MAIN ENVIRONMENTAL FACTORS AFFECTING CONCENTRATIONS OF CULTURABLE AIRBORNE BACTERIA IN INDOOR LABORATORIES OVER A PERIOD OF ONE YEAR

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Abstract. This study aimed to assess temporal changes in the concentration of culturable airborne bacteria (CAB) in three microbiology laboratories to determine the environmental factors that affects CAB concentration. The CAB concentration was determined once per month from March 2011 to February 2012 in the three laboratories. An Andersen one-stage sampler was used to collect CAB for 5 min, three times per day. CAB concentrations demonstrated an increasing tendency in summer and fall, but it was difficult to detect consistent seasonal patterns. Temperature, relative humidity (RH), number of people, and activity of people were associated with CAB concentrations. The overall CAB concentrations were significantly greater in the study rooms than that in the laboratory. CAB concentrations in indoor microbiology laboratories varied greatly depending on the number of people and whether or not a humidifier was used.
Keywords: *airborne bacteria, temperature, relative humidity, humidifier, seasonality*

Introduction

Air quality in closed environments is an important factor for human health because people tend to spend most of their time in various indoor environments, such as the home, the workplace, or other microenvironments (Klepeis et al., 2001). Exposure to indoor microbial airborne particles, especially fine (<1 mm) and ultrafine (<0.1 mm) particles, has been identified as an important factor affecting human health, and is known to cause adverse pulmonary effects, headache, and allergies (Douwes et al., 2003; Dockery, 2009). Airborne bacteria are known to cause infectious diseases, hypersensitivity pneumonitis, and lung functions (Pastuszka et al., 2000).

People who work with microbes or in the field of biotechnology have greater potential to be directly exposed to microorganisms than those in other occupations. Most currently operating clinical microbiology laboratories are busy and crowded. They were often designed several decades ago, before molecular diagnostic methods or bioterrorism preparedness became commonplace. Their air-handling systems have become overloaded or unstable as instruments and personnel have multiplied (Baron and Miller, 2008). Characterization and measurement of the concentration of airborne microorganisms in a laboratory is difficult because of the diversity of infectious microorganisms handled, variation in the efficiencies of air

sampling equipment, differences in the viability of infectious microorganisms, and lack of a standardized method for measuring individual microorganisms in the air (Hwang et al., 2014a). Many positive changes have been made in standard safety practices, and most laboratory workers feel that they are safer than they were earlier, but evidence-based research is lacking to confirm that supposition. Therefore, maintenance of a sanitary environment is crucial for laboratories dealing with microorganisms.

The purpose of this study was to assess temporal changes in CAB concentrations in three microbiology laboratories to determine which environmental factors, such as temperature, relative humidity (RH), number of people, and activity of people, are associated with CAB concentrations.

Materials and methods

Characteristics of the laboratories and environmental factors

This study was conducted for a 12-month period from March 2011 to February 2012. A total of 212 samples were collected once a month in the three microbial laboratories. *Table 1* summarizes the characteristics of the laboratories. The environmental factors such as temperature, RH, Number of people, and activity of people were measured in the three microbial laboratories. Temperature and RH were recorded by VelociCalc Air Velocity Meter (Model 9555, TSI Inc., USA). Number of people was counted only during CAB sampling times and activity of people was measured by counting of people who were passing by the sampling site within one meter during CAB sampling times.

Sampling and analysis

CAB was collected three times a day (9:00- 10:00, 13:00 - 14:00, and 16:00- 17:00), for once a month in each laboratory. Every sampling was conducted at the same location: the center of the each room. A single-stage viable cascade impactor (SKC Inc., USA) connected with a pump (Quick Take 30, SKC Inc., USA) was operated at a flow rate of 28.3 l min^{-1} for 5 min. Before the sampling operation, the sampling equipment was sterilized with a 70 % ethyl alcohol swab, and nutrient media in Petri dishes were placed on the one-stage impactor. The CAB samples were collected on trypicase soy agar (TSA) plates. The sampled media were sealed with laboratory film to prevent contamination and desiccation during the incubation. The samples were transferred to the laboratory in a sterilized ice box with refrigerant packs to keep the samples below 4°C. TSA plates were incubated at 35 °C for up to 2 days. The positive hole correction table was used to adjust colony counts (ACGIH, 1999). The concentrations of CAB was calculated by dividing the number of colonies by air volume and written as colony-forming unit per cubic meter of air (CFUm^{-3}).

