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Airborne bacterial contamination and disinfection efficacy of a portable hypochlorous acid spray system in dog cafés in South Korea*

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Summary

Dog cafés have grown in popularity but lack standardized hygiene regulations, raising concerns about airborne microbial exposure. A promising disinfection system has been developed, but its effectiveness in indoor spaces shared by humans and animals remains unclear. In this study, we evaluated the efficacy of a portable high-pressure spray device (Vi-Killer®) utilizing neutral electrolyzed water containing hypochlorous acid. Bacterial concentrations were measured in air from three dog cafés in South Korea before and after disinfection. ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) were identified and tested for antibiotic resistance. The average airborne bacterial concentration before disinfection was 193.2 CFU/m^3, which significantly decreased by 57.3% to 80.9 CFU/m^3 after disinfection ($p < 0.05$). A total of 466 bacterial isolates were obtained, of which 315 (67.6%) were identified. *Staphylococcus* spp., *Bacillus* spp., and *Micrococcus luteus* were dominant with minimal changes in community composition after disinfection. Six (1.3%) isolates were identified as ESKAPE pathogens; two exhibited multidrug resistance. The Vi-Killer® system effectively reduces microbial load in dog cafés and may mitigate the risk of airborne transmission of opportunistic and resistant bacteria. Regular air disinfection in animal–human shared facilities could improve public hygiene strategies.

Keywords: airborne pathogen, animal–human shared space, public health

The companion animal industry in South Korea has grown rapidly, with surging demand for leisure and travel activities among pet owners (27, 36). Alongside this trend, the popularity of dog cafés has rapidly expanded, particularly among residents of large cities who tend to have smaller residential spaces. Initially intended as spaces to enjoy food and beverages with dogs, dog cafés have evolved into multicare centers that incorporate grooming salons, hotels, daycare, and training centers. This trend has also emerged in other Asian countries, such as China and Thailand, where the presence of pets in cafés and restaurants is often restricted (53, 60).

Dog cafés, as places where people and animals are densely gathered, may pose public health threats, including a heightened risk of airborne microbe transmission, especially for immunocompromised groups (44, 60). In environments shared between humans and other animals, bioaerosols are continuously released due to barking, sneezing, shedding, movement, and excretion. These aerosols can carry bacteria, viruses, and fungi, some of which may be pathogenic, that can remain airborne or settle on indoor surfaces (12, 25). Repeated exposure to such pathogens can lead to allergic reactions, opportunistic infections, and

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antibiotic-resistant infections (4, 25, 33, 35). With the growing number of commercial animal–human shared spaces such as dog cafés, there is increasing awareness and emerging discussions about the need to extend the concept of healthcare-associated infections (HAIs) beyond clinical settings to include nonclinical environments as well (49).

Opportunistic and antibiotic-resistant pathogens with the potential for nosocomial transmission have been detected in the air in veterinary hospitals (21, 38, 71). However, to our knowledge, few studies have monitored airborne bacteria or assessed the microbial risks in non-medical commercial spaces where healthy pets and people coexist. In South Korea, certain multiuse facilities, such as childcare centers and nursing homes, are legally required to measure airborne bacterial levels annually ($\leq$ 800 CFU/m^3) (32), but no such regulation exists for pet-related facilities. Although veterinary clinics often follow standardized disinfection protocols, dog cafés and similar establishments lack such strategies and typically limit cleaning practices to surface hygiene, with little focus on air sanitation.

Developing standardized disinfection protocols for dog cafés is important because the presence and potential transmission of antibiotic-resistant and opportunistic pathogens have been reported in non-clinical spaces where healthy dogs are present (17, 49, 53, 60). Furthermore, dog cafés frequently host "shared dogs" for customers without pets, resulting in repeated close contact with multiple individuals (60). These environments differ from those of typical households and may function as unique hubs for the spread of antimicrobial resistance genes and infections (17, 53, 60). Although previous studies have identified hands, staff, and surfaces as transmission routes, little attention has been paid to bioaerosols in indoor air.

Spray disinfection gained attention during the COVID-19 pandemic as a strategy for combating indoor air contamination (6, 44). However, such sprays are only effective when the particles remain airborne, and the droplet size directly affects their efficacy. The World Health Organization (WHO) recommends particle sizes of 10-30 μm for optimal disinfection (67). However, generating such particles typically requires large-scale equipment, reducing portability (6). To address this, our team developed Vi-Killer®, a high-pressure portable spray system that uses neutral electrolyzed water (NEW) based on hypochlorous acid (HOCl). Compared to traditional chlorine-based disinfectants, NEW is neutral, safe, stable, and does not irritate the skin and mucosa, making it an eco-friendly solution. Several NEW-based products have been listed by the US EPA as effective disinfectants against SARS-CoV-2 (64), and Vi-Killer® has been demonstrated to effectively reduce airborne bacteria in laboratory and public-use environments (6). However, the effectiveness of this technology in enclosed, non-clinical commercial spaces shared by people and healthy animals, such as dog cafés, has not yet been fully evaluated.

