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Original Article

A Study on Concentration, Identification, and Reduction of Airborne Microorganisms in the Military Working Dog Clinic

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ABSTRACT

Background: The study was planned to show the status of indoor microorganisms and the status of the reduction device in the military dog clinic.
Methods: Airborne microbes were analyzed according to the number of daily patient canines. For identification of bacteria, sampled bacteria was identified using VITEK®2 and molecular method. The status of indoor microorganisms according to the operation of the ventilation system was analyzed.
Results: Airborne bacteria and fungi concentrations were 1000.6 ± 800.7 CFU/m^3 and 324.7 ± 245.8 CFU/m^3. In the analysis using automated identification system, based on fluorescence biochemical test, VITEK®2, mainly human pathogenic bacteria were identified. The three most frequently isolated genera were *Kocuria* (26.6%), *Staphylococcus* (24.48%), and *Granulicatella* (12.7%). The results analyzed by molecular method were detected in the order of *Kocuria* (22.6%), followed by *Macrococcus* (18.1%), *Glutamicibacter* (11.1%), and so on. When the ventilation system was operated appropriately, the airborne bacteria and fungi level were significantly decreased.
Conclusion: Airborne bacteria in the clinic tend to increase with the number of canines. Human pathogenic bacteria were mainly detected in VITEK®2, and relatively various bacteria were detected in molecular analysis. A decrease in the level of bacteria and fungi was observed with proper operation of the ventilation system.

1. Introduction

Military dogs are an essential presence in the military, which is used in various fields such as drug detection, missing persons detection, and explosives detection [1]. Korean military operates a dedicated military dog medical facility to support them, and veterinary officers trained in the military medical facility provide medical care. Veterinary officer in the facility can be exposed to various microorganisms during medical process. Microbes causing nosocomial infection among both human and animals such as *Klebsiella*, *Serratia*, *Acinetobacter*, and *Staphylococcus* have been detected in the samples from animal hospitals [2,3]. The risk of exposure to virulent bacteria such as Methicillin-resistant *Staphylococcus aureus*, *Clostridioides difficile*, and *Chlamydiosis*, among animal practitioners is constantly being warned [4]. A study of Korean military dogs reported that antibiotic-resistance *Enterococcus faecalis* and *Enterococcus faecium* were detected in the feces of dogs [5].

In the previous study, the association of high bacterial concentration exposure and respiratory symptoms in animal house was reported [6], so it is necessary to understand the concentration and identification of indoor bacteria in the office. Also, reduction measures of indoor microorganisms in the dog care facility to preserve the health of the veterinarian officer are needed. Therefore, a study is needed to create a sanitary environment of veterinarian facility to protect the veterinarians. This study aims to show the concentration level, identification of microorganisms and check the effect of operation of installed ventilation system on airborne microbes in the military dog clinic.

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2. Materials and methods

2.1. Participants

This study was conducted in one military examination room in the military between September and December 2019, and July 2020. Generally, in the military dog clinic room, the 1–6 patient canines were treated in one room simultaneously. The treatment of canine was done by two session per day (morning session and afternoon session). First, airborne bacteria and airborne fungi were sampled, cultured, and counted in only clinic room, 1 hour after the starting treatment session of military dogs without operating ventilation system. Then, the identification of bacteria was done with two samples.

To evaluate the effect of the ventilation facility, we re-collected airborne bacteria and fungi samples from treatment room, aisle, and dog kennels, according to operating conditions of ventilation system. Two bacteria and fungi sample were collected per each session, before and after operating the ventilation system. We collected the first airborne bacteria and fungi samples for each session ("before" sample) after canines were treated for an hour in clinic room. The first sample was collected in the clinic room, aisle, and dog kennels. Then, the ventilator system in clinic room was operated for an hour, and second samples ("after" sample) were collected. The second samples were collected in the clinic room and aisle. The difference of pressure was measured between clinic room, aisle, and dog Kennels. Sampling conditions such as temperature and humidity are attached in Appendix A.

2.2. Sampling and identification method

Airborne bacteria and airborne fungi were carried out at the center of each measurement site, 1.5 meters high. Each one agar plate of bacteria and fungi sample was collected per each session. To use a method of inhaling a certain amount of air and colliding with the medium (collision method), an airborne bacteria meter (Air ideal, bioMérieux, Marcy-l'Étoile, France) was used. Range from 5 to 15 μm size of particles, with a median size of particles at ~ 13 μm can be collected [7]. Tryptic soy agar medium (Kisanbio, Seoul, Korea) was used for floating bacteria, and Potato Dextrose Agar medium (Kisanbio, Seoul, Korea) containing antibiotic (streptomycin) was used for airborne fungi. A total of 200 L was inhaled at a flow rate of 100 ml/min each. Bacteria were cultured at 25 °C for 48 hours and fungi at 20 °C for 120 hours. After cultivation, colonies were counted and divided by the amount of air, expressed as the concentration of total suspended bacteria and fungi (CFU/m^3).

