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#### Original article

## Exposure Assessment of Airborne Bacteria and Fungi in the Aircraft

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#### ABSTRACT

*Objective:* The exposure levels of disease-causing bacteria and germs were assessed on aircraft cleaning workers on multiple different aircrafts.

*Method:* Five measuring points were selected depending on the aircraft types. Four aircraft cleaning agencies were selected for the test. Aircraft cleaning work was classified as intensive cleaning and general cleaning work. Ventilation in aircraft when sampling during the cleaning operation was categorized into forced ventilation and natural ventilation. The collection of airborne microorganisms was made through inertial impactors which were installed 1.5 meters above the bottom of the aircraft. The airborne bacteria and fungus growth badges were selected by Trytpic Soy Agar and Sabouraud Dextrose Agar.

*Results:* The average concentrations of bacteria in the air were higher in the order of small, medium, and large airplanes. Rainy days had higher concentrations inside and outside the aircraft as compared to those in sunny days. Regarding ventilation, concentrations in natural ventilation were higher than concentrations in forced ventilation. According to the type of work, the concentrations in the intensive cleaning groups (cleaning one plane a day) were lower than those of the ordinary cleaning groups (cleaning several planes per day).

*Conclusion:* The concentration levels of airborne bacteria and fungi in the aircraft surveyed were lower than the indoor environmental standards of Korea (800 cfu/m<sup>3</sup> and 500 cfu/m<sup>3</sup>). The average concentrations of bacteria in the air and fungi in the air were highest in small aircraft owned by Company D. © 2022 Occupational Safety and Health Research Institute, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

#### 1. Introduction

Unlike ordinary cleaning workers, in-flight cleaning workers are exposed to potential work risks due to the limited working space and tight work schedules. Airlines usually entrust room cleaning to outside companies. The types of cleaning service provided depend on the duration of the aircraft stay. Psychological factors, such as the work pressure caused by these factors, act as job stress for aircraft cleaning workers [1].

Pollution caused by airborne microorganisms is a key factor in indoor air pollution and is a rapidly emerging area in terms of working environments as it plays an important role in the spread of certain infectious diseases and allergies [2].

Research on infectious diseases in buses, trains, and airplanes is actively progressing. The importance of bioaerosols to indoor enclosed spaces is increasing [3].

A bioaerosol is defined as organic pollen, bacteria, fungi, and viruses, as well as metabolites such as allergens, (1-3)- $\beta$ -D-glucan, and toxins such as endotoxins and mycotoxins [4]. Airborne microorganisms reportedly cause respiratory problems such as asthma, rhinitis, sinusitis, and bronchitis [5–8].

Until now, most of the studies on workers inside aircraft have been on cabin crew [9]. On-site exposure assessment of bioaerosol has been reported in other transportation systems such as subways and buses, indoor multiuse facilities, and work facilities [10-13], but there is no information related to bioaerosol on airplanes to date.

Previous studies have shown that 30% of museum workers developed allergic symptoms while working with potentially fungal contaminated objects [14].

Evaluation was also conducted in various indoor environments. Fungi concentrations were measured in medical schools, hospitals,

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paper works, food works, poultry farms, and bakeries. The results were 5,437.6 cfu/m<sup>3</sup>, 3,871.7 cfu/m<sup>3</sup>, 3,802.7 cfu/m<sup>3</sup>, 3,402.7 cfu/m<sup>3</sup>, and 1796.8 cfu/m<sup>3</sup>. On average, the concentration at the hospital was the highest [15].

Bacterial and fungal concentrations were measured for various spaces in the hospital. The concentrations of bacteria were 152 cfu/m<sup>3</sup> in neonatal intensive care unit, 127 cfu/m<sup>3</sup> in cardiac treatment unit, 160 cfu/m<sup>3</sup> in cancer ward, 92 cfu/m<sup>3</sup> in otolaryngology operation room, and 243 cfu/m<sup>3</sup> in eye operation room. The concentration in the eye operating room was the highest. Other previous studies recommend that the limit of acceptable bacterial concentrations for operating room was measured to be higher than this.