Across studies conducted in various regions, total airborne bacterial (TAB) concentrations in animal-related indoor environments have generally ranged from several tens to over 1,000 CFU/m^3 depending on occupancy and ventilation conditions (21, 42, 50). Similar findings have also been reported across European countries. In Poland, Sitkowska et al. (57) reported mesophilic bacterial concentrations between 39 and 5,034 CFU/m^3 across 44 veterinary clinics, dominated by Gram-positive cocci such as *Staphylococcus* spp. In Portugal, Viegas et al. (65) observed airborne bacterial loads of 84-328 CFU/m^3 and fungal loads up to 504 CFU/m^3 in a small-animal clinic, identifying azole-resistant *Aspergillus* strains and emphasizing the need for improved ventilation and spatial separation of animals and procedure areas. From a One Health perspective, airborne transmission of infectious agents is recognized as a cross-sectoral public health issue requiring integrated management across human and animal environments (14). Collectively, these international findings demonstrate that airborne microbial contamination in animal-related facilities is a globally recognized concern requiring standardized air hygiene management.

Building on this global and quantitative context, our previous field validation using a portable pH-neutral hypochlorous acid (HOCl) spray system achieved a 70.3% reduction ($p < 0.05$) in airborne bacterial counts across various operational indoor environments – including senior-care hospitals and childcare centers – and successfully lowered values that exceeded 800 CFU/m^3 to within regulatory limits in some facilities. However, its effectiveness has not yet been evaluated in non-clinical mixed-use spaces where humans and healthy animals coexist, such as dog cafés. Therefore, we hypothesized that application of the portable pH-neutral HOCl spray system would significantly reduce airborne bacterial concentrations and alter microbial diversity in dog cafés under real-world operating conditions.

In this study, we aimed to assess the air disinfection efficacy of the Vi-Killer® system in dog cafés in South Korea by analyzing airborne bacterial concentrations and species diversity. In addition, we focused on the detection and antimicrobial resistance characteristics of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), which are major contributors to healthcare-associated infections, including those potentially linked to animal contact in therapeutic and nonclinical settings (52, 54). Although these pathogens have been well studied in clinical settings, their presence and transmission in commercial animal–human shared spaces remain insufficiently characterized.

Material and methods

Vi-Killer® spray system and aerosol particle size analysis. We employed a portable Vi-Killer spray disinfection system (Tracoworld Ltd., Gwangmyeong, Korea) that integrates a high-pressure pump (UHP S1, Tracoworld Ltd.), LYOHM® nozzles (H. Ikeuchi & Co., Ltd., Osaka, Japan), and rechargeable G-MAX® battery (Greenworks, Mooresville, CA, USA). The disinfectant applied was pH-NEW (Deger®, LGS Corporation, Gwangmyeong, Korea), containing approximately 100 ppm of free chlorine in the form of HOCl. The hypochlorous acid solution used in this study was a neutral electrolyzed water (NEW) product (DEGER). DEGER was produced by electrolyzing high-purity salt (≥ 99.99%) in deionized water. According to the certificate of analysis of the solution used in this study, DEGER had an average pH of 7.28, a free available chlorine concentration of 112 ppm, and an oxidation–reduction potential (ORP) of 872 mV. The sodium concentration of the solution was approximately 180 mg/L. To verify that the device consistently produced droplets within the WHO's recommended size range for effective airborne disinfection (10-30 μm), aerosol particle size distribution was measured using a laser diffraction spray particle size analyzer (2000S, Bettersize Instruments Ltd., Dandong, China). Five independent runs were conducted to ensure reproducibility of the results.

Air sampling and disinfection procedure in dog cafés. From December 2024 to July 2025, field experiments were conducted at three dog cafés located in Gyeonggi Province, South Korea. Each café was visited three times on separate days during normal business hours, following prior coordination with the facility operators to reflect typical real-world operating conditions. Dog café A is a large-scale facility that offers not only food and beverages but also grooming, pet boarding, and training services. The café refused approval for disinfection experiments in customer service areas where food and beverages were consumed. Instead, the experiments were conducted only in the pet boarding areas. Dog café B is another large establishment with integrated grooming, pet boarding, and training services. Disinfection experiments were performed in the customer service area, where food and drinks are served, and the pet grooming area. Dog café C functions primarily as a traditional dog café and allowed experiments in two spatially separated customer service areas.

Following the protocol established in our previous study (5), air sampling and disinfection procedures were performed accordingly. Air samples were collected at a flow rate of 300 L/min using an AES Chemunex air sampler (BRUZ Cedex, France), positioned at a fixed height of 1.2 m above the floor within each sampling area to reflect the breathing zone. This sampling height corresponds to the average human breathing zone and is consistent with recommendations from the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA), and is widely adopted in indoor bioaerosol monitoring studies (10, 24, 63, 66). Initial baseline air samples were collected under normal operating conditions with animals present in the space, reflecting typical occupancy in dog cafés. Subsequently, 540 mL of NEW (Deger®, LGS Corporation) was dispersed per 89.1 m^3 of space using the Vi-Killer® device. Spraying was performed along a standardized walking path to ensure uniform distribution of disinfectant aerosols. For safety considerations, disinfection was conducted during short, pre-arranged intervals when customer access to the target area was temporarily restricted, and animals were managed by staff according to routine operational procedures. The HVAC (Heating, Ventilation, and Air Conditioning) system was temporarily deactivated during the disinfection procedure. Air sampling after disinfection was performed 5 min following spraying using the same sampling configuration, while ventilation remained unchanged to ensure comparability between pre- and post-disinfection measurements.

