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Research Paper

Effects of Seasonal Changes on the Concentration of Airborne Bacteria and Fungi in Indoor Laboratory Animal Rooms

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ABSTRACT

The purpose of this study was to assess temporal changes in the concentrations of bioaerosols in a laboratory mouse room (LMR) and a laboratory rabbit room (LRR) and to determine environmental factors associated with the total bacteria and fungi concentrations. The concentrations of total airborne bacteria and fungi in the LMR and LRR were sampled once a month from March, 2011 to February, 2012. The total bacteria concentrations in the LMR showed a gradual increase during the summer, while the highest concentrations in the LRR were noted in July and November. The total fungi concentrations showed a similar seasonal pattern of change in the LMR and LRR with a noticeable increase during the summer. Relative humidity (RH) was the environmental factor most associated with the concentrations of total bacteria and fungi. Temperature, CO_2 and illuminance were also slightly associated with total bacteria and fungi concentrations. The overall airborne microbe concentrations were significantly greater in the LRR than in the LMR. Airborne microbe concentrations in the LMR and LRR varied greatly depending on seasonality, and these changes were affected by environmental factors.

Key words: Laboratory animal room, total bacteria, total fungi, relative humidity, illuminance, seasonality.

INTRODUCTION

Indoor particulate matter is linked with detrimental health impacts (Goyal and Kumar, 2013). People spend the majority of their time indoors and indoor environments have sometimes been considered to be intrinsically different from outdoor environments (Smith, 2008). In an indoor air environment, the most common bacteria are Gram-negative bacteria and Gram-positive bacteria, such as *Micrococcus* and *Staphylococcus*. Airborne bacteria are known to cause infectious diseases, hypersensitivity pneumonitis and the impairment of lung function (Gomy et al., 1999; Pastuszka et al., 2000). *Cladosporium, Penicillium* and *Aspergillus* are fungi that are commonly found in high concentrations on farms and at composting facilities. These fungi cause allergic rhinitis, extrinsic allergic alveolitis, and bronchial asthma (Chang et al., 2001; Lugauskas et al., 2004; Douwes et al., 2003).

Indoor airborne microbe problems are induced by environmental factors, such as microbial contaminants from outdoor air, the type of ventilation used and the presence of moisture (Burger, 1990). Temperature and relative humidity (RH) are the most critical factors for the survival of bioaerosols (Takahashi, 1997; Tang, 2009). As a result, the RH in an indoor environment can contribute to health problems such as respiratory infections and allergies (Arundel et al., 1986).

Individuals who work with animals or conduct animal biotechnology studies have a greater potential of being directly exposed to micro-organisms than those with other occupations. Therefore, the maintenance of a sanitary environment is crucial for workplaces that deal with animals. Several health risk factors exist in laboratory animal rooms. For example, there are airborne

Table 1: The characteristics of the laboratory animal rooms.

Variables	Laboratory mouse room	Laboratory rabbit room
Room volume (m^3) (W × D × H)	81.4 (5.3 × 6.4 × 2.4)	61.4 (3.3 × 6.2 × 3.0)
Ventilation	HVAC system	Conventional ventilation system
Air exchange rate	14 - 18 times per hour	-
Temperature (°C)	23 ± 3	19 ± 6
RH (%)	40 ± 20	47 ± 15
CO_2 (ppm)	635 ± 104	384 ± 61
Illuminance (lux)	682 ± 109	239 ± 111
Cage volume (m) (W × D × H)	0.03 (0.3 x 0.4 x 0.3)	0.06 (0.4 × 0.5 × 0.41)
Number of animal per cage	2 - 10 mice	One rabbit
Number of cage	140	30
Bedding type	Aspen bedding	None
Feed	Purina diet	Purina diet
Cleaning cycle (floor)	Every weekday	Twice a week

contaminants or animal allergens originating from animal fur, faeces, urine, food, or bedding materials (Kaliste et al., 2002).