Statistical analysis

Kolmogorov-Smirnov test was used to determine whether the data were normally or log-normally distributed. Arithmetic means (AM) of CAB concentrations and their standard deviation (SD) were calculated. Independent *t*-test was used to compare the differences between environmental factors such as divided room by structure of laboratory, use of humidifier, and use of air-conditioner. CAB concentrations in the three microbial laboratories, and p value less than 0.05 was considered to be statistically

significant. Correlation analysis was used to identify the association between CAB concentrations and environmental factors. All statistical analyses were performed by SPSS (version 23.0; IBM Inc., USA).

Results

Table 2 summarizes the CAB concentrations in the three microbiology laboratories each month. The AM of CAB concentrations over the year ranged from 7–5823 CFUm^{-3} in the three microbiology laboratories. The highest AM concentration of CAB was found in August for laboratories A (945 CFUm^{-3}) and B (1162 CFUm^{-3}) and in October in laboratory C (2396 CFUm^{-3}), whereas the lowest AM concentration of CAB was observed in February in laboratory A (60 CFUm^{-3}), in March in laboratory B (91 CFUm^{-3}), and April in laboratory C (319 CFUm^{-3}). The AM of CAB concentrations differed significantly between laboratories A and B and C (p < 0.05).

To show seasonal changes in CAB concentrations, the 12 months were grouped into four seasons: March to May were grouped as spring, June to August as summer, September to November as fall, and December to February as winter. *Figure 1* presents seasonal changes in CAB concentrations. Laboratories A and B showed similar seasonal changes in CAB concentrations, gradually increasing from summer to fall and then decreasing from fall to winter. However, the CAB concentrations in laboratory C increased from fall to winter and decreased from spring to summer.

Spearman correlation analyses were performed to identify relationships between CAB concentrations, temperature, RH, number of people, and activity (*Table 3*). A significant positive correlation was observed between CAB concentration and temperature (r = 0.269, p < 0.001), RH (r = 0.451, p < 0.001), number of people (r = 0.328, p < 0.001), and activity of people (0.321, p < 0.001).

Table 4 shows the CAB concentrations by location and by factors within laboratories. Two groups were defined for each factor, as follows: laboratory rooms and study rooms, off and on for the humidifier, laboratory room and study room among rooms with the humidifier off, off and on for the air conditioner. The CAB concentrations were significantly higher in study rooms than in laboratory rooms; they were significantly higher in the laboratories in which the humidifier was on than in laboratories in which the humidifier was off; when the humidifier was on, they were higher in study rooms than in laboratories; they were higher in the laboratories in which the air conditioner was on than in the laboratories in which it was off. However, no significant difference in CAB concentrations was observed between groups when the humidifier was off or when the air-conditioner was on (p > 0.05).

Fig. 2 shows associations between CAB concentrations and number of people in each study room. CAB concentrations in laboratory C (r = 0.451), which contained more people, were significantly more strongly associated with number of people than those of laboratories A or B, although CAB concentrations in laboratories A (r = 0.137) and B (r = 0.331) were also associated with number of people.

Table 1. The characteristics of environmental factors in the three microbial laboratories