The collected and cultured bacteria on triptic soy agar medium were screened according to the visual characteristics, inoculated into a new culture medium (Brain Heart Infusion Agar Plate, Kisanbio, Seoul, Korea), and cultured at 37 °C for 24 hours, followed by Gram staining. The identification of bacteria cultured in two Brain Heart Infusion agar was analyzed by two methods using automated susceptibility test systems (VITEK®2, bioMérieux, Marcy-l'Étoile, France) and molecular identification, respectively.

VITEK®2 system is antimicrobial susceptibility testing, which automatically performs all the steps required for identification after a primary treatment has been prepared and standardized. VITEK®2 read each kinetic analysis every 15 min. Also, optical system with multichannel fluorimeter, photometer was used to analyze fluorescence, turbidity, and colorimetric signals [8]. Among the automated susceptibility test with VITEK®2, the result with excellent, very good, good, and acceptable (>85% probability) was collected. Gram-negative and Gram-positive bacteria were analyzed using ID-GN (version 5.01) and ID-GP VITEK card (version 5.01), respectively.

Sanger sequencing is performed for molecular identification. Genomic DNA isolation was performed by Chelex boiling method, using Chelex bead (Chelex® 100 Chelating Resin, Bio-Rad, Hercules, CA, USA) with water bath boiling [9]. Polymerase chain reaction (PCR) with amplification and sequencing of the 16S ribosomal RNA gene was applied. All PCR assays were performed on a Verti R TM 96-well Thermal Cycler (Thermo Fisher Scientific, BRIMS, Cambridge, MA, USA), with 27F primer (AGA GTT TGA TCC TGG CTC AG) and 1492R primer (GGT TAC CTT GTT ACG ACT T). PCR mixture was made using Solg™ EF-Taq DNA polymerase (Solgent, Daejeon, Korea). The amplification was carried out under the following conditions: 95 °C for 15 minutes (initial denaturation) and 30 cycles of 95 °C for 20 seconds (denaturation), 50 °C for 40 seconds (annealing), 72 °C for 90 seconds (extension), and one cycle of 72 °C for 5 minutes (final extension). PCR products were sequenced by dye-terminator sequencing (BigDye® Terminator v3.1 Cycle Sequencing kits, Thermo Fisher Scientific) and DNA analyzer (capillary 50cm) (ABI PRISM 3730XL, Thermo Fisher Scientific). Initial denaturation was done at 96 °C for 1 minute by 1 cycle. Denaturation, annealing, and final extension were performed in 30 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 seconds. Sequence assembly and The Basic Local Alignment Search Tool (BLAST) was performed for sequence searching. The GenBank database was used. The result is described as trimmed data with collect value over 99%.

2.3. Facility structure and ventilation system

The military dog clinic room has a structure in which clinic room, aisle, and dog kennels are in a straight line. Each space is separated by doors, generally used with the entire door open. The room size is 6 m × 6 m × 2.5 m. The size of aisle is 12 m × 1.5 m × 2.5 m. Ventilation facilities are installed on the ceiling of the treatment room and aisle, and there is no separate ventilation system for dog kennels. A total of six blowers (3,000 m^3/hour) are installed in the ventilation system, and three are connected to the air supply and three to the exhaust (Fig. 1). There is no air purification system installed in the ventilation system. Outdoor air is directly blown through the ventilation system. The difference of pressure was measured by differential pressure measuring instrument (Testo 521, Testo, Lenzkirch, Germany) between clinic room, aisle, and dog kennels. When the passage was blocked, the pressure difference was measured by placing a Pitot tube under the blocked door.

2.4. Statistical analysis

The differences of airborne bacteria and fungi according to the number of treatments when the ventilation system was not operated was analyzed using ANOVA. The differences between airborne bacteria and fungi levels were compared by Wilcoxon signed-rank test. A p value was calculated. A p value of less than 0.05 were regarded as statistically significant. R 3.5.3 was used for statistical analysis.

3. Results

3.1. Airborne microorganism level in clinic room

Initially, we sampled and counted airborne bacteria and fungi for nine sessions, three times each for the numbers of patient canines in the clinic room. The results are shown in Table 1. The average total bacteria and fungi in clinic room was 1000.6 ± 800.7 CFU/m^3 and 324.7 ± 245.8 CFU/m^3. When the number of canine patients was 1–2, the airborne bacteria level was

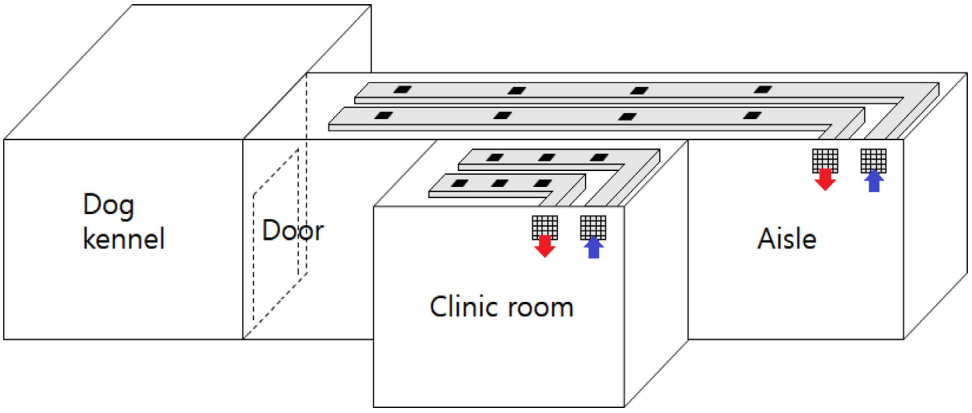

Fig. 1. Schematic diagram of the military dog clinic. The clinic room is connected to the outside dog cage through the aisle, and ventilation system is installed on the ceiling of the room.