Individuals who work with laboratory animals often experience allergic symptoms, such as allergic rhinoconjunctivitis with nasal congestion, rhinorrhoea, sneezing and itching. These allergies are caused by laboratory mice and rabbits (Bush et al., 1998). Bioaerosols in the laboratory animal rooms might also cause respiratory symptoms, including asthma and infectious diseases (Douwes et al., 2003).

Many previous studies were carried out on allergic diseases in laboratory animal care personnel (Bush et al., 1998; Jang et al., 2009). However, airborne microbe concentrations in laboratory animal rooms, or their negative effects on human health, have not been fully investigated. In public facilities, such as medical institutions, nursery facilities, senior centres and postpartum care centres, there is a domestic threshold value for airborne micro-organisms (a total of 800 CFU/m^3 suspended bacteria) to maintain indoor air quality (Ministry of Environment, Korea). There is no such exposure limit for animal laboratories, despite the great risks of micro-organisms in these laboratories on human health.

The purpose of this study was to assess temporal changes in the concentrations of total airborne bacteria and fungi in a laboratory mouse room (LMR) and a laboratory rabbit room (LRR) and to determine environmental factors, such as temperature, RH, carbon dioxide (CO_2) and illuminance associated with these total bacteria and fungi concentrations.

MATERIALS AND METHODS

Laboratory animal rooms

This study was conducted for a 12-month period from March, 2011 to February, 2012. Samples were collected once a month in the LMR and LRR with Ethics Committee that granted the approval for the animal studies. Table 1 summarizes the characteristics of the laboratory animal rooms. The LMR (5.3 m × 6.4 m × 2.4 m) and the LRR (3.3 m × 6.2 m × 3.0 m) are located in the College of Pharmacy University. Indoor air quality was controlled by heating, ventilation and air-conditioning (HVAC) system, with high efficiency air filters and RH control, in the LMR, and by a conventional ventilation system in the LRR. The temperatures of the LMR and LRR were 23 ± 3°C and 19 ± 6°C, respectively, and the RHs were 40 ± 20% and 47 ± 15%, respectively. CO_2 and illuminance were 635 ± 104 ppm and 682 ± 109 lux in the LRM and 384 ± 61 ppm and 239 ± 111 lux in the LRR.

The cleaning cycle was every weekday in the LMR and twice a week in the LRR. This involved the following process: cleaning the floor with a broom followed by a mop; changing the cage, tray, bedding material, food, water bottle and removing animal fur, faeces and urine. In particular, the water bottle was reported as a potential source of the growth of micro-organisms (Kaliste et al., 2002).

Environmental factors

The environmental factors such as temperature, RH, CO_2 and illuminance were measured in the LMR, LRR and outdoor using real time monitor. Temperature and RH were recorded by VelociCalc Air Velocity Meter (Model 9555, TSI Inc., USA). CO_2 concentrations were measured by the IAQ-Calc Indoor Air Quality Meter (Model IM-2D, Topcon Co., Japan). The outdoor illuminance was too high, as such; the illumination meter was not able to measure the illuminance. These environmental factors were recorded during the total bacteria and total fungi sampling.

Sampling and analysis

Airborne total bacteria and total fungi were collected thrice a day (9:00 to 10:00, 13:00 to 14:00, and 16:00 to 17:00), for once a month in the LMR and LRR. Sampling was conducted at the same location each time, at the centre of

Table 2: Monthly concentrations of total bacteria and fungi in laboratory animal rooms.