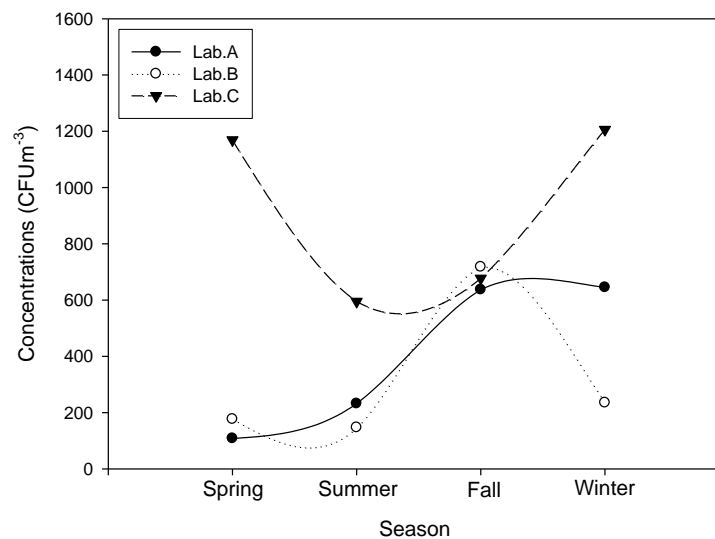|	Laboratory A	Laboratory B	Laboratory C
Room volume (m^{-3})	380	393	390
(Area × Height)	(146 × 2.6)	(151 × 2.6)	61.4 (151 × 2.6)
Temperature (°C) (Mean ± SD)	23.6 ± 2.4	24.2 ± 2.5	24.5 ± 1.4
RH (%) (Mean ± SD)	37.7 ± 20.5	34.3 ± 18.3	35.7 ± 18.2
No. of people (Mean ± SD)	0.98 ± 1.38	1.10 ± 1.56	2.90 ± 3.40
Laboratory room	0.4 ± 0.6	0.4 ± 0.6	0.8 ± 0.70
Study room	1.6 ± 1.7	1.8 ± 1.9	5.0 ± 1.25
Activity of people (Mean ± SD)	1.00 ± 1.30	0.84 ± 1.31	1.46 ± 1.67
Experiment	Production of amino acid with microorganism	Production of amino acid with microorganism	Waste purification with microorganism

Table 2. *Monthly concentrations of CAB in three microbial laboratories*

Months									
		CAB (CFUm^{-3})							
		Lab. A			Lab. B			Lab. C	
	N	Mean(SD)	Range	N	Mean(SD)	Range	N	Mean(SD)	Range
Jan.	6	107 (55.6)	50 – 213	6	120 (112.1)	28 – 329	6	1780 (2434.0)	108 – 5823
Feb.	6	126 (117.2)	7 – 337	6	122 (112.5)	35 – 346	6	594 (383.2)	190 – 1175
Mar.	5	60 (32.4)	21 – 101	6	91 (44.6)	35 – 130	5	1191 (1158.2)	290 – 2572
Apr.	6	335 (165.4)	138 – 543	6	110 (94.1)	28 – 251	6	319 (159.2)	86 – 587
May	6	273 (235.3)	115 – 729	6	241 (167.2)	64 – 435	6	375 (238.4)	175 – 842
Jun.	6	576 (293.4)	213 – 988	6	485 (183.4)	314 – 823	6	767 (490.5)	167 – 1317
Jul.	6	387 (187.5)	220 – 693	6	507 (163.6)	329 – 712	6	623 (360.2)	220 – 1273
Aug.	6	945 (1117.7)	190 – 3177	6	1162 (896.1)	451 – 2888	6	641 (373.1)	71 – 1196
Sept.	4	510 (59.7)	451 – 570	6	290 (199.6)	93 – 596	6	706 (211.1)	329 – 968
Oct.	6	748 (1210.5)	64 – 3177	6	206 (73.3)	86 – 314	6	2396 (2013.6)	306 – 5180
Nov.	6	630 (661.0)	130 – 1931	6	210 (68.0)	123 – 298	6	517 (166.1)	306 – 739
Dec.	6	91 (44.2)	42 – 153	6	290 (151.4)	115 – 493	6	1133 (1369.2)	259 – 3754
Total	69	401 (571.3)	7 – 3177	72	320 (391.5)	28 – 2888	71	916 (1157.6)	71 – 5823

Table 3. *Spearman correlation analysis between CAB and environmental factors in the microbial laboratories*

	Conc. (CFUm⁻³)	Temperature (°C)	RH (%)	No. of people	Activity of people
Conc. (CFUm⁻³)	1.000				
Temperature (°C)	0.269**	1.000			
RH (°C)	0.451**	0.301**	1.0000		
No. of people (%)	0.328**	0.120	-0.038	1.000	
Activity of people	0.321**	0.232**	0.218**	0.232**	1.000