The concentration of fungi was  $26 \text{ cfu/m}^3$  in neonatal intensive care unit,  $30 \text{ cfu/m}^3$  in cardiac treatment unit,  $8 \text{ cfu/m}^3$  in cancer ward,  $16 \text{ cfu/m}^3$  in otolaryngology operation room, and  $17 \text{ cfu/m}^3$  in eye operation room.

The outdoor concentrations were  $1052 \text{ cfu/m}^3$  and  $22 \text{ cfu/m}^3$ , respectively. Bacterial concentrations were found to be much higher outdoors than indoors [16].

Aerosol exposure evaluation in various types of indoor air was conducted in China. As a result, the office (bacteria: 206-3733 cfu/m<sup>3</sup>; fungi:  $29 \sim 1779$  cfu/m<sup>3</sup>), school (bacteria:  $72.5 \sim 7500$  cfu/m<sup>3</sup>; fungi:  $12 \sim 9730.3$  cfu/m<sup>3</sup>), and residence (bacteria:  $93 \sim 3808$  cfu/m<sup>3</sup>; fungi:  $38 \sim 9672.1$  cfu/m<sup>3</sup>). In particular, the concentration of bacteria and fungi in old buildings was high, and on average, the concentration of fungi in schools was the highest [17].

In this way, research on the evaluation of exposure to bioaerosol in various indoor environments is being conducted. Among previous studies, aerosol concentrations above the standard were often detected. Therefore, it is necessary to actively study aerosol concentration in the indoor environment and secure basic data.

Therefore, the purpose of this study is to assess the exposure levels of aircraft cleaning workers by performing on-site measurements of the biohazardous factors that occur inside aircraft at in-flight cleaning work sites.

#### 2. Methods

#### 2.1. Objective of the study

In order to determine the status of aircraft cleaning workers, four airlines which rely on airline cleaning services classified as full service carriers and low-cost carriers were selected and on-site surveys were conducted among the airlines operating air transport services at two airports (Incheon and Gimpo airports) in the capital of Korea.

An exposure assessment of airborne microorganisms was carried out considering the ventilation methods and types of cleaning work. Ventilation methods used during cleaning operations on the aircraft were divided into two types: natural ventilation with doors open and forced ventilation connected to cars that supply air. Aircraft cleaning work was classified as intensive cleaning and general cleaning work according to the aircraft schedule.

The types of aircraft cleaning work were divided into intensive work (cleaning one aircraft per day) and general cleaning (cleaning many aircraft per day).

Therefore, the collection of airborne microorganisms was measured repeatedly three times, with three trials at intervals of two hours at sites where intensive work was carried out, and three times with three trials at 20-minute intervals per aircraft at sites where normal work was carried out.

The measurement locations for airborne bacteria and airborne fungi consisted of five points: the front, middle, rear, center of the second floor, and the toilet, depending on the structure of the aircraft (single floor or two floors). At the same time, the temperature and relative humidity, which affect the occurrence of airborne bacteria and fungi, were also measured.

#### 2.2. Measurement method

#### 2.2.1. Airborne bacteria and airborne fungi

Concentration measurements of airborne bacteria and airborne bacteria were conducted in accordance with the indoor air quality process test standards of the Ministry of the Environment of Korea.

N-6 Andersen impaction head (one-stage viable particulate cascade impactor, Model 10-800 Andersen Inc., USA) was used to collect airborne bacteria and fungi. Air samples were taken from a position 1.2 meters above the floor.

The sampling period lasted for 2–5 minutes depending on the environmental conditions of the site, and sampling was conducted at a flow rate of 28.3 L per minute. After collecting air samples by inserting sterilized agar plates into the measuring equipment, the agar plates were immediately sealed with laboratory film at the site and transported to the microbiological analysis room to prevent contamination by external factors.

In the case of bacteria, Tryptic Soy Agar (Diffco Bacto, Kansas, USA) was selected to suppress fungi due to its higher nitrogen content than carbohydrate nutrient availability.

In the case of airborne fungi, Sabouraud Dextrose Agar (Diffco Bacto, Kansas, USA) containing 100 mg of chloramphenicol was used to inhibit bacterial growth.