Month	Total bacteria (CFU/m³)				Total fungi (CFU/m³)			
	N	LMR		LRR	N	LMR		LRR
		GM (GSD)	N	GM (GSD)		GM (GSD)	N	GM (GSD)
January	3	13.5 (3.8)	3	400.7 (1.5)	3	10.2 (2.6)	3	32.5 (1.7)
February	3	24.7 (9.9)	3	289.2 (1.5)	3	5.6 (2.2)	3	15.2 (2.2)
March	3	10.2 (3.6)	3	56.0 (1.3)	3	7.0 (2.0)	3	16.2 (2.1)
April	3	138.2 (4.7)	3	186.2 (1.4)	3	8.8 (2.9)	3	43.6 (2.7)
May	3	80.0 (2.8)	2*	380.6 (1.1)	3	50.5 (1.6)	2*	178.5 (3.2)
June	3	121.4 (1.4)	3	264.6 (1.9)	3	80.9 (1.8)	3	111.6 (1.2)
July	3	754.7 (2.5)	2*	1017.7 (1.2)	3	311.2 (1.2)	3	1582.8 (1.1)
August	3	511.6 (1.9)	3	658.4 (4.6)	3	88.2 (5.2)	3	50.1 (5.6)
September	3	276.5 (2.5)	3	780.1 (1.2)	3	54.5 (1.2)	3	110.6 (1.6)
October	3	54.5 (1.8)	3	256.6 (2.9)	3	22.5 (2.7)	3	46.0 (3.1)
November	2*	135.7 (5.2)	3	1110.1 (3.5)	2	53.9 (1.8)	2	73.4 (1.9)
December	3	352.9 (1.1)	3	512.0 (1.2)	3	55.7 (1.3)	3	86.2 (1.8)
Total	35	101.2 (5.3)	34	369.9 (2.6)	36	32.5 (3.9)	34	69.2 (4.1)

*; One sample missing.

the 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 for 5 min. The sampling equipment was sterilized with a 70% ethyl alcohol swab prior to operation and nutrient media in Petri dishes placed on the one-stage impactor. The total bacteria samples were collected on trypticase soy agar (TSA) plates and the fungal samples collected on Sabouraud dextrose agar with chloramphenicol (SDA_C) plates. Outdoor concentrations of bioaerosols were collected between 13:30 and 14:30 on the rooftop of the same building and two other rooftops on campus. When it rains, outdoor samplings were performed one or two days later. The exposed plates were then sealed with laboratory film to prevent contamination and desiccation during 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, and SDA_C plates incubated at 35°C for about 5 days. Colonies of total bacteria and total fungi were counted daily. The positive-hole correction table was used to adjust colony counts (Macher, 1999). The concentrations of total bacteria and total fungi were calculated by dividing the number of colonies by air volume and written as colony-forming units per cubic meter of air (CFU/m³) (NIOSH Manual of Analytical Methods, 1998).

Statistical analysis

The Kolmogorov-Smirnov test was used to determine whether the data were normally or log-normally distributed. The geometric means (GM) of the airborne microbe concentrations and their geometric standard deviations (GSD) were calculated, since the data were log-normally distributed. The independent *t*-test was used to compare the differences in the GM of airborne microbe concentrations in the LMR and LRR and a p value of less than 0.05 was considered to be statistically significant. Correlation analysis was used to identify the association between airborne microbe concentrations and environmental factors. Simple linear regression analyses were conducted to determine the effects of the environmental factors on the total bacteria and total fungi concentrations. All statistical analyses were performed using SPSS (version 19.0; IBM Inc., USA).

RESULTS

Table 2 summarizes the monthly concentrations of airborne total bacteria and fungi in the LMR and LRR. The concentrations of bioaerosols varied greatly depending on time. The range of the GM of the total bacteria concentrations in the laboratory animal rooms over the year was 10 to 1,110 CFU/m³. The highest concentration of total bacteria was found in July (755 CFU/m³) in the LMR and in November (1,110 CFU/m³) in the LRR, while the lowest concentration of total bacteria was observed in March for both rooms (10 CFU/m³ in the LMR and 56 CFU/m³ in the LRR). The results showed that the GM of the LMR and LRR total bacteria concentrations were significantly different ($p < 0.05$). The GM of the total fungi concentrations in the laboratory animal rooms over the year ranged from 6 to 1583 CFU/m³. The highest total fungi concentration was seen in July for both rooms (311 CFU/m³ in the LMR and 1,583 CFU/m³ in the LRR). The lowest total fungi concentration was seen in February for both rooms (6 CFU/m³ in the LMR and 15 CFU/m³ in the LRR). The GM of the LMR and LRR total fungi concentrations were found to be significantly different ($p < 0.05$).