*: $p < 0.05$, **: $p < 0.001$, N = 204

Figure 1. *Seasonal changes in concentrations of CAB in microbial laboratories*

Table 4. *Comparisons of CAB concentrations between the categorized groups by the environmental factors of the three microbial laboratories*

	Categorized groups	No. of sample	CAB concentrations (CFUm^{-3})				*p*-value
			Mean $\pm$ SD [b]	Min	Median	Max	
[a] Separation	Laboratory room	106	384 $\pm$ 382	21	298	3177	< 0.01
	Study room	105	712 $\pm$ 1077	7	329	5823	
	Total	211	547 $\pm$ 821	7	314	5823	
Humidifier	Turn off	198	423 $\pm$ 508	7	298	3754	< 0.001
	Turn on	13	2432 $\pm$ 1861	123	2337	5823	
	Total	211	2432 $\pm$ 1861	7	314	5823	
Turn off humidifier	Laboratory room	106	384 $\pm$ 382	7	298	3177	> 0.05
	Study room	92	469 $\pm$ 621	7	314	3754	
	Total	198	2432 $\pm$ 1861	7	298	3754	
Air-conditioner	Turn off	77	426 $\pm$ 622	21	290	4191	> 0.05
	Turn on	132	621 $\pm$ 917	7	329	5823	
	Total	209	549 $\pm$ 825	7	314	5823	

[a] These laboratories were made up of one space divided by two spaces into laboratory room and study room with a partition wall [b]; Standard deviation

(a) Laboratory A (r =0.137) (b) Laboratory B (r =0.331) (c) Laboratory C (r =0.451)
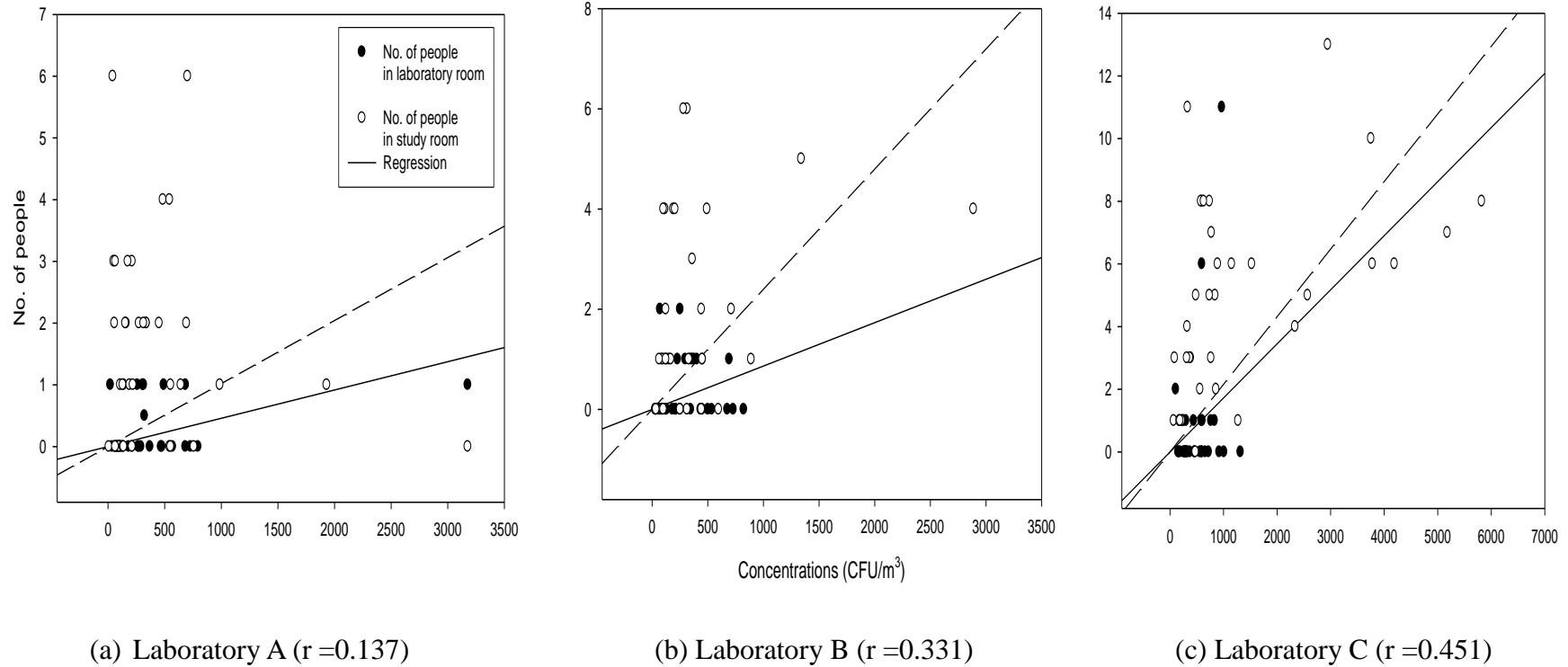
Figure 2. Association between CAB concentrations and the number of people both for laboratory room and study room of laboratory A, B, and C