As for cultural condition, the airborne bacteria were cultured for 24-48 hours within a temperature range of  $30-37^{\circ}$ C. For the airborne fungi, the time was 72 hours or more at room temperature ( $15 \sim 25^{\circ}$ C).

The concentrations of airborne bacteria and fungi were calculated in units of  $cfu/m^3$  divided by the number of colonies cultivated in each medium according to the volume of air sampled (m<sup>3</sup>) (see Equations 1 and 2).

cfu (colony forming unit)/ $m^3$  = Colony counted on agar plate/Air volume ( $m^3$ ) (1)

Air volume (m<sup>3</sup>) =  $28.3\ell/\min \times \text{sampling time (min)}/10^3$  (2)

#### 2.2.2. Temperature and relative humidity

During the collection of airborne microorganisms, the temperature and relative humidity were measured at a location 1.2 to 1.5 m above the center point of the aircraft. Gray Wolf (IQ-610, USA) was used as the measuring equipment for this case. After installing the equipment at each measurement point and allowing a stabilization time of more than three minutes, the measurement was conducted.

#### 2.2.3. Data analysis

For each measurement item, the normal distribution was reviewed through a Q-Q plot. For a statistical analysis, the SPSS program (IBM, USA) was used. The data were not normally distributed. Therefore, data log conversion was performed to create normality. After that, statistical analysis was conducted. Regarding the concentration of airborne microorganisms in the airplane, Student *t*-test was applied in the comparison part according to sunny and rainy days, indoor and outdoor locations, along with the work type and ventilation method. A correlation analysis (Pearson's correlation) was used to determine the effects of the temperature and relative humidity on the concentrations of airborne microorganisms generated inside and outside of the aircraft with or without rain. ANOVA was applied to compare the concentrations of airborne microorganisms according to the aircraft size (small, medium, and large).

#### 3. Results

3.1. Distributions of airborne bacteria and fungi concentration and physical factors by company

As a result of measuring airborne bacteria and airborne fungi levels, the measured median value of airborne bacteria was found to be between 34.7 and 143.6 (cfu/m<sup>3</sup>), and the median value of airborne fungi was between 27.8 and 118.1 (cfu/m<sup>3</sup>). All four companies surveyed showed distributions below the standards of the Ministry of the Environment of the Republic of Korea (bacteria 800 cfu/m<sup>3</sup>, fungi 500 cfu/m<sup>3</sup>) <Fig. 1>.

According to the results of measuring the temperature and relative humidity, the maximum value of the temperature was 31.0°C, the minimum value was 23.2°C, the maximum value of the relative humidity was 93.1%, and the minimum value was 24.3% <Table 1>.

# 3.2. Correlation analysis between the airborne microbial concentrations and physical factors (temperature and humidity) in the air inside and outside of the aircraft according to the external climatic conditions (with or without rain)

Fig. 2 shows the differences in the concentrations of airborne bacteria and airborne fungi inside and outside the aircraft on rainy and sunny days. During rainy days, both airborne bacteria and fungi had higher mean concentrations in outside (183.3 cfu/m<sup>3</sup> and 285.6 cfu/m<sup>3</sup>) than inside (139.5 cfu/m<sup>3</sup> & 127.7 cfu/m<sup>3</sup>). Statistically, there was no significant difference for airborne bacteria (p > 0.05), though there were significant differences were found for airborne fungi (p < 0.05). Even on clear days, the concentration in outdoor air (143.0 cfu/m<sup>3</sup> & 190.0 cfu/m<sup>3</sup>) was higher than indoor air (106.8 cfu/m<sup>3</sup> & 95.1 cfu/m<sup>3</sup>) for both airborne bacteria and fungi, respectively. The statistical analysis result was also not statistically significant differences were found for airborne fungi (p < 0.05), though statistical malysis result was also not statistically significant differences were found for airborne fungi (p < 0.05), as in rainy days.