Table 1
Level of indoor airborne fungi and bacteria in the clinic room according to the number of patient dogs

Canines (numbers)	Microorganisms				Condition of sampling point				
	Airborne bacteria (CFU/m^3)*		Airborne fungi (CFU/m^3)		Temperature (°C)		Relative humidity (%)		Samples (Numbers)
1–2	284.0	±46.1	328.7	±181.1	21.4	±2.1	65.5	±6.7	3
3–4	855.0	±778.5	295.3	±131.0	21.7	±2.8	43.8	±4.8	3
5–6	1,862.70	±198.7	459.3	±173.9	21.1	±1.3	45.9	±8.2	3
Total	1,000.60	±800.7	324.7	±245.8	21.4	±1.9	51.1	±11.0	9

Numbers are presented as mean ± standard deviation.
* Airborne bacteria was statistically different according to the number of patient dogs ($p < 0.05$). ANOVA was used to calculated p value.

284.0 ± 46.1 CFU/m^3, and the airborne fungi was 328.7 ± 181.1 CFU/m^3. When the number of dog patients was 3–4, airborne bacteria and fungi level was 855.0 ± 778.5 CFU/m^3 and 295.0 ± 131.0 CFU/m^3, and for 5–6, it was 1,862.7 ± 198.7 CFU/m^3 and 459.3 ± 173.9 CFU/m^3. There was a statistical relationship between the number of patient dog and floating bacteria ($p < 0.05$).

3.2. Bacterial identification

Tables 2 and 3 shows the results of analyzing the collected bacteria by VITEK®2 and molecular identification. The three most frequently isolated genera were *Kocuria* (26.6%), *Staphylococcus* (24.5%), and *Granulicatella* (12.7%). The three most frequently isolated species were *G. elegans* (12.7%), *S. sciuri* (11.4%), and *K. kristinae* (10.1%). As a result of molecular identification, the genera with the highest concentration were *Kocuria* (22.6%) followed by *Macrococcus* (18.1%) and *Glutamicibacter*, (11.1%). The most frequent species was *K. salina* (10.4%), followed by *C. oceanosedimentum* (7.3%), *G. protophormiae* (7.0%), and so on.

3.3. Evaluation of the ventilation system to reduce airborne microorganisms

Comparing the concentration of airborne bacteria and fungi before and after operating ventilation system treatment of the canines without operating the ventilation system, the difference of airborne bacteria and fungi level in the treatment was for -89.5 ± 374.08 and 97.5 ± 439.0 CFU/m^3, although statistically not significant. The bacteria and fungi concentration of the aisle was increased by 227.5 ± 443.49 CFU/m^3 and 307.5 ± 302.0 CFU/m^3. The pressure differences between clinic room and aisle was -0.4 ± 0.7. The pressure differences between aisle and kennels was -0.3 ± 0.7.

Table 2
The results of identification of bacteria in the military dog clinic analyzed using VITEK®2

Genus	Species	Colony Count (numbers)		Differential Fraction (%)	
Kocuria		63		26.6	
	K. kristinae		24		10.1
	K. rhizophila		24		10.1
	K. rosea		9		3.8
	K. varians		6		2.5
Staphylococcus		58		24.5	
	S. cohni		3		1.3
	S. kloosii		5		2.1
	S. lentus		20		8.4
	S. sciuri		27		11.4
	S. vitulinus		3		1.3
Granulicatella		30		12.7	
	G. elagans		30		12.7
Micrococcus		24		10.1	
	M. luteus		24		10.1
Sphingomonas		6		2.5	
	S. paucimobiills		6		2.5
Alloiococcus		3		1.3	
	A. otis		3		1.3
Bacillus		3		1.3	
	B. simplex		3		1.3
Photobacterium		3		1.3	
	P. damselae		3		1.3
Rhizobium		3		1.3	
	R. radiobacter	3			1.3
Unidentified		44		18.6	18.6
Total		237	237	100.0	100.0

Table 3
The results of identification of bacteria in the military dog clinic analyzed by molecular identification