Table 3 shows the outdoor concentrations of bioaerosols and the indoor/outdoor ratios (I/O ratios) for the LMR and LRR. The range of the GM of the outdoor total bacteria concentrations over the year was 45 to 331 CFU/m³. The

Table 3: Outdoor concentrations of total bacteria and fungi and indoor/outdoor ratios.

Month	Total bacteria (CFU/m³)				Total fungi (CFU/m³)			
	N	GM (GSD)	I/O ratio		N	GM (GSD)	I/O ratio	
			LMR	LRR			LMR	LRR
January	3	65.9 (1.5)	0.2	6.1	3	14.6 (1.9)	0.7	2.2
February	3	51.6 (1.8)	0.5	5.6	3	17.0 (2.7)	0.3	0.9
March	3	55.2 (2.3)	0.2	1.0	3	14.0 (1.0)	0.5	1.2
April	3	77.1 (2.6)	1.8	2.4	3	70.6 (6.1)	0.1	0.6
May	3	45.1 (5.6)	1.8	8.4	3	29.4 (4.3)	1.7	6.1
June	3	124.9 (3.0)	1.0	2.1	3	174.3 (4.9)	0.5	0.6
July	3	331.2 (1.3)	2.3	3.1	3	301.9 (2.2)	1.0	5.2
August	3	178.1 (1.2)	2.9	3.7	3	58.8 (1.2)	1.5	0.9
September	3	97.3 (1.2)	2.8	8.0	3	84.7 (1.2)	0.6	1.3
October	3	233.2 (2.6)	0.2	1.1	3	105.9 (3.0)	0.2	0.4
November	3	116.5 (2.8)	1.2	9.5	3	147.7 (1.1)	0.4	0.5
December	3	151.5 (1.3)	2.3	3.4	3	33.1 (4.3)	1.7	2.6
Total	36	105.6 (2.5)	1.4	4.5	36	56.2 (3.8)	0.8	1.9