Discussion

CAB concentrations were measured in three microbiology laboratories to assess monthly and seasonal changes and to investigate the effects of several environmental factors (temperature, RH, number of people, and activity of people) to determine whether there are any associations between these factors and CAB concentrations.

In 29 of a total of 212 samples (13.7%) from the three microbiology laboratories, CAB concentrations exceeded 800 $CFUm^{-3}$, according to Korean guidelines (Ministry of Environment of Korea, 2014). Among the 29 samples that exceeded 800 $CFUm^{-3}$, 21 were from laboratory C. The overall mean concentration (916 $CFUm^{-3}$) of CAB in laboratory C was threefold higher than the Indoor Air Quqlity Association recommendation. A previous study showed that the mean concentration of total airborne bacteria in indoor environments, including occupational environments, was 308 $CFUm^{-3}$ in subway stations (Hwang et al., 2015), 684 $CFUm^{-3}$ (median) in homes, 222 CFU/m^{-3} (median) in elderly car centres (Madureira et al., 2015), 105 CFU/m^{-3} in swine confinement buildings (Douwes et al., 2003), 113 $CFUm^{-3}$ in a feedstock manufacturing factory (Kim et al., 2007), 198 $CFUm^{-3}$ during a pelleting and powdering process, and 281 $CFUm^{-3}$ (maximum level) in 100 U.S. office buildings (Tsai and Macher, 2005). These concentrations of total airborne bacteria were relatively low compared to that measured in laboratory C in this study. Airborne bacteria levels exceeding 600 $CFUm^{-3}$ can be associated with insufficient ventilation or abnormal sources of microorganisms (Salonen et al., 2007).

The CAB concentrations in laboratories A and B presented similar seasonal patterns, whereas the CAB concentrations in laboratory C showed a contrasting pattern (*Fig. 1*). In previous studies of indoor air quality conducted in Chicago homes, culturable bacteria were highest in summer and fall (Moschandreas et al., 2003), whereas in Finland, only a slight yet significant difference was observed between summer and winter bacterial levels (Reponen et al., 1992). However, other studies in homes have shown a large decline from spring to summer, an increase in fall, followed by a decrease toward winter (Frankel et al., 2012). These discrepancies might be caused by other factors, which can influence CAB concentrations, rather than by seasonal changes themselves. Sources of bacteria in outdoor air can change over short periods of time, in relation to climatic conditions (Rintala et al., 2008; Womack et al., 2010); however, indoor air bacterial concentrations are less strongly related to climatic conditions than those of outdoor air.

To identify factors influencing CAB concentrations in microbiology laboratories, Spearman's correlation analyses were used to identify correlations between CAB concentrations, temperature, RH, number of people, and activity of people (*Table 3*). Positive correlations were observed between CAB concentrations, temperature (r = 0.269), RH (r = 0.451), number of people (r = 0.328), and activity of people (r = 0.321). Temperature is an environmental factor that typically influences biological agents (WHO, 2009). In our study, higher CAB concentrations were significantly related to higher temperature. This result is consistent with previous studies (Guo et al., 2004; Jo et al., 2005; Hwang et al., 2011a; Hwang et al., 2015). However, other studies did not show associations, positive or negative, between CAB concentrations and temperature (Frankel et al., 2012; Madureira et al., 2015). The authors hypothesized that the