Genus	Species	Colony Count (numbers)	Differential Fraction (%)
Kocuria		65	22.6
	K. arsenatis	10	3.5
	K. gwangalliensis	10	3.5
	K. rhizophila	15	5.2
	K. salina	30	10.4
Macrococcus		52	18.1
	M. bovicus	12	4.2
	M. carouselicus	3	1.0
	M. epidermidis	3	1.0
	M. esteraromaticum	6	2.1
	M. foliorum	6	2.1
	M. hydrocarbonoxydans	6	2.1
	M. testaceum	6	2.1
	M. aloeverae	5	1.7
	M. yunnanensis	5	1.7
Glutamicibacter		32	11.1
	G. protophormiae	20	6.9
	G. soli	12	4.2
Curtobacterium		27	9.4
	C. plantarum	3	1.0
	C. albidum	3	1.0
	C. oceanosedimentum	21	7.3
Bacillus		18	6.3
	B. drentensis	9	3.1
	B. infantis	3	1.0
	B. niacini	6	2.1
Dietzia		12	4.2
	D. kunjamensis	12	4.2
Corynebacterium		9	3.1
	C. xerosis	6	2.1
	C. efficiens	3	1.0
Streptomyces		7	2.4
	S. flavoviridis	3	1.0
	S. hirsutus	4	1.4
Psychrobacter		6	2.1
	P. faecalis	3	1.0
	P. pulmonis	3	1.0
Planococcus		6	2.1
	P. halocryophilus	3	1.0
	P. versutus	3	1.0
Pseudomonas		6	2.1
	P. coleopterorum	6	2.1
Terrabacter		6	2.1
	T. tumescens	6	2.1
Janibacter		6	2.1
	J. limosus	6	2.1
Acinetobacter		6	2.1
	A. lwoffii	6	2.1
Brevundimonas		6	2.1
	B. vesicularis	6	2.1
Dyella		6	2.1
	D. japonica	3	1.0
	D. kyungheensis	3	1.0
Lactobacillus		3	1.0
	L. thailandensis	3	1.0
Pantoea		3	1.0
	P. ananatis	3	1.0
Pseudarthrobacter		3	1.0
	P. chlorophenolicus	3	1.0
Unidentified		9	3.1
Total		288 288	100.0 100.0

Airborne microbe level was measured in the same way after blocking the passage because of suspected influx of bacteria from kennels. As a result of the measurement, it was confirmed that the concentration of bacteria and fungi in the treatment room decreased for 950 ± 730.3 CFU/m^3 ($p < 0.05$) and 633.5 ± 724.8 CFU/m^3 ($p < 0.05$) after ventilation in clinic room and 315.3 ± 498.9 CFU/m^3 ($p < 0.01$) and 363.5 ± 417.8 CFU/m^3 ($p < 0.05$) in the aisle. The pressure difference between clinic room and aisle was -1.8 ± 1.6. The pressure difference between blocked aisle and kennels was -2.2 ± 1.0 (Table 4).

4. Discussion

This study is about indoor air quality, focused on airborne microorganisms in the military dog clinic facility where research has not been actively conducted. In this study, the measurement and analysis of suspended microbial concentrations in military care facilities were conducted. The airborne bacteria in the military dog treatment facility were correlated with the number of the treatment of canines. The maximum concentration of the bacteria was over 1,000 CFU/m^3, the recommended reference for indoor air in many countries [10]. As a result of identification of bacteria by VITEK®2, mostly human pathogenic bacteria were identified. In a molecular identification, relatively diverse bacteria such as nonhuman virulent dog pathogens, plant pathogen, etc. have been also identified. The ventilation system operation cannot decrease the concentration of the airborne bacteria level when the passage to the kennels where the concentration of the floating bacteria was higher was opened. The operation of the ventilation after blocking the passage successfully decreased the concentration of the airborne microbes in the clinic room.

In the past study of indoor air quality in animal hospitals, *Micrococcus* (36.6%), *Corynebacterium* (16.8%), *Bacillus* (16.0%), and *Staphylococcus* (14.5%), etc. were detected as a result of identification of airborne bacteria and up to 500 CFU/m^3 [2]. Chen et al. [3] reported that an average of 635–1,554 CFU/m^3 fungi and 458–1,672 CFU/m^3 bacteria were detected in each hospital in Taiwan's animal hospital. Studies on animal living facilities have been reported on concentrations of suspended bacteria in stables, barns, and swine, and high concentrations of airborne bacteria were detected [11,12]. Several studies have shown that high airborne microbe level can be harmful to the human health. Heederik et al. [6] showed a negative correlation between endotoxin exposure and forces expiratory volume in one second (FEV_1) in pig farmers. Also, when exposed to total high concentrations of bacteria in the air, increasing the frequency of shortness of breath, heavy perspiration, and clogged noise was shown. Studies conducted on automobile production, plant machine operators showed significant correlation between total airborne bacteria and phlegm [6]. Therefore, it should be taken into account that exposure to high bacteria may adversely affect the health, especially the respiratory system. In this study, the mean of the airborne bacteria level was relatively high compared with reference value in the clinic room without ventilation system. Therefore, for veterinarian health, appropriate reduction measures of microbes are necessary.

