron oxide) are the most common metals [12,

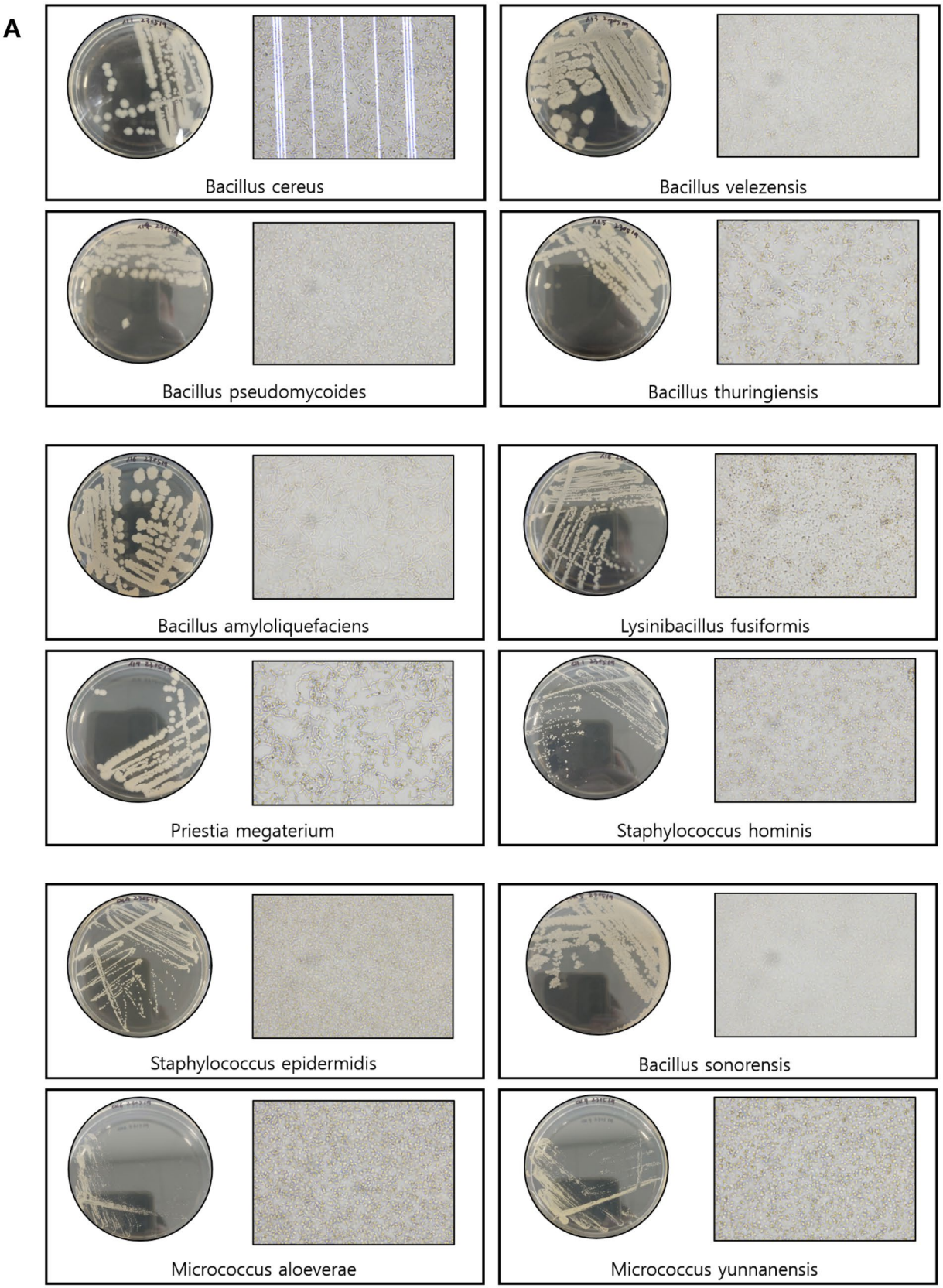
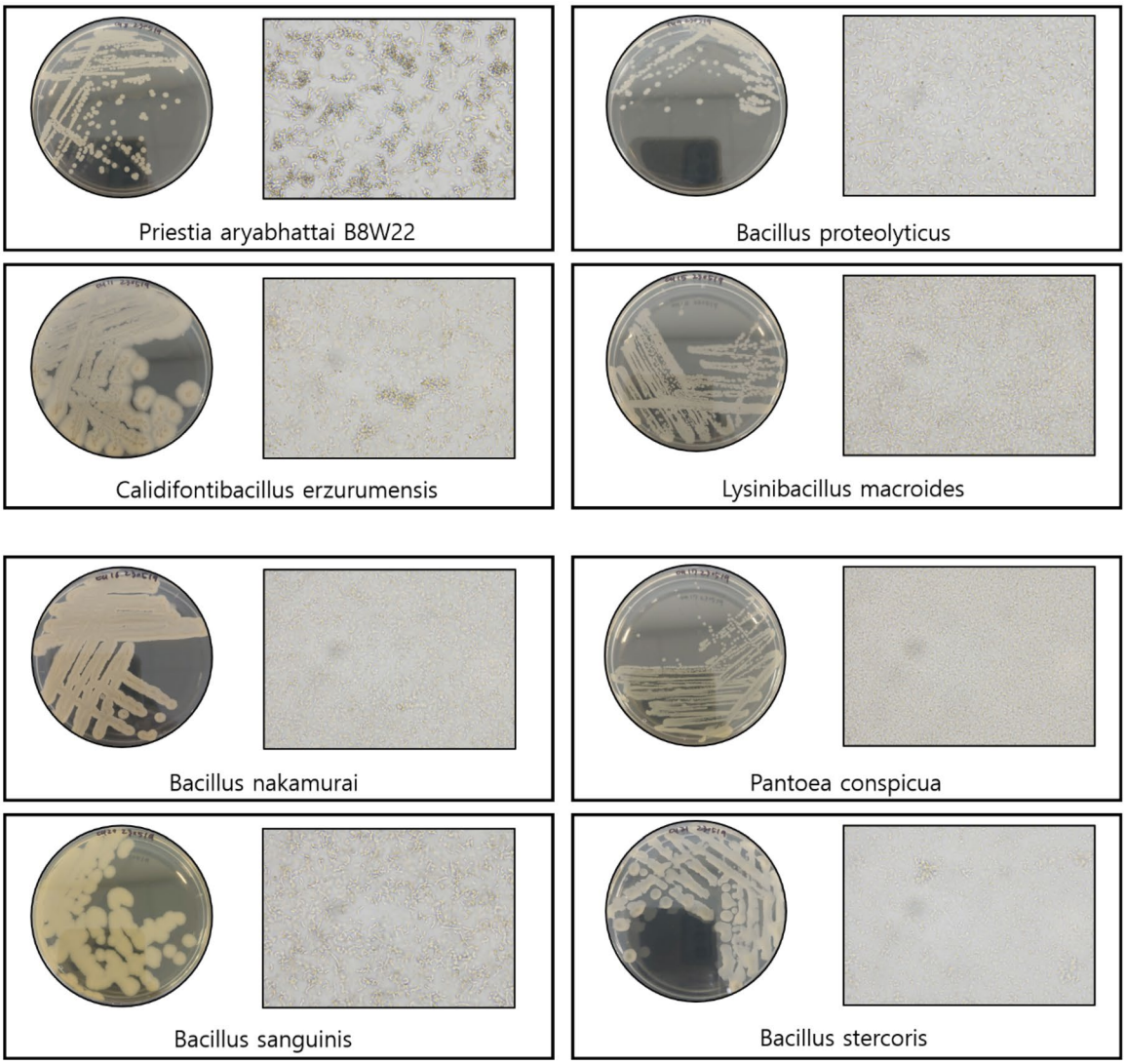