#### Table 1

Temperature and relative humidity by cleaning company

Company	Item	Max	Min	Mean
А	Temperature (°C)	27.1	23.2	24.9
	Relative humidity (%)	55.6	24.3	32.6
В	Temperature (°C)	31.0	24.8	21.2
	Relative humidity (%)	64.2	38.9	30.4
С	Temperature (°C)	30.7	29.5	27.2
	Relative humidity (%)	74.2	63.9	46.5
D	Temperature (°C)	30.9	28.5	25.3
	Relative humidity (%)	93.1	78.9	68.4

As shown in Table 2, a correlation analysis was conducted using the concentrations of airborne microorganisms according to the temperature and relative humidity. As a result of this analysis, it was found that the temperature and relative humidity had a stronger correlation with airborne fungi than with airborne bacteria both inside and outside the aircraft. Both concentrations of airborne fungi inside and outside the aircraft showed a negative correlation with the temperature and a positive correlation with the relative humidity.

In addition, the concentration of airborne bacteria inside the aircraft showed a statistically significant correlation with the internal and external relative humidity levels (p < 0.05), and the concentration of airborne fungi inside the aircraft was statistically significant correlated with the internal and external temperature and relative humidity levels (p < 0.01).

## 3.3. Comparison of the concentrations of airborne microorganisms according to the ventilation method

Fig. 3 shows the differences in the concentrations of airborne bacteria and fungi according to the ventilation method applied to cleaning. As a result of the measurement, the mean concentrations of airborne bacteria and fungi by natural ventilation (124.9 cfu/m<sup>3</sup> and 113.2 cfu/m<sup>3</sup>) were higher than those by forced ventilation (58.3 cfu/m<sup>3</sup> and 39.7 cfu/m<sup>3</sup>), respectively. As a result of statistical analysis, there was a statistically significant difference of airborne bacteria and fungi between natural ventilation and forced ventilation (p < 0.05).



Fig. 1. Concentration distribution of airborne bacteria and fungi by company.



Fig. 2. Airborne microbial concentrations inside and outside the aircraft on rainy and sunny days.

# 3.4. Comparison of the concentrations of airborne microorganisms according to the type of cleaning work

Fig. 4 shows the difference between airborne bacteria and fungi according to the type of cleaning work. The type of cleaning work is divided into the intensive task of cleaning one aircraft per day and the general task of cleaning multiple aircraft per day. On average, general work takes one to two hours. The intensive work takes six to nine hours.

As a result of the measurement, the mean concentrations of airborne bacteria and fungi during general cleaning (94.8 cfu/m<sup>3</sup> and 71.0 cfu/m<sup>3</sup>) were significantly higher than those during intensive cleaning (58.1 cfu/m<sup>3</sup> and 49.8 cfu/m<sup>3</sup>) (p < 0.05).

# 3.5. Comparison of airborne microbial concentrations according to the aircraft size

Fig. 5 shows the differences in the concentrations of airborne bacteria and fungi by different aircraft sizes. The mean concentrations of airborne bacteria and fungi were found to be highest in small aircraft (124.9 cfu/m<sup>3</sup> and 113.2 cfu/m<sup>3</sup>) and lowest in large aircraft (51.3 cfu/m<sup>3</sup> and 42.0 cfu/m<sup>3</sup>), showing a statistically significant difference (p < 0.05).



Fig. 3. Airborne microbial concentrations according to the ventilation method.

#### 4. Discussion

In the past, research in the field of aviation service was limited to that on chemical substances and flight attendants. Moreover, research on airborne microorganisms in the aviation industry has been incomplete. However, studies of microorganism exposure levels in the air in various industries have continued. Studies of airborne microorganisms have been conducted in relation to various industrial groups, such as occupational groups in agriculture [13]. General urban air environments [14] and studies on airborne bacteria at hospitals [15].

Recently, various studies related to occupational health have been conducted in the field of aviation services as well. Examples include a study of aircraft manufacturing worker deaths [16,17], a study of aircraft maintenance worker deaths [18]. Research on the fatigue and stress of air traffic controllers, and work on the mortality rate of aircraft maintenance workers due to chemical exposure [19]. Other studies, such as work on cumulative traumatic disorders with high scalability among aircraft manufacturing workers [20], have concentrated on workers in other fields related to aircraft. The interest and importance of research on the safety and health of aviation service workers is growing both at home and abroad.