At each visit, air samples were collected in triplicate at the same sampling location under identical conditions, representing three independent air collections rather than internal instrument replicates or spatially distinct sampling points. Each café was sampled on three separate days, and mean values were calculated across visits for subsequent analyses. The collected air samples were cultured on tryptic soy agar (TSA; Oxoid, Basingstoke, UK) at 37°C for 48 h, and bacterial counts are expressed as CFU/m^3.

Bacterial isolation and identification. Total airborne bacteria (TAB) were counted via direct enumeration of colonies on TSA plates. To determine the bacterial diversity, colonies with distinct morphologies (e.g., size, pigmentation, and texture) were selectively isolated. These colonies were subcultured and identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) system (bioMérieux, Marcy-l'Étoile, France). This subset of isolates was used only for qualitative diversity analysis and not for the extrapolation of TAB levels.

Confirmation and antimicrobial susceptibility testing of ESKAPE pathogens. Bacterial isolates presumed to be members of the ESKAPE group were confirmed via 16S rRNA gene sequencing using the primer pairs F: CCAGCAGCCGCGGTAATACG and R: TACCAGGGTATCTAATCC (Macrogen Inc., Seoul, South Korea). The sequencing results were analyzed using NCBI BLAST for species confirmation.

Antibiotic susceptibility was evaluated using the disc diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (13). The following antibiotics were tested: ampicillin (AMP, 10 μg), cephalothin (CEP, 30 μg), cefoxitin (FOX, 30 μg), ceftriaxone (CRO, 30 μg), chloramphenicol (30 μg), ciprofloxacin (CIP, 5 μg), colistin (COL, 10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), rifampin (5 μg), streptomycin (100 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (SXT, 1.25 and 23.75 μg), and vancomycin (30 μg). Mueller-Hinton agar plates (Becton Dickinson, Franklin Lakes, NJ, USA) were inoculated with bacterial suspensions adjusted to a McFarland standard of 0.5, and antibiotic discs were applied within 15 min of inoculation. The plates were incubated at 37°C for 18 h, after which inhibition zones were measured. *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control strains.

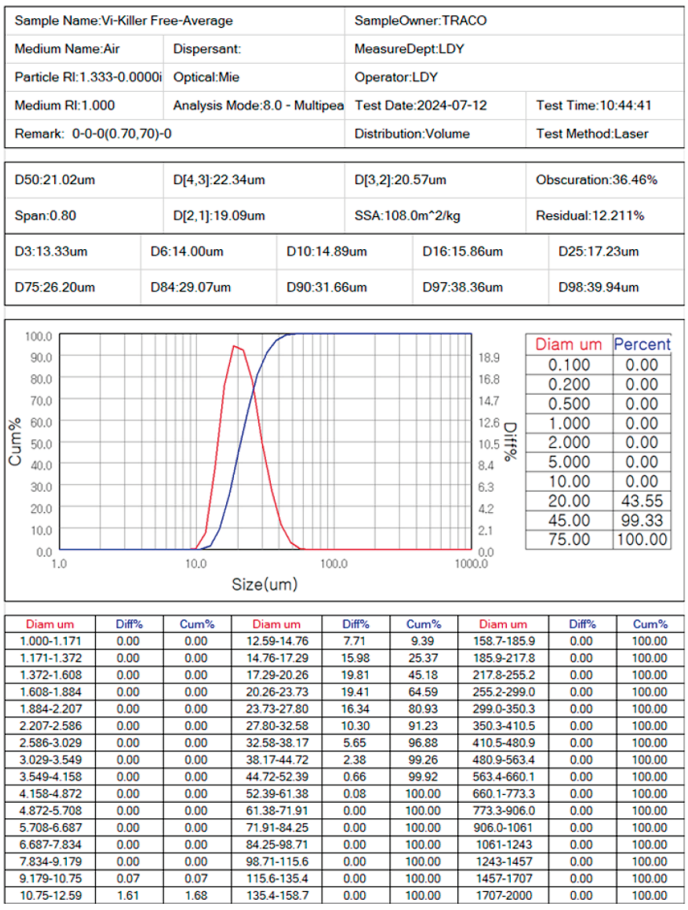
Statistical analysis. Differences in the bacterial counts before and after disinfection were evaluated using paired *t*-tests. Significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 29 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Characteristics of aerosolized particles. According to data from the aerosol particle size distribution, more than 85% of the NEW disinfectant particles were 10-30 µm in size, with a median diameter of 21.02 µm (Fig. 1). This particle size considered adequate for sufficient airborne retention time, is consistent with the WHO's recommended range for effective airborne disinfection. Furthermore, a key advantage of the spray system is that contact surfaces do not get wet after spraying, a feature highly valued by users. Therefore, the data demonstrate that surface and airborne disinfection is optimized by controlling the spray particle size.