Fig. 1 Bacterial identification in control EPA and test antimicrobial filters. Bacteria in the filters were extracted with PBS, and cultured in TSB medium and identified using 16S rRNA sequencing. **A** Morphological differences by the type of detected bacterial species; **B** types of bacterial species detected in EPA filters and antimicrobial filters, respectively



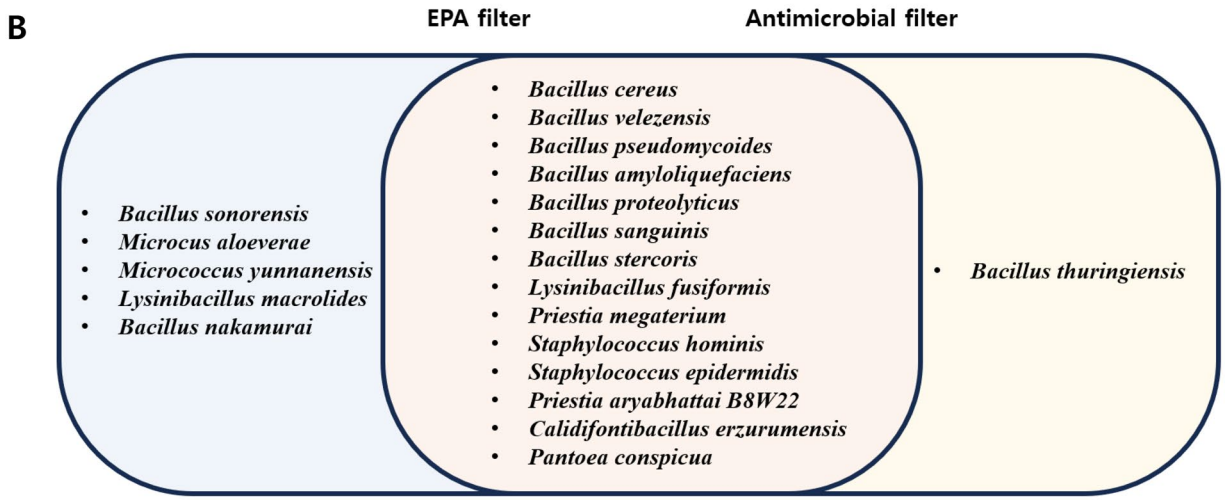


Fig. 1 (continued)

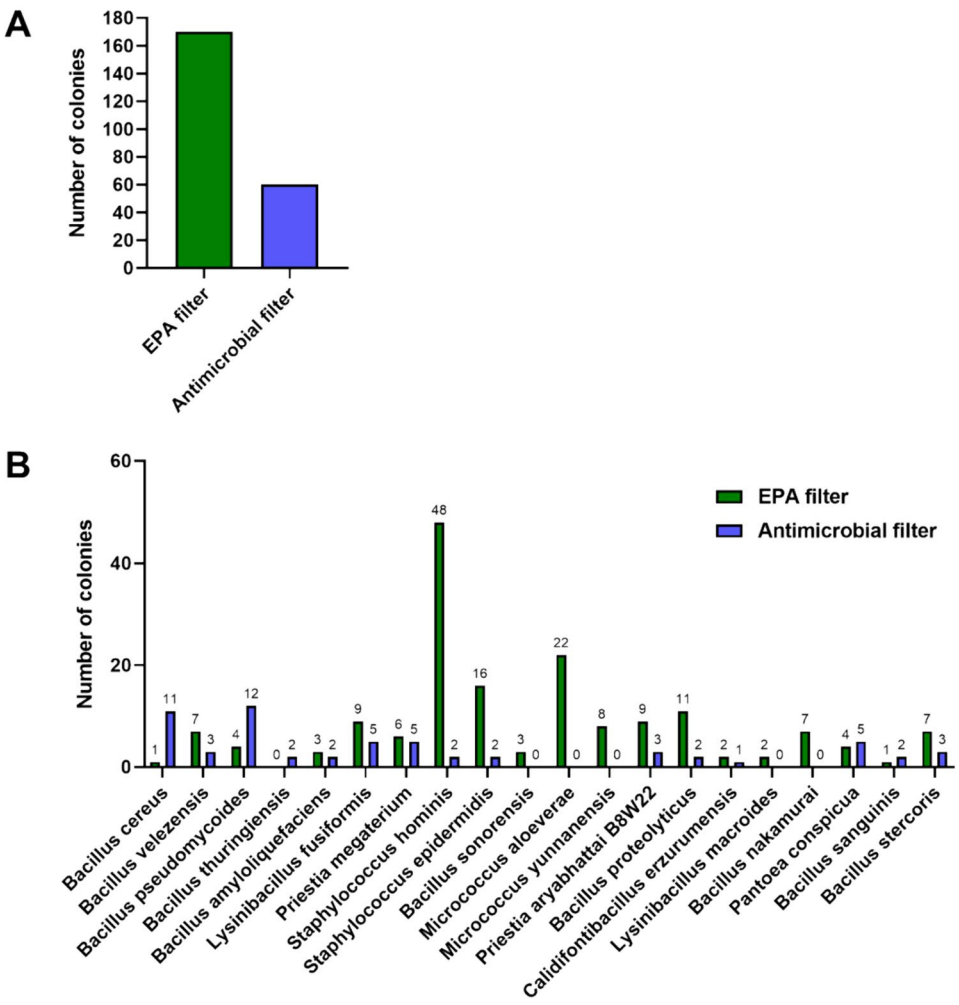


Fig. 2 Comparison of **A** the number of total colonies and **B** the number of colonies by bacterial species detected in EPA and antibacterial filters. Bacteria were cultured by smearing 1 mL solution of filter extraction onto five TSB agarose plates, and the number of colonies shown is the sum of the numbers identified on the five plates

26] in metropolitan subways. In addition, the ionic components of EPA filters, such as SO_4^{2-}, Na^+, NH^{4+}, and Ca^{2+}, originate from tunnel and outdoor sources, as demonstrated in previous studies [26, 27].