Table 4
Levels of airborne microorganisms before and after operation of the ventilation system in military dog clinic

Door status	Sampling Location	Sample (number)	Pressure difference (Pa)	Ventilation status	Airborne bacteria (CFU/m^3)*	*p value*†	Airborne Fungi (CFU/m3)*	*p value*†
Open	Dog Kennel	8	—	Before	1400.0 ± 1057.6	—	1156.0 ± 708.3	
	Clinic room	8	-0.4 ± 0.7	Before	1811.5 ± 1395.8		1190.8 ± 695.2	
		8		After	1722.0 ± 1122.4		1288.3 ± 867.2	
				After-Before	-89.5 ± 374.08	0.69	97.5 ± 439.0	0.41
	Aisle	8	-0.3 ± 0.7	Before	853.5 ± 609.9		594.0 ± 344.7	
		8		After	1081.0 ± 860.4		901.5 ± 515.4	
				After-Before	227.5 ± 443.49	0.20	307.5 ± 302.0	0.01
Closed	Dog Kennel	8	—	Before	1928.8 ± 1007.9	—	1235.8 ± 675.5	
	Clinic room	8	-1.8 ± 1.6	Before	1720.0 ± 1142.7		1087.5 ± 857.3	
		8		After	770.0 ± 519.87		454.0 ± 230.6	
				After-Before	-950.0 ± 730.3	<0.05	-633.5 ± 724.8	<0.05
	Aisle	8	-2.2 ± 1.0	Before	1037.0 ± 886.66		850.0 ± 533.5	
		8		After	721.8 ± 520.0		486.5 ± 281.0	
				After-Before	-315.3 ± 498.9	<0.01	-363.5 ± 417.8	<0.05

* Each number is presented as mean ± standard deviation.
† A *p* value was calculated by Wilcoxon signed rank test.

In several studies that conducted the purification of suspended bacteria in indoor air quality in Korea, an automated identification method using VITEK®2 was used for bacteriological identification [13–15]. However, Wolmarance et al. [16] reported that VITEK®2 has unstable results for microbial analysis of environmental samples in Gram-positive bacteria and is particularly difficult to qualitatively bacillus. Delmas et al. [17] reported that 93.3% of clinical specimens could be identified in the identification of bacteria in *Staphylococci* using VITEK®2 gram positive card, but only 73% of environmental specimens. To complement VITEK®2, molecular identification was also performed. In our study, most of the bacteria detected with VITEK®2 were human pathogens. For example, *Kocuria* were detected in both VITEK®2 and molecular identification. However, four identified species detected in VITEK®2, *K. kristinae* [18], *K. rhizophilia* [19], *K. rosea* [20] and *K. varians* [21], have been reported as human pathogens. However, *K. gwangalliensis*, *K. arsenates, and K. salina*, the species identified by molecular analysis, have little evidence as human pathogen. Most of the other bacteria detected in VITEK®2, including *S. cohni*, *S. kloosi* [22], *S. lentus* [23], *S. sciuri* [24], *G. elagans* [25], *M. luteus* [26], and so on, have evidence of human infection. In the molecular identification, human pathogen like *K. rhizophilia* was also detected. However, nonhuman pathogenic bacteria, such as *Macrococcus* [27], were also detected. Interestingly, *M. bovicus* and *M. carouselicus* [28] were known as dog pathogen. Also, *Glutamicibacter* [29], not human pathogenic but often found in ecosystems, or *Curtobacterium* [30], mostly found in plants, etc., were identified. Species such as *Macrococcus*, *Glutamicibacter*, and *Curtobacterium* identified by molecular analysis did not exist in the database of VITEK®2. Although it is difficult to directly compare the results because the sampling was conducted at the different time, the identification result of VITEK®2 shows mostly human pathogenic bacteria comparing to molecular identification. Also, it has a higher unidentified rate than PCR (18.6 % vs. 3.1 %). It seems likely that nonhuman pathogenic bacteria from indoor air can be misclassified or unclassified among VITEK®2 analysis. More attention should be paid to interpreting the results of bacterial analysis of environmental samples using VITEK®2 than molecular identification.

Recently, interest in a reduction device through a ventilation facility for indoor microorganisms is increasing [31–34]. The office is adjacent to the kennels where the canines reside. When the door of the kennels is opened with operating the ventilation system of the clinic room, the concentration of airborne bacteria and fungi increased. This seems to be due to the introduction of air from the kennels with high concentration of airborne microorganisms because of the imbalance of air supply and exhaust. The clinic room seems to form negative pressure by operating the ventilation system. After the passage is blocked, the inflow of outside air from kennels decreases, and the negative pressure in the room and aisle appears to increase. When the door was closed and the ventilation system is operated, the concentration of microorganisms was reduced properly. Therefore, in the operation of ventilation facility, it is necessary to consider preventing the inflow of contaminated air considering the direction of air inflow. One study on the air conditioning facilities in the medical operating room reported that it is possible to supply fresh air only by changing the design without increasing the large facility cost [35]. The results of this study also showed that the effect of the ventilation system can be improved through subtle and appropriate management. The average airborne bacterial and fungi after the proper ventilation operation was confirmed statistically significant, also the level to be below the standard reference value.