Airborne bacterial concentrations across dog cafés. The TAB levels before and after disinfection are summarized in Table 1. Across the three participating dog cafés, the mean bacterial concentration before disinfection was 193.2 ± 187.1 CFU/m^3, which decreased significantly to 80.9 ± 78.0 CFU/m^3 after disinfection, corresponding to an overall reduction of 57.3% ($p < 0.05$). In dog café A, the bacterial concentration in the large-dog boarding area declined from 125.7 ± 65.6 CFU/m^3 to 51.7 ± 36.7 CFU/m^3 (63.8% reduction), whereas that in the small-dog boarding area decreased from 104.3 ± 19.6 CFU/m^3 to 44.0 ± 14.5 CFU/m^3 (57.6% reduction). In dog café B, the grooming area had the highest pre-disinfection bacterial load (354.0 ± 384.6 CFU/m^3), which declined to 147.3 ± 169.5 CFU/m^3 after disinfection (60.4% reduction). Similarly, in the adjacent customer service area, the concentration decreased from 272.7 ± 183.7 CFU/m^3 to 106.7 ± 62.5 CFU/m^3 (59.2% reduction). Both locations showed high baseline contamination, which consistently decreased to less than half following disinfection. In dog café C, the bacterial concentration in customer service area 1 decreased from 170.3 ± 145.3 CFU/m^3 to 64.0 ± 40.3 CFU/m^3 (57.5% reduction). In customer service area 2, the concentration decreased from 132.0 ± 129.6 CFU/m^3 to 71.7 ± 66.4 CFU/m^3 (45.5% reduction).

Bacterial community distribution. A total of 295 colonies were isolated before disinfection and 171 colonies afterward, and the community composition was elucidated (Fig. 2 and Tab. 2). The overall genus-level composition remained largely consistent between pre- and post-disinfection samples. Prior to disinfection, the predominant taxa were *Staphylococcus* spp. (54 isolates, 18.3%), *Bacillus* spp. (52 iso-



Diam um	Diff%	Cum%	Diam um	Diff%	Cum%	Diam um	Diff%	Cum%
1.000-1.171	0.00	0.00	12.59-14.76	7.71	9.39	158.7-185.9	0.00	100.00
1.171-1.372	0.00	0.00	14.76-17.29	15.98	25.37	185.9-217.8	0.00	100.00
1.372-1.608	0.00	0.00	17.29-20.26	19.81	45.18	217.8-255.2	0.00	100.00
1.608-1.884	0.00	0.00	20.26-23.73	19.41	64.59	255.2-299.0	0.00	100.00
1.884-2.207	0.00	0.00	23.73-27.80	16.34	80.93	299.0-350.3	0.00	100.00
2.207-2.586	0.00	0.00	27.80-32.58	10.30	91.23	350.3-410.5	0.00	100.00
2.586-3.029	0.00	0.00	32.58-38.17	5.65	96.88	410.5-480.9	0.00	100.00
3.029-3.549	0.00	0.00	38.17-44.72	2.38	99.26	480.9-563.4	0.00	100.00
3.549-4.158	0.00	0.00	44.72-52.39	0.66	99.92	563.4-660.1	0.00	100.00
4.158-4.872	0.00	0.00	52.39-61.38	0.08	100.00	660.1-773.3	0.00	100.00
4.872-5.708	0.00	0.00	61.38-71.91	0.00	100.00	773.3-906.0	0.00	100.00
5.708-6.687	0.00	0.00	71.91-84.25	0.00	100.00	906.0-1061	0.00	100.00
6.687-7.834	0.00	0.00	84.25-98.71	0.00	100.00	1061-1243	0.00	100.00
7.834-9.179	0.00	0.00	98.71-115.6	0.00	100.00	1243-1457	0.00	100.00
9.179-10.75	0.07	0.07	115.6-135.4	0.00	100.00	1457-1707	0.00	100.00
10.75-12.59	1.61	1.68	135.4-158.7	0.00	100.00	1707-2000	0.00	100.00

Fig. 1. Droplet size profile of neutral electrolyzed water (NEW) emitted from the Vi-Killer® spraying device. Particle size distribution was measured in air using the Bettersizer 2000S (Dandong Bettersize Instruments Ltd.), a laser diffraction particle size analyzer. The analysis applied Mie scattering theory to a multimodal distribution model. Results showed that the median diameter (D50) was 21.02 µm, with more than 85% of the generated particles ranging between 10 and 30 µm.