In the microbial identification of the control and test EPA filters, 20 bacterial species belonging to seven genera were detected. Although there may be several reasons why half of the 20 species of microorganisms detected were *Bacillus*, we presume that its ability to survive in a relatively harsh environment by forming spores could be a major factor [28]. The test environment (e.g., humidity and temperature) was deduced to have worked favorably for the survival of *Bacillus*. The antibacterial efficiencies of the test filter against each bacterial genus were 15.91% for *Bacillus*, 54.55% for *Lysinibacillus*, 93.75% for *Staphylococcus*, 100% for *Micrococcus*, 46.67% for *Priestia*, and 0% for other (*Pantoea*, *Calidifontibacillus*). Notably, the colony numbers of *S. hominis*, *S. epidermidis*, and *M. aloeverae*, which are related to anthropogenic sources [13], reduced more considerably than those of other microorganisms in the antimicrobial filters. *Staphylococcus* and *Micrococcus*, non-spore-forming bacteria, demonstrated a significant reduction rate in this field study [29, 30]. On the other hand, *Bacillus*, *Lysinibacillus*, and *Priestia*, all of which are spore-forming bacteria with high resistance to environmental factors exhibited a low reduction rate in this study [31–33]. Therefore, the reduced antimicrobial effect against spore-forming bacteria is more likely attributed to the inherent high resistance to an environment, rather than the coating materials any shortcomings in the coating materials' ability to kill bacteria. While the actual detected bacteria are known to cause mild disease, *Staphylococcus* can cause diseases through direct tissue invasion or exotoxin production [34–38]. Direct tissue invasion represents the primary mechanism for staphylococcal diseases, such as cellulitis, lymphangitis, otitis media, pneumonia, skin infections, osteomyelitis, endocarditis, and infectious arthritis. Staphylococcal diseases related exotoxin encompass staphylococcal scalded skin syndrome, food poisoning, and toxic shock syndrome. This may be considered as an indicator of antibacterial efficacy against pathogenic microorganisms. *Micrococcus* is generally regarded as a commensal organism; however, it can be an opportunistic pathogen,

Table 2 Distribution of the number of colonies in EPA and antibacterial filters according to bacterial genera

Genus	Species	Colony counts	
		Control group	Test group
Bacillus	*cereus*	1	11
	velezensis	7	3
	pseudomycoides	4	12
	thuringiensis	0	2
	amyloliquefaciens	3	2
	sonorensis	3	0
	proteolyticus	11	2
	nakamurai	7	0
	sanguinis	1	2
	stercoris	7	3
Staphylococcus	*hominis*	48	2
	epidermidis	16	2
Lysinibacillus	*fusiformis*	9	5
	macroides	2	0
Micrococcus	*aloeverae*	22	0
	yunnanensis	8	0
Priestia	*megaterium*	6	5
	aryabhattai B8W22	9	3
Pantoea	*conspicua*	4	5
Calidifontibacillus	*erzurumensis*	2	1

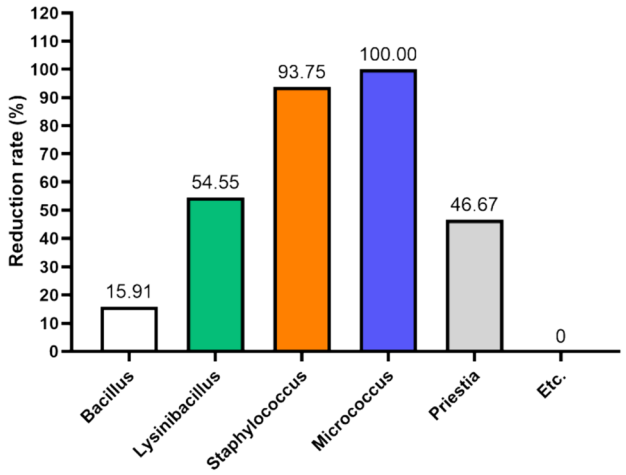


Fig. 3 Reduction rate of antimicrobial filters against each bacterial genus. *Pantoea* and *Calidifontibacillus* genera, each represented by only one detected species, were consolidated into a single group, resulting in the organization of bacterial genera into six categories

particularly in immunosuppressive patients. For instance, a specific species of *Micrococcus* has been reported as a potential cause of infections such as septic arthritis, bacteremia, brain and hepatic abscesses, endocarditis in immunosuppressive individuals [39]. While *Staphylococcus* and *Micrococcus* infections may not present a serious threat to healthy individuals, they can pose serious risks to vulnerable populations such as children, elderly, and patients in multi-use facilities. Thus, the scope of the antimicrobial EPA filter extends beyond subways to encompass the various multi-used facilities such as hospitals and daycare centers.