The measured concentrations of airborne bacteria and airborne fungi in the aircraft utilized in this study here were found to be

#### Table 2

Correlation analysis between airborne microorganisms according to temperature and relative humidity inside and outside the aircraft

Sortation	Indoor temperature	Indoor relative humidity	Outdoor temperature	Outdoor relative humidity	Indoor airborne bacteria concentration	Indoor airborne fungi concentration	Outdoor airborne bacteria concentration	Outdoor airborne fungi concentration
Indoor temperature	1	_	_	_	_		—	_
Indoor relative humidity	-0.546*	1	—	-	—	—	—	_
Outdoor temperature	0.346†	-0.593*	1	_	—	—	—	—
Outdoor relative humidity	-0.346†	0.692*	-0.800*	1	—	—	—	-
Indoor airborne bacteria concentration	0.008	0.352*,†	-0.252	0.331 <sup>†</sup>	1	_	_	_
Indoor airborne fungi concentration	-0.370†	0.573*	-0.729*	0.755*	0.324	1	—	_
Outdoor airborne bacteria concentration	0.252	-0.288	0.114	-0.135	-0.141	-0.117	1	_
Outdoor airborne fungi concentration	-0.276	0.432*	-0.515*	0.597*	0.157	0.281	-0.152	1

Note: Each represents a correlation between variables.

\* Significant at the 1% level.

<sup>†</sup> Significant at the 5% level.



Fig. 4. Airborne microbial concentrations according to the type of cleaning work.

below the standard values set by the Republic of Korea (airborne bacteria 800 cfu/m<sup>3</sup>, airborne fungi 500 cfu/m<sup>3</sup>) and are very low when compared to the concentrations associated with other occupational groups. The measurement period here was from July to September, and the air conditioning system that operated during the cleaning of the aircraft appeared to have an effect.

In addition, for most aircraft, a ventilation system using a highefficiency HEPA filter is applied, unlike the ventilation systems in general workplaces. Therefore, it is believed that maintaining a high ventilation volume compared to those in other industries is the main cause of the decreased levels of airborne microorganisms.

Also the work is carried out without passengers due to the nature of the cleaning work, which is considered to be the principal reason for the low concentration of airborne microorganisms measured in this study.

As a result of the correlation analysis between the airborne microorganisms according to the presence or absence of rain and the thermal factors of the temperature and relative humidity, the correlation between the humidity inside the aircraft and the airborne bacteria levels was found to be most significant on a sunny day. In general, it has been reported that the relative humidity has a significant effect on the generation of airborne microorganisms. It is known that a relative humidity of 60% or more has an effect on the growth of airborne fungi [21]. In this study, it was found that airborne fungi levels were higher with higher relative humidity levels than airborne bacteria in sunny weather.

It is judged that the relative humidity of 60% or less has a significant influence on the increase in the occurrence of airborne fungi, and related additional studies need to be conducted to clarify the correlation between airborne microorganisms and temperature along with relative humidity.



Fig. 5. Airborne microbial concentrations by aircraft size.

Among the aircrafts that has been measured, the concentration of airborne microorganisms was the highest in Company D.

In the case of Company D, natural ventilation is adopted, and due to the general type of work (due to the cleaning of many aircraft per day, they are constantly transferred to other aircraft), dust serving as a nutrient source and carrier of microorganisms is generated on the floor in large amounts. This is presumed to be the reason for scattering.

In the case of Company D, all of the aircraft of the company were small aircraft, and for most of the small aircraft, the form of natural ventilation was used.

As the scale is small, dust is easily scattered in the aircraft, which affects the concentrations of airborne microorganisms.

On the other hand, Company B adopts the forced ventilation method, and their use of intensive cleaning operation (there is no movement to another aircraft because the workers only cleaned one aircraft per day) reduces the level of airborne microorganisms, resulting in a relatively low concentration.

In this study, the conventional method of evaluating the exposure level of airborne microorganisms of measuring at a height of 1.2 to 1.5m from the floor was adopted.