Tab. 1. Number of aerobic bacteria in various indoor multiuse facilities and reduction afterair disinfection

Dog Café	Room type	Condition	No. of aerobic bacteria (CFU/m^3)			R*(%)
			Mean ± SD	Min	Max	
Dog Café A	Large Dog Hoteling Area	Before	125.7 ± 65.5	50	165	63.8
		After	51.7 ± 36.7	10	79	
	Small Dog Hoteling Area	Before	104.3 ± 19.6	86	125	57.6
		After	44.0 ± 14.5	30	59	
Dog Café B	Customer Service Area	Before	272.7 ± 183.7	99	465	59.2
		After	106.7 ± 62.5	43	168	
	Pet Grooming Area	Before	354.0 ± 384.6	122	798	60.4
		After	147.3 ± 169.5	46	343	
Dog Café C	Customer Service Area 1	Before	170.3 ± 145.3	46	330	57.5
		After	64.0 ± 40.3	20	99	
	Customer Service Area 2	Before	132.0 ± 129.6	20	274	45.5
		After	71.7 ± 66.4	10	142	
Total		Before	193.2 ± 187.1	20	798	57.3**
		After	80.9 ± 78.0	10	343	

Explanations: * – Average reduction rate; ** – $P < 0.05$

Tab. 2. Bacterial species identified before and after disinfection

Bacteria	No. of bacteria		
	Before	After	Total
Acinetobacter johnsonii	2	1	3
Acinetobacter junii	1	0	1
Acinetobacter lwoffii	5	2	7
Acinetobacter pittii	4	1	5
Acinetobacter ursingii	0	1	1
Aerococcus viridans	1	0	1
Bacillus altitudinis	9	4	13
Bacillus cereus	19	9	28
Bacillus circulans	3	2	5
Bacillus clausii	1	0	1
Bacillus firmus	1	1	2
Bacillus idriensis	1	0	1
Bacillus licheniformis	0	1	1
Bacillus megaterium	10	5	15
Bacillus simplex	3	1	4
Bacillus subtilis	5	4	9
Brevundimonas diminuta	0	1	1
Brucella sp.	1	0	1
Corynebacterium amycolatum	1	0	1
Corynebacterium auriscanis	1	0	1
Corynebacterium stationis	1	0	1
Cronobacter sakazakii	1	0	1
Empedobacter brevis	0	1	1
Enhydrobacter aerosaccus	2	0	2
Enterobacter cloacae	0	1	1
Enterococcus hirae	1	1	2
Escherichia coli	0	1	1
Exiguobacterium acetylicum	3	4	7
Exiguobacterium aurantiacum	1	1	2
Finegoldia magna	2	0	2
Klebsiella pneumoniae	0	3	3
Leclercia adecarboxylata	1	0	1
Lysinibacillus fusiformis	1	2	3
Macrococcus caseolyticus	8	3	11
Micrococcus luteus	49	22	71
Micrococcus lylae	1	0	1
Moraxella catarrhalis	1	0	1
Moraxella osloensis	1	0	1
Moraxella ovis	1	0	1
Neisseria animaloris	0	1	1
Neisseria flava	1	0	1
Neisseria weaveri	1	1	2
Neisseria zoodegmatis	1	0	1
Pantoea agglomerans	1	0	1
Paracoccus yeei	1	0	1
Paenibacillus provencensis	0	1	1
Pseudomonas aeruginosa	0	2	2
Rhizobium radiobacter	1	0	1
Solibacillus silvestris	0	1	1
Staphylococcus arlettae	1	0	1
Staphylococcus capitis	2	1	3
Staphylococcus cohnii	2	1	3
Staphylococcus epidermidis	4	4	8
Staphylococcus felis	3	1	4
Staphylococcus haemolyticus	2	4	6
Staphylococcus hominis	10	6	16
Staphylococcus intermedius	16	6	22
Staphylococcus pseudintermedius	10	6	16
Staphylococcus saprophyticus	1	0	1
Staphylococcus sciuri	0	1	1
Staphylococcus simulans	2	1	3
Staphylococcus urealyticus	1	0	1
Staphylococcus xylosus	0	3	3
Streptococcus equi	0	1	1
Unknown	93	58	151
Total	295	171	466

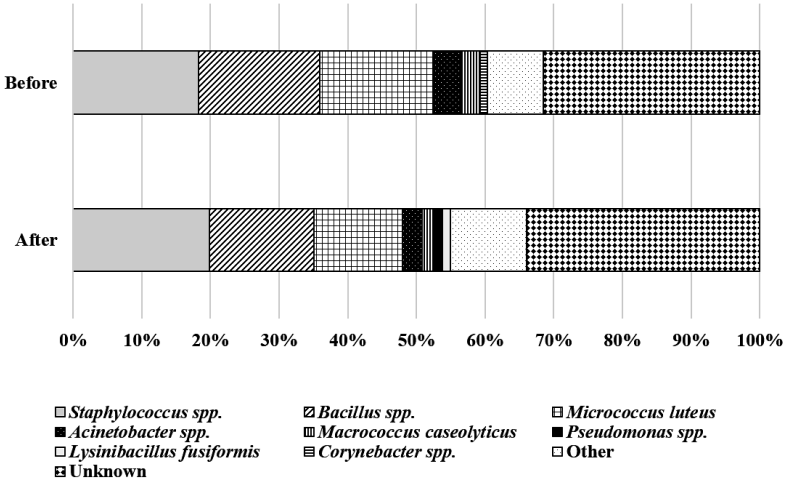


Fig. 2. Comparative analysis of airborne bacterial communities in three dog cafés pre- and post-disinfection. The stacked bar graphs present the relative abundances of airborne bacterial genera detected in the three dog cafés, comparing the samples collected before and after disinfection. Each bar represents the proportion of the dominant taxa within the bacterial community.