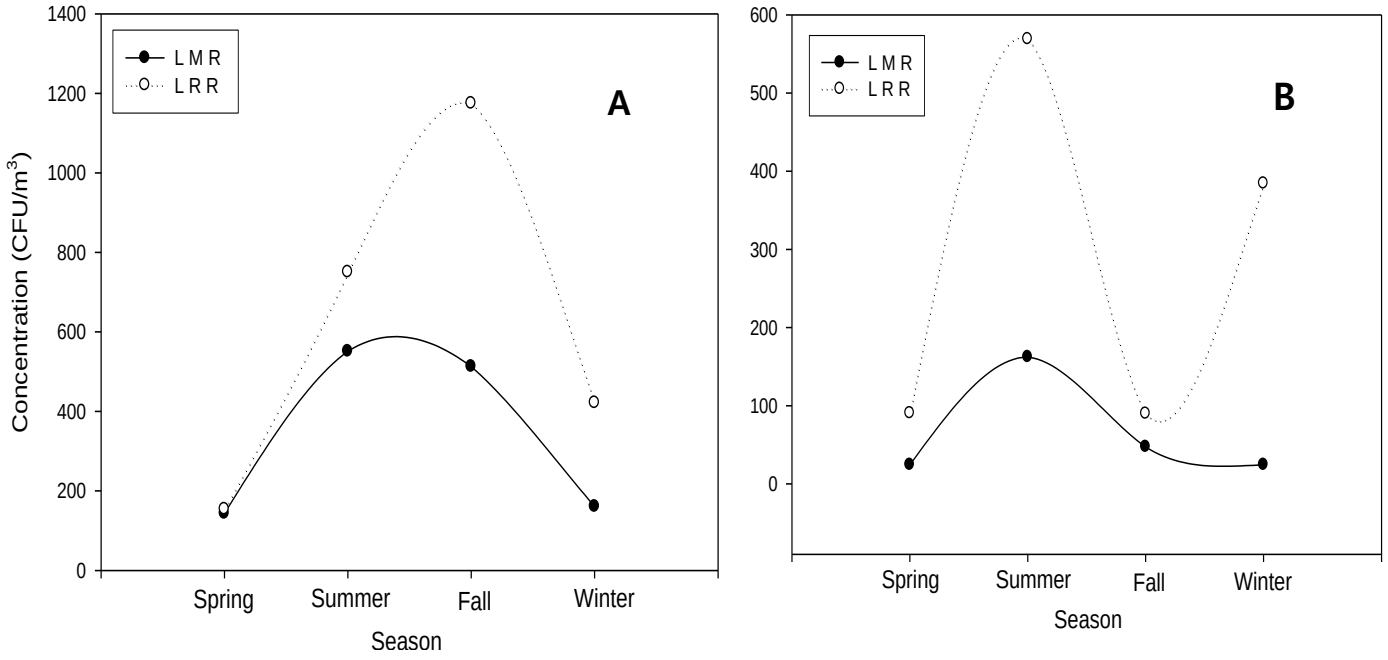

Figure 1. Seasonal changes in concentrations of total bacteria and total fungi in LMR and LRR rooms. (a) Total bacteria and (b) Total fungi.

average I/O ratio of the total bacteria was 1.4 in the LMR and 4.5 in the LRR. The I/O ratios of the total bacteria in the laboratory animal rooms were higher than 1 for all months, except from January to March and October in the LMR. The GM of the outdoor total fungi concentrations throughout the year ranged from 14 to 302 CFU/m³. The average I/O ratio of the total fungi in the LMR and the LRR was 0.8 and 1.9, respectively. The highest I/O ratio for total fungi was observed in May and December for the LMR (1.7) and in May for the LRR (6.1).

To demonstrate seasonal changes in the concentrations of bioaerosols, the 12-month period was divided into four seasons: spring, March to May; summer, June to August; fall, September to November; and winter, December to February. Figure 1 presents the seasonal changes in airborne microbe concentrations. The total bacteria concentrations in the LMR and outdoors showed similar seasonal changes, increasing gradually from spring to summer and then decreasing from summer to winter. The concentrations of total bacteria in the LRR increased from spring to summer and remained constant through the other seasons. Although none of the seasonal concentrations for both total bacteria and total fungi in the LMR and LRR were significantly different ($p > 0.05$), the concentrations in the LRR were higher than the concentrations in the LMR

Simple linear regression analysis was performed to determine the effect of various environmental factors on the concentrations of total bacteria and total fungi in the LMR, the LRR, and these laboratory animal rooms

Table 4: Simple linear regression analysis of the effect of environmental factors on total bacteria and total fungi concentrations.