The limitations of this study are that analysis using VITEK®2 and analysis molecular analysis are performed by separate samples collected on different days, so that results cannot be directly compared. However, through the literature review of the identified bacteria and comparing unidentified ratios from the results of the two methods, it can be indirectly estimated that the proportion of misclassified and unidentified bacteria sampled in indoor air using VITEK®2 method was higher than molecular method. In the further research, the result of VITEK®2 and molecular identification must be compared by same sample and colony. If possible, MALDI-TOF/MS, next-generation sequencing, or various biochemical identification methods should be done for more precise identification. In addition, in the initial evaluation of the military dog clinic, the concentration of airborne bacteria was shown to be relatively higher than fungi. Therefore, we focused on the analysis of bacteria, and we did not carry out the identification of fungi. In further studies, identification fungi in various ways should be done. In this study, only the reduction of bacteria in the military dog clinic through appropriate operation of the installed ventilation system was shown. In the future study, it is necessary to propose an engineering method that can improve indoor air quality with in depth analysis of ventilation systems such as indoor air quality modeling or simulation method. Comparative analysis of research on indoor environments in animal treatment facilities other than military dog treatment facilities should also be conducted.

In conclusion, we measured high levels of airborne microbes, especially bacteria, and identified several human pathogenic bacteria in the military dog clinic. Relatively high airborne bacteria level exceeding the reference value were observed. Human pathogenic bacteria were mainly identified in VITEK®2, and various bacteria, including nonhuman pathogenic bacteria, were identified in molecular identification. Therefore, it was considered that countermeasures for reducing airborne microbes are needed. Because ventilation system operation with inappropriate way, with opening the passage through dog kennels, the level of airborne microbes is not decreased. After performing a simple manage to prevent the influx of microbes from the kennels, the effectiveness of the ventilation system was re-evaluated. As a result, statistically significant reduction of airborne bacteria and fungi level was observed, and it was possible to reduce bacteria below a reference value in the current system.

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Conflicts of interest

All authors have no conflict of interest including financial or consultant, institutional and other relationship in this study.

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None.

Appendix A. The sampling environment and condition of every sample used for the study

Sample ID	Ventilation	Site	Temperature (°C)	Relative humidity (%)	Canines (numbers)	Door	Date	Plate
1	None	Clinic	23.8	67.8	1	Opened	17th, September 2019	TSA
2	None	Clinic	23.8	67.8	1	Opened	17th, September 2019	PDA
3	None	Clinic	24.8	41.1	3	Opened	23rd, September 2019	TSA
4	None	Clinic	24.8	41.1	3	Opened	23rd, September 2019	PDA
5	None	Clinic	22.3	55.2	6	Opened	6th, October 2019	TSA
6	None	Clinic	22.3	55.2	6	Opened	6th, October 2019	PDA
7	None	Clinic	21.2	42.4	6	Opened	8th, October 2019	TSA
8	None	Clinic	21.2	42.4	6	Opened	8th, October 2019	PDA
9	None	Clinic	20.8	49.4	4	Opened	14th, October 2019	TSA
10	None	Clinic	20.8	49.4	4	Opened	14th, October 2019	PDA
11	None	Clinic	19.8	67.0	2	Opened	12th, November 2019	TSA
12	None	Clinic	19.8	67.0	2	Opened	12th, November 2019	PDA
13	None	Clinic	20.5	55.8	2	Opened	25th, November 2019	TSA
14	None	Clinic	20.5	55.8	2	Opened	25th, November 2019	PDA
15	None	Clinic	19.5	41.0	4	Opened	3rd, December 2019	TSA
16	None	Clinic	19.5	41.0	4	Opened	3rd, December 2019	PDA
17	None	Clinic	19.8	40.0	6	Opened	5th, December 2019	TSA
18	None	Clinic	19.8	40.0	6	Opened	5th, December 2019	PDA
19	Before	Kennel	28.0	65.0	—	Opened	20th, July 2020 Morning Session	TSA
20	Before	Kennel	28.0	65.0	—	Opened	20th, July 2020 Morning Session	PDA
21	Before	Clinic	26.0	65.0	1	Opened	20th, July 2020 Morning Session	TSA
22	Before	Clinic	26.0	65.0	1	Opened	20th, July 2020 Morning Session	PDA
23	Before	Aisle	25.8	65.0	1	Opened	20th, July 2020 Morning Session	TSA
24	Before	Aisle	25.8	65.0	1	Opened	20th, July 2020 Morning Session	PDA
25	After	Clinic	25.9	65.0	1	Opened	20th, July 2020 Morning Session	TSA
26	After	Clinic	25.9	65.0	1	Opened	20th, July 2020 Morning Session	PDA
27	After	Aisle	26.0	65.0	1	Opened	20th, July 2020 Morning Session	TSA
28	After	Aisle	26.0	65.0	1	Opened	20th, July 2020 Morning Session	PDA
29	After	Kennel	27.0	68.0	1	Closed	20th, July 2020 Morning Session	TSA
30	After	Kennel	27.0	68.0	1	Closed	20th, July 2020 Morning Session	PDA
31	Before	Clinic	26.0	67.5	1	Closed	20th, July 2020 Morning Session	TSA
32	Before	Clinic	26.0	67.5	1	Closed	20th, July 2020 Morning Session	PDA
33	Before	Aisle	26.0	67.5	1	Closed	20th, July 2020 Morning Session	TSA
34	Before	Aisle	26.0	67.5	1	Closed	20th, July 2020 Morning Session	PDA
35	After	Clinic	26.0	67.5	1	Closed	20th, July 2020 Morning Session	TSA
36	After	Clinic	26.0	67.5	1	Closed	20th, July 2020 Morning Session	PDA
37	After	Aisle	26.0	67.5	1	Closed	20th, July 2020 Morning Session	TSA
38	After	Aisle	26.0	67.5	1	Closed	20th, July 2020 Morning Session	PDA
39	Before	Kennel	27.0	60.0	—	Opened	20th, July 2020 Afternoon Session	TSA
40	Before	Kennel	27.0	60.0	—	Opened	20th, July 2020 Afternoon Session	PDA