lates, 17.6%), *Micrococcus luteus* (49 isolates, 16.6%), *Acinetobacter* spp. (12 isolates, 4.1%), *Macrococcus caseolyticus* (8 isolates, 4.1%), and *Corynebacter* spp. (3 isolates, 2.7%). Minor taxa (< 1% each) and unidentified isolates accounted for 24 (8.1%) and 93 (31.5%) of the isolates, respectively. The major taxa detected after disinfection were *Staphylococcus* spp. (34 isolates, 19.9%), *Bacillus* spp. (26 isolates, 15.2%), *M. luteus* (22 isolates, 12.9%), *Acinetobacter* spp. (5 isolates, 2.9%), *M. caseolyticus* (3 isolates, 1.8%), *Pseudomonas* spp. (2 isolates, 1.2%), and *Lysinibacillus fusiformis* (2 isolates, 1.2%). Minor taxa (< 1% each) and unidentified isolates accounted for 24 (14.0%) and 58 (33.9%) of the isolates, respectively. These findings indicate that, although the total bacterial abundance declined, the relative community composition remained stable, suggesting that some taxa possessed higher environmental persistence.

ESKAPE pathogens and antimicrobial resistance. The antibiotic resistance profiles of the isolated ESKAPE pathogens are summarized in Table 3. Among 466 isolates, six colonies (1.3%) were identified as ESKAPE pathogens (Tab. 3), all in post-disinfection samples. These included *P. aeruginosa* (n = 2; PAF7-3 and PAC5-4) from dog café C and both *Enterobacter cloacae* (n = 1; ECD8-2) and *K. pneumoniae* (n = 3; KPD8-1, KPD9-1, and KPC2-6) from dog café B. Antimicrobial susceptibility testing revealed that each isolate was resistant to at least one antibiotic (Tab. 3). Both *P. aeruginosa* strains were resistant to AMP, CEP, FOX, and SXT. The *E. cloacae* isolates were resistant to CEP. Among the three *K. pneumoniae* strains, one was resistant (KPC2-6) only to AMP and COL, whereas the other two (KPD8-1 and KPD9-1) demonstrated multidrug resistance (MDR), with resistance profiles including β-lactams (CEP, FOX, and CRO) and fluoroquinolones (CIP) (Tab. 3).

In typical indoor environments, the TAB level is considered a key indicator of indoor air quality (50). The TAB concentrations vary depending on the purpose of the space, occupant density, hygiene conditions, temperature, ventilation levels, and frequency of access (1, 21, 41, 47). In vet clinics, concentrations of 57.1 to 291.3 CFU/m^3 (21), > 500 CFU/m^3 (50), and 1,000 ± 800 CFU/m^3 (31) have been reported. However, research on indoor air quality in non-medical commercial facilities, such as dog cafés, is scarce. In the present study, TAB concentrations prior to disinfection ranged from 104.3 to 354.0 CFU/m^3 and varied among cafés, with dog café B having higher levels (Tab. 1). These levels fall within a range generally considered to represent moderate microbiological contamination of indoor air, rather than severe contamination. Although there were differences in the contamination levels between spaces, we only included three cafés, limiting the generalizability of the results.

In environments where humans and companion animals coexist, airborne particulates originating from animal hair, saliva, and feces may be resuspended and act as major contributors to air contamination (11, 20, 60). In such settings, surface disinfection alone may be insufficient. Conventional air disinfection technologies such as ozone, steam and chemical sprays are of limited efficacy and may pose toxicity risks to humans and other animals under certain conditions (2, 46, 69). In contrast, the Vi-Killer® system disperses HOCl particles in the 10-30 μm range, allowing prolonged interaction with airborne pathogens. The system requires no installation and is safe to operate in occupied spaces, making it a practical solution for complex high-density environments such as dog cafés. In this study, application of the Vi-Killer® system resulted in a significant average TAB reduction rate of 57.3% ($p < 0.05$, Tab. 1), demonstrating its practical efficacy in improving air hygiene in real-world commercial spaces shared by humans and animals.