Place	Environmental factors	Total bacteria				Total fungi			
		β[a]	Standard error	95% CI[b]	p-value	β	Standard error	95% CI	p-value
Laboratory mouse room	Temperature	1.13	1.14	0.87-1.46	0.34	1.13	0.01	0.96-1.33	0.14
	RH	1.04	1.01	1.02-1.07	0.001	1.05	0.00	1.03-1.07	< 0.001
	CO_2	1.01	1.00	1.00-1.01	0.001	1.01	0.00	1.00-1.01	< 0.001
	Illuminance	1.00	1.00	1.00-1.01	0.23	1.00	0.00	1.00-1.01	0.23
Laboratory rabbit room	Temperature	1.02	1.00	0.97-1.07	0.45	1.12	0.04	1.04-1.20	< 0.001
	RH	1.02	1.01	0.99-1.04	0.15	1.05	0.01	1.03-1.08	< 0.001
	CO_2	1.00	1.00	0.99-1.01	0.73	1.01	0.00	1.00-1.01	0.10
	Illuminance	1.00	1.00	0.99-1.00	0.82	1.00	0.00	1.00-1.01	0.62
Laboratory mouse room + Laboratory rabbit room	Temperature	0.97	1.03	0.91-1.04	0.42	1.08	0.03	1.01-1.16	0.02
	RH	1.04	1.01	1.02-1.06	< 0.001	1.05	0.01	1.04-1.07	< 0.001
	CO_2	1.00	1.00	0.99-1.00	0.20	1.00	0.00	0.99-1.00	0.74
	Illuminance	1.00	1.00	0.99-0.99	< 0.001	1.00	0.00	0.99-1.00	0.12

a; Regression coefficients, b; 95 % confidence interval

combined (Table 4). In the LMR, the environmental factors found to be associated with total bacteria and total fungi concentrations were RH (β = 1.04, p = 0.001 and β== 1.05, p < 0.001, respectively) and CO_2 (1.01, p = 0.001 and β = 1.01, p < 0.001, respectively). In the LRR, none of the environmental factors were associated with total bacteria concentrations. However, temperature (β = 1.12, p < 0.001) and RH (β = 1.05, p < 0.001) were significantly associated with total fungi concentrations. The combined results for the LMR and LRR were analyzed to find out the differences between this and each laboratory. The results revealed that RH (β = 1.04, p < 0.001 and β = 1.05, p < 0.001) was a common environmental factor that affected both total bacteria and total fungi concentrations in the combined laboratory animal rooms. In addition, illuminance (β = 1.00, p < 0.001) affected total bacteria concentrations and temperature (β = 1.081, p = 0.02) affected the total fungi concentrations in the combined laboratory animal rooms. The results of simple linear regression analysis revealed that RH was the environmental factor most associated with the concentrations of total bacteria and total fungi.

DISCUSSION

In the current study, the concentrations of bioaerosols in the LMR and LRR were measured in order to assess the monthly and seasonal changes. The effects of environmental factors were also investigated (temperature, RH, CO_2 and illuminance) on total bacteria and total fungi concentrations to examine whether there was any relationship between these factors and concentrations of airborne microbe.

Airborne microbe samples at the end of each month, over a 12-month period were measured. Total bacteria and total fungi were collected thrice a day throughout the month. The total bacteria concentrations in the LMR gradually increased during the summer, while in the LRR, the concentrations were higher than 1,000 CFU/m^3 in July and November. In summer, the microbe concentrations tended to increase due to high temperature and relative humidity. Therefore, the total bacteria concentrations showed the highest concentrations in July in both rooms. The mean concentration of total bacteria in the study was 271 CFU/m^3 in the LMR and 575 CFU/m^3 in the LRR. The highest concentration of total bacteria found in each room was 1,677 CFU/m^3 in the LMR and 4,550 CFU/m^3 in the LRR. These were lower than the values reported by a previous study, which were 6,400 CFU/m^3 and 9,000 CFU/m^3 in the LRRs (Kaliste et al., 2002). This is because the previous study measured a high level of airborne bacteria in the room during working activities and the bacteria concentrations rapidly increased after these activities.

The total fungi concentrations showed a similar seasonal pattern of change in the LMR and LRR and noticeably increased in summer. Summer in South Korea is a rainy season, with the relative humidity typically being higher than during the other seasons, which contributes to the growth of microbes. According to a previous study, total fungi concentrations varied within the maximal detection value of 120 CFU/m^3 in the LRRs (Kaliste et al., 2002). The mean total fungi concentration in this study was 72 CFU/m^3 in the LMR and 214 CFU/m^3 in the LRR and the concentration ranges were 4 to 370 CFU/m^3 and 34 to 1,782 CFU/m^3 for each room. The total fungi concentrations in our study were higher than those in the previous study.