(*continued*)

Sample ID	Ventilation	Site	Temperature (°C)	Relative humidity (%)	Canines (numbers)	Door	Date	Plate
41	Before	Clinic	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	TSA
42	Before	Clinic	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	PDA
43	Before	Aisle	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	TSA
44	Before	Aisle	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	PDA
45	After	Clinic	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	TSA
46	After	Clinic	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	PDA
47	After	Aisle	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	TSA
48	After	Aisle	26.0	65.0	1	Opened	20th, July 2020 Afternoon Session	PDA
49	Before	Kennel	27.0	66.0	1	Closed	20th, July 2020 Afternoon Session	TSA
50	Before	Kennel	27.0	66.0	1	Closed	20th, July 2020 Afternoon Session	PDA
51	Before	Clinic	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	TSA
52	Before	Clinic	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	PDA
53	Before	Aisle	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	TSA
54	Before	Aisle	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	PDA
55	After	Clinic	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	TSA
56	After	Clinic	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	PDA
57	After	Aisle	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	TSA
58	After	Aisle	25.5	64.0	1	Closed	20th, July 2020 Afternoon Session	PDA
59	Before	Kennel	24.2	61.0	—	Opened	21st, July 2020 Morning Session	TSA
60	Before	Kennel	24.2	61.0	—	Opened	21st, July 2020 Morning Session	PDA
61	Before	Clinic	23.9	62.0	2	Opened	21st, July 2020 Morning Session	TSA
62	Before	Clinic	23.9	62.0	2	Opened	21st, July 2020 Morning Session	PDA
63	Before	Aisle	23.9	62.0	2	Opened	21st, July 2020 Morning Session	TSA
64	Before	Aisle	23.9	62.0	2	Opened	21st, July 2020 Morning Session	PDA
65	After	Clinic	23.9	62.0	2	Opened	21st, July 2020 Morning Session	TSA
66	After	Clinic	23.9	62.0	2	Opened	21st, July 2020 Morning Session	PDA
67	After	Aisle	23.9	62.0	2	Opened	21st, July 2020 Morning Session	TSA
68	After	Aisle	23.9	62.0	2	Opened	21st, July 2020 Morning Session	PDA
69	Before	Kennel	24.2	61.0	2	Closed	21st, July 2020 Morning Session	TSA
70	Before	Kennel	24.2	61.0	2	Closed	21st, July 2020 Morning Session	PDA
71	Before	Clinic	24.0	60.0	2	Closed	21st, July 2020 Morning Session	TSA
72	Before	Clinic	24.0	60.0	2	Closed	21st, July 2020 Morning Session	PDA
73	Before	Aisle	24.0	60.0	2	Closed	21st, July 2020 Morning Session	TSA
74	Before	Aisle	24.0	60.0	2	Closed	21st, July 2020 Morning Session	PDA
75	After	Clinic	24.0	60.0	2	Closed	21st, July 2020 Morning Session	TSA
76	After	Clinic	24.0	60.0	2	Closed	21st, July 2020 Morning Session	PDA
77	After	Aisle	24.0	60.0	2	Closed	21st, July 2020 Morning Session	TSA
78	After	Aisle	24.0	60.0	2	Closed	21st, July 2020 Morning Session	PDA
79	Before	Kennel	24.2	61.0	—	Opened	22nd, July 2020 Afternoon Session	TSA
80	Before	Kennel	24.2	61.0	—	Opened	22nd, July 2020 Afternoon Session	PDA
81	Before	Clinic	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	TSA
82	Before	Clinic	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	PDA
83	Before	Aisle	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	TSA
84	Before	Aisle	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	PDA
85	After	Clinic	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	TSA
86	After	Clinic	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	PDA
87	After	Aisle	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	TSA
88	After	Aisle	24.0	62.0	3	Opened	22nd, July 2020 Afternoon Session	PDA
89	Before	Kennel	24,2	61.0	—	Closed	22nd, July 2020 Afternoon Session	TSA
90	Before	Kennel	24,2	61.0	—	Closed	22nd, July 2020 Afternoon Session	PDA
91	Before	Clinic	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	TSA
92	Before	Clinic	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	PDA
93	Before	Aisle	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	TSA
94	Before	Aisle	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	PDA
95	After	Clinic	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	TSA
96	After	Clinic	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	PDA
97	After	Aisle	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	TSA
98	After	Aisle	25.5	64.0	3	Closed	22nd, July 2020 Afternoon Session	PDA
99	Before	Kennel	25.0	60.0	—	Opened	22nd, July 2020 Morning Session	TSA
100	Before	Kennel	25.0	60.0	—	Opened	22nd, July 2020 Morning Session	PDA
101	Before	Clinic	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	TSA