Previous studies have reported that HOCl dry fog does not induce cytotoxic or genotoxic effects at concentrations up to approximately 300 ppm, and that repeated exposure to aerosolized HOCl at approximately 100 ppm is not associated with adverse health outcomes in animal models (6, 37). However, adverse biochemical and oxidative responses following HOCl inhalation have also been reported under specific experimental conditions, including pronounced oxidative stress-related markers after single exposure to gaseous HOCl in animal models (43). These findings indicate that inhalation effects may vary depending on exposure form and conditions, and that the currently available inhalation safety data remain limited. Based on the overall body of evidence, the present study employed HOCl at an approximately 100 ppm, representing a conservative concentration considered effective and compatible with practical indoor application. Consistent with this view, recent reviews have highlighted the potential of HOCl as a respiratory antiseptic while emphasizing the need for continued accumulation of long-term inhalation safety data across diverse exposure scenarios (68).

The system generates aerosol particles within the WHO-recommended size range (10-30 μm) while maintaining the non-toxic,

Tab. 3. Antibiotic resistance profiles of ESKAPE isolates

ID	Species	Isolated sites	Before/After	Antibiotic resistance[1]
PAF7-3	*Pseudomonas aeruginosa*	Dog Café C	After	AMP, CEP, FOX, SXT
PAC5-4	*Pseudomonas aeruginosa*	Dog Café C	After	AMP, CEP, FOX, SXT
ECD8-2	*Enterobacter cloacae*	Dog Café B	After	CEP
KPD8-1	*Klebsiella pneumoniae*	Dog Café B	After	AMP, CEP, FOX, CRO, CIP
KPD9-1	*Klebsiella pneumoniae*	Dog Café B	After	AMP, CEP, CRO, CIP, COL
KPC2-6	*Klebsiella pneumoniae*	Dog Café B	After	AMP, COL

Explanations: [1] – AMP – ampicillin; CEP – cephalothin; FOX – cefoxitin; CRO – ceftriaxone; CHL – chloramphenicol; CIP – ciprofloxacin; COL – colistin; KAN – kanamycin; SXT – trimethoprim-sulfamethoxazole

non-corrosive, and non-irritating properties of HOCl. NEW containing HOCl exhibits natural, non-toxic, broad-spectrum antimicrobial activity and can significantly reduce the number of pathogenic viruses and bacteria within a short period (61, 70). In previous studies, we confirmed the bacterial inactivation efficacy of this system in confined chambers and public-use facilities (5, 6). Reducing the bacterial load not only improves hygiene but also contributes to the control of opportunistic and antibiotic-resistant pathogens.

Bacterial community analysis of air samples from the three dog cafés revealed that *M. luteus*, *Bacillus* spp., and *Staphylococcus* spp. were the dominant taxa before and after disinfection (Fig. 2 and Tab. 2). The relative composition of the bacterial communities showed minimal changes between the pre- and post-disinfection states (Fig. 1). This may be attributed to the non-selective antimicrobial spectrum of HOCl, which reduces the overall bacterial density rather than targeting specific taxa (40, 51). The dominant taxa, *M. luteus*, *Bacillus* spp., and *Staphylococcus* spp., are all Gram-positive bacteria that exhibit high survivability under dry indoor conditions owing to the high peptidoglycan content and endospore formation in *Bacillus* (7, 12, 62). These three taxa were also found to be dominant in veterinary hospitals (21, 42). *Micrococcus*, although not highly pathogenic, is a common skin commensal that readily disperses in the air (21, 26). *Staphylococcus* is a core HAI pathogen associated with dermatological infections in both humans and companion animals and is frequently associated with antibiotic resistance (29, 48, 55). *Bacillus cereus* accounted for 28 of the 466 isolates in our study (6.0%, Tab. 2) and is widely recognized as a foodborne pathogen of clinical relevance (15). Unlike animal hospitals, dog cafés provide food and beverages for humans, highlighting the risk of cross-contamination with foodborne bacteria (60).

The WHO has designated ESKAPE pathogens as priority targets for antimicrobial resistance surveillance owing to their high clinical relevance and MDR potential (52). Given the detection of ESKAPE pathogens in dogs involved in nonclinical, dog-related contact activities such as animal-assisted interventions, recent studies have highlighted the importance of considering regular monitoring and infection control in such settings (52). ESKAPE organisms are globally recognized as major MDR pathogens (8). Aerosols are increasingly acknowledged for their role as vectors in the transmission of antibiotic-resistant genes and opportunistic pathogens, making the airborne presence of MDR ESKAPE bacteria a serious public health concern (25, 56).