Currently, a fixed-type local sample collection method is used to evaluate airborne microorganisms, but the development and distribution of personal measuring equipment and related products to workers is relatively insufficient compared to local sample collection equipment.

In addition, regarding the previously developed personal sample collector, concentrations must be collected by sampling through filter paper or an absorbent solution, followed by transportation to an analysis room and culturing in a medium with a pretreatment.

Due to these cumbersome processes, these methods are not well utilized in the field of environmental and occupational health.

If a collector capable of measuring individual worker exposure levels was used instead in this study, the level of exposure to airborne microorganisms of aircraft cleaning workers could have been evaluated more accurately.

In this study, due to the limited field survey period and airborne microbial measurement equipment, extended measurements were not performed, i.e., seasonal measurements, flight schedule measurements, and different aircraft types and corresponding sizes.

In addition, measurements of aircraft galleys, where the concentrations of airborne microorganisms were estimated to be high due to food handling, were not conducted.

Therefore, in order to identify the nature of aircraft cleaning workers' exposure to biological hazards accurately, further studies focusing on the selection of work types involving exposure to microorganisms at high concentrations in the air and during the work of all workers exposed to biological hazards in aircraft should be conducted.

This study can serve as basic data for researchers concentrating on aircraft cleaning workers so that they may accurately measure the biohazardous factors of aircraft cleaning workers. Moreover, it is necessary to expand research on the characteristics of aircraft cleaning workers and their job characteristics.

This study has the following limitations. Ventilation volume and ventilation efficiency were not mentioned. There is no distinction other than the classification by aircraft size. In addition, the types of work of cleaning workers were not standardized, so the types of work were not mentioned.

In the case of French cheese factories, 104 to  $2 \times 108$  cfu/m<sup>3</sup> for Fungi and from 103 to 106 cfu/m<sup>3</sup> for bacteria, and in the compost plants in Korea, bacteria averaged 7728 (±1024) cfu/m<sup>3</sup> for Italy's timber mill, the bacteria were found to be 130-2000 cfu/m.<sup>3</sup> [22–24]

As such, aircraft showed a lower level of aerosol concentration than other places. This seems to reflect of the characteristics of the high-performance HEPA filter applied to the aircraft and the cleanly managed aircraft for passengers.

In this study, concentrations of airborne bacteria and fungi were measured in the absence of passenger, which is one of the main generation sources of them, due to aircraft cleaning operations. Therefore, in the future, it is necessary to measure airborne bacteria and fungi during the flight operation in which passengers are present in order to evaluate practical exposure to them in aircraft.

There are several other agents as well as airborne bacteria and fungi in bioaerosol, but this study has limitations in evaluating exposure to only airborne bacteria and fungi. Thus, additional studies should be considered on various characteristics of bioaerosol such as identification of airborne microorganisms and their infectivity and toxicity potentially present in aircraft.

#### 5. Conclusion

In this study, differences in concentrations of airborne bacteria and airborne fungi according to the work conditions of aircraft cleaning workers were compared. The concentrations of airborne bacteria and airborne fungi in domestic aircraft were found to be below the standard values of the Republic of Korea (airborne bacteria 800 cfu/m<sup>3</sup>, airborne fungi 500 cfu/m<sup>3</sup>). The mean concentrations of airborne bacteria and fungi according to climatic conditions were higher outside and on rainy days than inside and on sunny days. Based on the results obtained from this study, it was found that rainy weather conditions, i.e. high humidity conditions, would act as a factor in increasing the concentrations of airborne bacteria and fungi. Regarding the ventilation type used, it was found that the concentration of airborne microorganisms was low in aircraft adopting the forced ventilation method. It was found that the concentrations of airborne microorganisms according to the type of work by the aircraft cleaning workers were lower during intensive cleaning (cleaning one aircraft per day) as compared to general cleaning (cleaning multiple aircraft per day). In terms of the aircraft scale, concentrations were highest in small aircraft and lowest in large aircraft. In this study, the concentration of bioaerosol in aircraft was low, but bioaerosol research on various environments should be conducted.

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#### **Conflicts of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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