Simple linear regression analysis was used to evaluate the association between environmental factors and the total bacteria and fungi concentrations. First, correlations between airborne microbe concentrations and environmental factors were investigated. In the LMR, RH and CO_2 were found to be associated with the concentrations of total bacteria and total fungi (p < 0.01). Several studies showed that RH is an important factor for the survival of bioaerosols (Takahashi, 1997; Tang, 2009).

However, only a few studies showed an association between CO_2 and microbe concentrations (Bartlett et al., 2004; Mentese et al., 2009). These studies reported a much higher CO_2 concentration in the LMR than in the LRR, which is not in agreement with the findings of our study.

However, there were more than five hundred different sizes of mice in the room. CO2 would be released into the air from the expiration of the animals, which would therefore affect the total bacteria and total fungi concentrations in the room. In the LRR, temperature and RH were the only environmental factors found to be associated with total fungi concentrations ($p < 0.01$). Indoor air quality was controlled by a heating, ventilating and air conditioning (HVAC) system in the LMR, while temperature and RH were maintained at consistent levels. However, indoor air quality in the LRR was controlled by a conventional ventilation system, which might have caused variations in environmental factors over time. This may be the reason for the finding that total fungi concentrations were affected by temperature and RH in the LRR. Interestingly, combining the results from the LMR and LRR, illuminance and RH were found to be associated with total bacteria concentrations ($p < 0.01$). For animals shown to be susceptible to phototoxic retinopathy, light in the room should be maintained between 130 and 325 lux at the cage level (National Research Council, Guide for the care and use of laboratory animals, 2010).

Temperature and RH were significantly associated with total fungi concentrations, when combining the results from the LMR and LRR ($p < 0.05$). Therefore, RH was the environmental factor most associated with both total bacteria and total fungi concentrations. The overall airborne microbe concentrations in the LRR were higher than those in the LMR. The most distinguishable differences between the two rooms were the ventilation systems and cleaning cycles. The indoor air quality of the LMR was controlled using an HVAC system, whereas the LRR was controlled using a conventional ventilation system. Ventilation systems affect indoor airborne microbe concentrations since they prevent outdoor micro-organisms from being transported inside the buildings (Burge et al., 2000; Wu et al., 2005).

There are several limitations in this study. First, the incubation temperature of the total fungi was high. The optimum temperature range for the growth of total fungi is known to be 25 to 30°C, but in our study, the total fungi were incubated at 35°C. However, the optimum temperature differed depending on the species. In the LRR, the common genera of total fungi were *Cladosporium, Penicillium*, yeasts and *Aspergillus* (Kaliste et al., 2002). The optimum temperature range is 23 to 32°C for *Penicillium*, 40 to 53°C for *Aspergillus* and 30 to 37°C for yeast (Ayersi, 1969; Pitt et al., 1983). Some species of fungi are not able to grow at 35°C and as such the total fungal concentrations were underestimated. Secondly, the species of the total bacteria and fungi were not identified, and as a result we are unable to identify the genera that existed in the laboratory animal rooms.

The concentrations of bioaerosols changed over time and were affected by environmental factors. Thus, airborne microbe concentration levels need to be controlled to prevent negative influences on the health of individuals who work in animal laboratory rooms. Furthermore, these findings should be used to determine the optimal level of bio-safety for airborne microbe concentrations in laboratory animal rooms.

Conclusions

In this study, we assessed monthly and seasonal changes in the concentrations of bioaerosols in the LMR and LRR and determined the environmental factors associated with total bacteria and total fungi concentrations. RH was found to be the environmental factor most associated with the total bacteria and fungi concentrations. Temperature, CO2 and illuminance were also found to be slightly associated with total bacteria and fungi concentrations. Overall, airborne microbe concentrations were significantly higher in the LRR than in the LMR.

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