In the present study, three ESKAPE pathogens were identified: *P. aeruginosa*, *K. pneumoniae*, and *E. cloacae* (Tab. 3). All MDR isolates identified were strains of *K. pneumoniae*, a nosocomial pathogen that often exhibits MDR phenotypes against β-lactams and other broad-spectrum antibiotics, along with *A. baumannii* (55). Similar to *Staphylococcus* spp., *Pseudomonas* is a common cause of skin infections and otitis externa in companion animals and is strongly associated with antibiotic resistance, leading to poor treatment response and recurrence (29, 52). Notably, *P. aeruginosa* is highly persistent in healthcare environments (69) and is frequently isolated from dogs with post-grooming furunculosis (49). *Enterobacter* spp., harboring MDR genes, are widely distributed in the environment and have been linked to a range of nosocomial infections (19, 30, 52). Although *S. aureus* was not isolated in this study, 88 strains of *Staphylococcus* spp., including *S. epidermidis*, *S. felis*, and *S. pseudintermedius* (Tab. 2), which are associated with pet-related infections, were identified. Non-*aureus Staphylococcus* species are not classified as ESKAPE; however, they are closely related to antibiotic resistance and skin or ear infections in dogs and may pose health risks to both humans and companion animals (29, 48). Thus, further risk assessments, including toxin gene analysis, antibiotic resistance profiling, and subtyping, are required for these isolates. Interestingly, all ESKAPE isolates in this study were detected only after disinfection. However, as previously discussed, HOCl has a non-selective broad-spectrum antimicrobial effect, and this observation should be interpreted with caution.

Companion animal-related HAIs have been predominantly attributed to transmission by clinical staff, surface contact, and host-associated microbes within veterinary hospitals (21, 48, 55, 59, 69). However, airborne transmission has consistently been reported (16). As airborne particles that carry microorganisms can remain suspended in the air for several days (16), effective infection control strategies must include air disinfection in addition to surface cleaning. Antibiotic-resistant pathogens such as MRSA and *Clostridium difficile* can disseminate through the air (9, 34), and airborne exposure to MRSA in particular has been linked to nasal colonization (3).

In the present study, opportunistic and MDR pathogens, including ESKAPE, were detected in the air sampled from dog cafés (Tab. 3). Previous studies have reported the presence and transmission of harmful or antibiotic-resistant bacteria in pet daycare, grooming salons, and cafés (17, 49, 53, 60). However, these studies mostly focused on surfaces or fomites, leaving gaps in our understanding regarding air contamination. The detection of such pathogens in non-clinical pet facilities highlights the need for tailored hygiene and cleaning protocols (17, 45). Disinfection strategies that go beyond basic cleaning significantly reduce bacterial levels (22). Considering the airborne detection of MDR organisms in this study, indoor air should also be considered in the hygiene management strategies of these facilities. The surface disinfection methods commonly used in veterinary settings are limited in their ability to eliminate pathogens given the structural complexity of bacterial communities (39, 58). In contrast, spray-based technologies can more comprehensively distribute disinfectants over entire spaces that persist as aerosols for extended durations (6). Therefore, spray-based air disinfection is a viable alternative.

This study provides meaningful understanding of the practical assessment of airborne pathogens and application of an HOCl-based air disinfection system in real-world commercial environments shared by humans and companion animals. However, the study had some limitations that should be acknowledged. First, the spaces investigated were not standardized, making direct comparisons between the facilities challenging. Unlike veterinary hospitals with clearly defined zones, such as examination rooms, treatment areas, and operating theaters, dog cafés vary widely in their spatial layout. Furthermore, owing to the operational constraints of running businesses, disinfection experiments were conducted only in areas approved by the facility operators during business hours. As modern dog cafés increasingly integrate services such as hoteling, grooming, and training, researchers should aim to adopt more structured experimental designs that cover diverse subspaces. Second, environmental factors, such as visitor density, animal occupancy, and ventilation, which may have influenced TAB levels, were not controlled for. Third, this study relied on culture-based analyses, which may have failed to detect low-abundance or non-culturable bacteria. Furthermore, even among culturable isolates, 32.4% (151 out of 466; Tab. 2) remained unidentified after MALDI-TOF analysis, highlighting limitations in the identification step of culture-based approaches. Given the recent shift toward next-generation sequencing methods in indoor microbiome research (23), future studies should incorporate molecular techniques for more comprehensive community profiling.

Overall, this study provides field-based evidence that a portable hypochlorous acid spray system can effectively reduce airborne bacterial loads in dog cafés. However, a limitation should be acknowledged. Environmental parameters such as ambient temperature, relative humidity, occupancy density, and ventilation status were not quantitatively recorded during the sampling sessions. These factors are known to influence the persistence, dispersion, and removal of airborne microorganisms in indoor environments (1, 18, 41), and their omission may have contributed to variability in measured bacterial concentrations. Future studies incorporating continuous monitoring of these parameters would enable a more refined interpretation of air hygiene dynamics and strengthen causal inferences regarding disinfection performance.

We investigated the indoor air quality of three dog cafés in South Korea and evaluated the effectiveness of a portable HOCl-based spray disinfection system at reducing airborne bacterial concentrations. The system demonstrated practical potential for improving indoor air hygiene by producing uniform disinfectant aerosols that persisted in air and significantly reduced TAB concentrations. Although MDR ESKAPE organisms were detected during monitoring, their targeted efficacy against specific pathogens was not confirmed. The interaction structure in urban dog cafés facilitates the bidirectional transmission of resistant bacteria between humans and pets, potentially contributing to community-wide dissemination (28, 60). This study underscores that dog cafés, beyond being leisure spaces, may serve as environments for interspecies pathogen exchange, and our findings highlight the need for air disinfection to be included in infection control protocols in such facilities.

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