As mentioned above, this study analyzed the microorganisms captured in regular and antimicrobial filters operated in an occupied space and demonstrated the effectiveness of the antimicrobial filter against identified bacterial genera. Although lots of antimicrobial filters have been developed in order to reduce the microbial reservoir on the filters, thus addressing the issue of re-contamination in indoor air environments, few case studies have compared the microbiomes collected by regular and antimicrobial filters in the field trials. Generally, microbial suspension was inoculated on the filter surfaces, followed by plaque assay to evaluate the biocidal performance of antimicrobial filters. For example, Gedanken et al. reported antimicrobial activities of polyester filter media coated with CuO by a roll-to-roll coating method and filtering properties of car air-conditioning filters fabricated using the coated media [40]. According to in-solution assay, the coated polyester media showed the complete elimination of *E. coli* and *S. aureus* and 99.15% reduction in SARS-CoV-2 viability after 2 h of contact time. In another case, Kim et al. modified existing HEPA filter by incorporating tannic acid for 16 h immersion in the dark with shaking, resulting in the modified filters exhibiting approximately 90% in-solution capture of H1N1 virus compared to standard HEPA filters [41]. In another case, Cogan et al. tested modified MK3 filter on HVAC system of trains operating on the UK rail network, showing the absence of detectable bacterial and fungal organisms [42]. The MK3 filter underwent pre-treatment with a chlorhexidine digluconate solution utilizing an automated treatment system to produce coated filters. In this study, the antimicrobial filters incorporating roll-to-roll coated PET textiles with metal oxide, chitosan, and copper were applied in the occupied field trials to investigate their reduction effects on captured microorganisms. Microbial species captured on both the control and antimicrobial filters were identified and quantified, highlighting the efficacy of the antimicrobial filter against non-spore-forming bacteria, namely *Staphylococcus* and *Micrococcus,* associated with anthropogenic sources. While it was less effective against resistant spore-forming bacteria such as *Bacillus, Lysinibacillus*, and *Priestia* strains, the findings from the field study regarding representative bacterial genus, *Staphylococcus*, align to some extent the antibacterial efficacy assessed through the KS K 0693 test.

Although the detection of pathogenic microorganisms (e.g. SARS-CoV-2, influenza A) is limited depending on the conditions of collection and possibility of an infectious

disease outbreak, it is necessary to compare the performance of antimicrobial filters against more diverse strains of microorganisms under different seasonal and replacement intervals in the future. For example, antibacterial efficiency of the test antimicrobial filter operated for 2 months on a consistent subway platform was 36.89%. During the 2-month filter maintenance period, non-resistant strains may have become extinct, and the accumulation of fine dust on the coated antimicrobial agents may have hindered direct contact between the microorganisms and biocidal agents [43, 44]. Nonetheless, to ensure optimal utilization, such as the replacement cycle for actively developed antimicrobial filters, it may be necessary to explore their performance in relation to seasonal variations and the accumulation of suspended particulates, as mentioned above.

Conclusions

In summary, the effect of antimicrobial filters on the reduction of airborne microbiome was investigated through a field study in a metropolitan subway. The regular EPA and test antimicrobial filters were operated in air purifiers on the platform for 1 week. Iron (Fe) was the major element in particulate matter collected by the EPA filters, and tunnel- and outdoor-related ions such as SO_4^{2-}, Na^+, NH^{4+}, and Ca^{2+} were also detected. Through 16S rRNA sequencing, it was found that the antimicrobial filters contained 15 bacterial species, whereas 19 bacterial species were detected in the control filters. In comparison to EPA filters, most bacteria exhibited reduced colony counts in the antimicrobial filters, resulting in 64.71% of antimicrobial efficacy. Overall, this field investigation explored the microorganisms captured in both the regular filter and the antimicrobial filter operated in an occupied space, clearly demonstrating the effectiveness of the antimicrobial filter against certain bacterial genera, namely *Staphylococcus* and *Micrococcus*, associated with anthropogenic sources.

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