(*continued on next page*)

(*continued*)

Sample ID	Ventilation	Site	Temperature (°C)	Relative humidity (%)	Canines (numbers)	Door	Date	Plate
102	Before	Clinic	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	PDA
103	Before	Aisle	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	TSA
104	Before	Aisle	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	PDA
105	After	Clinic	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	TSA
106	After	Clinic	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	PDA
107	After	Aisle	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	TSA
108	After	Aisle	24.0	63.0	5	Opened	22nd, July 2020 Morning Session	PDA
109	Before	Kennel	25.0	60.0	—	Closed	22nd, July 2020 Morning Session	TSA
110	Before	Kennel	25.0	60.0	—	Closed	22nd, July 2020 Morning Session	PDA
111	Before	Clinic	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	TSA
112	Before	Clinic	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	PDA
113	Before	Aisle	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	TSA
114	Before	Aisle	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	PDA
115	After	Clinic	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	TSA
116	After	Clinic	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	PDA
117	After	Aisle	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	TSA
118	After	Aisle	24.9	67.0	3	Closed	22nd, July 2020 Morning Session	PDA
119	Before	Kennel	24.8	71.0	—	Opened	23rd, July 2020 Afternoon Session	TSA
120	Before	Kennel	24.8	71.0	—	Opened	23rd, July 2020 Afternoon Session	PDA
121	Before	Clinic	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	TSA
122	Before	Clinic	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	PDA
123	Before	Aisle	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	TSA
124	Before	Aisle	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	PDA
125	After	Clinic	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	TSA
126	After	Clinic	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	PDA
127	After	Aisle	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	TSA
128	After	Aisle	24.0	63.0	5	Opened	23rd, July 2020 Afternoon Session	PDA
129	Before	Kennel	25.0	60.0	5	Closed	23rd, July 2020 Afternoon Session	TSA
130	Before	Kennel	25.0	60.0	5	Closed	23rd, July 2020 Afternoon Session	PDA
131	Before	Clinic	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	TSA
132	Before	Clinic	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	PDA
133	Before	Aisle	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	TSA
134	Before	Aisle	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	PDA
135	After	Clinic	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	TSA
136	After	Clinic	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	PDA
137	After	Aisle	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	TSA
138	After	Aisle	24.9	67.0	5	Closed	23rd, July 2020 Afternoon Session	PDA
139	Before	Kennel	24.5	68.0	—	Opened	23rd, July 2020 Morning Session	TSA
140	Before	Kennel	24.5	68.0	—	Opened	23rd, July 2020 Morning Session	PDA
141	Before	Clinic	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	TSA
142	Before	Clinic	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	PDA
143	Before	Aisle	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	TSA
144	Before	Aisle	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	PDA
145	After	Clinic	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	TSA
146	After	Clinic	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	PDA
147	After	Aisle	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	TSA
148	After	Aisle	24.1	67.0	6	Opened	23rd, July 2020 Morning Session	PDA
149	Before	Kennel	25.0	65.0	—	Closed	23rd, July 2020 Morning Session	TSA
150	Before	Kennel	25.0	65.0	—	Closed	23rd, July 2020 Morning Session	PDA
151	Before	Clinic	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	TSA
152	Before	Clinic	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	PDA
153	Before	Aisle	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	TSA
154	Before	Aisle	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	PDA
155	After	Clinic	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	TSA
156	After	Clinic	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	PDA
157	After	Aisle	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	TSA
158	After	Aisle	24.6	63.0	6	Closed	23rd, July 2020 Morning Session	PDA
159	Before	Kennel	25.0	60.0	—	Opened	24th, July 2020 Afternoon Session	TSA
160	Before	Kennel	25.0	60.0	—	Opened	24th, July 2020 Afternoon Session	PDA
161	Before	Clinic	24.5	60.0	2	Opened	24th, July 2020 Afternoon Session	TSA
162	Before	Clinic	24.5	60.0	2	Opened	24th, July 2020 Afternoon Session	PDA

(*continued*)

Sample ID	Ventilation	Site	Temperature (°C)	Relative humidity (%)	Canines (numbers)	Door	Date	Plate
163	Before	Aisle	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	TSA
164	Before	Aisle	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	PDA
165	After	Clinic	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	TSA
166	After	Clinic	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	PDA
167	After	Aisle	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	TSA
168	After	Aisle	24.5	65.0	2	Opened	24th, July 2020 Afternoon Session	PDA
169	Before	Kennel	25.0	65.0	—	Closed	24th, July 2020 Afternoon Session	TSA
170	Before	Kennel	25.0	65.0	—	Closed	24th, July 2020 Afternoon Session	PDA
171	Before	Clinic	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	TSA
172	Before	Clinic	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	PDA
173	Before	Aisle	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	TSA
174	Before	Aisle	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	PDA
175	After	Clinic	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	TSA
176	After	Clinic	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	PDA
177	After	Aisle	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	PDA
178	After	Aisle	24.5	65.0	5	Closed	24th, July 2020 Afternoon Session	PDA

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