discrepancy might be caused by small variations in indoor parameters, which exclude any association with biological pollutants. RH was significantly associated with CAB concentrations ($p < 0.05$) and was the environmental factor most strongly associated with the CAB concentration ($r = 0.451$). RH is known to be crucial for microorganism growth, even at low temperatures (Tsai et al., 2009). However, no relationship was observed between comparatively low RH ($< 60\%$) and CAB concentration (Hwang et al., 2011a). The number of people ($r = 0.328$) and activity of people ($r = 0.321$) were associated with CAB concentrations. Other studies have found that the number of people is positively associated with the concentration of CABs in subway station environments, consistent with airborne microorganisms being dispersed into the air from subway passengers' clothing and hair (Boudia et al., 2006; Cho et al., 2006; Bogomolova and Kirtsideli, 2009; Hwang et al., 2014b). Moreover, changes in microbial communities between peak and nonpeak commuting hours can largely be attributed to increases in skin-associated genera (Leung et al., 2014). Sources of airborne bacteria in built environments include humans, pets, soils, and plants (Jo and Seo, 2005). Indoor human occupancy was found to be closely related to indoor microbial levels (Scheff et al., 2000), and settled spores were resuspended in indoor air by air movement caused by human activities, such as walking and running (Buttner and Stetzenbach, 1993). In classrooms, sampling time supports the effect of activity, as indoor bioaerosol ratios were higher during break times, when childrens' activity was higher than during class time (Jo and Seo, 2005).

CAB concentrations were higher in study rooms than in laboratory rooms, even in which the humidifier was turned off (*Table 4*). These results may be influenced by peoples' activity levels, which could contribute to increased CAB concentrations, as mentioned earlier. *Fig. 2* demonstrates that the CAB concentration increased as the number of people increased in three study rooms. Laboratories using a humidifier showed significantly higher concentrations of CAB than laboratories not using a humidifier. Humidifiers can introduce bacteria into the air, as many spray water, especially those that use recirculated water or water from stagnant indoor reservoirs; *Legionella* spp. can colonize warm to hot water systems, living in biofilms that develop on surfaces in contact with water (ACGIH, 1999). Higher concentrations of CAB were observed in laboratories with the air conditioner on than in laboratories with the air conditioner off. Ventilation systems affect indoor bioaerosol concentrations because they prevent outdoor microorganisms from being transported inside buildings (Wu et al., 2005). Good ventilation and hygiene decrease the concentrations of airborne contaminants. That is, a higher ventilation rate may lead to decreased exposure to inflammatory microbial components, as measured in a granulocyte assay (Frankel et al., 2012). However, it is well known that indoor facilities such as rooms, hallways, and underground parking lots show poor indoor air quality, and poor ventilation is known to be one of the most important causes of poor air quality (Fisk et al., 2009). In addition, condensation on filters and surfaces within heating, ventilating, and air conditioning systems can be a source of biological contamination (ACGIH, 1999).

Although we identified environmental factors that can affect CAB concentrations throughout our one-year assessment cycle, this study has several limitations. First, the incubation temperature was high. The optimum temperature range for growth of CAB is known to be 25–30°C, but in this study, CAB were incubated at 35°C. However, the optimum temperature differs depending on the species (Ayersi, 1969; Pitt et al., 1983).

Second, because of issues of accessibility, resources, and permits, we were unable to conduct the tests at the three laboratories at the same time. Finally, we were unable to identify the isolated CAB due to a funding shortage. However, we were able to predict the species of some CAB based on previous studies conducted in similar indoor microbiology laboratories (Hwang et al., 2011b; 2013).

Conclusion

We assessed the monthly and seasonal changes in CAB concentrations in three microbiology laboratories and determined which environmental factors, such as temperature, RH, number of people, and activity of people, were associated with CAB concentrations. CAB concentrations did not show consistent patterns of seasonal variation between laboratories. Temperature, RH, number of people, and activity of people were associated CAB concentrations. The overall CAB concentrations were significantly greater in the study rooms than in the laboratory rooms. CAB concentrations varied greatly depending on the number of people and use of a humidifier. Therefore, it is important to clean humidifiers regularly to prevent overgrowth of CAB in indoor environments.

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