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Relationship between culturable airborne bacteria concentrations and ventilation systems in underground subway stations in Seoul, South Korea

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Abstract In this study, we evaluated the concentrations of culturable airborne bacteria (CABs) in the underground environment of 16 subway stations in Seoul, South Korea. The effects of environmental factors on CAB distributions and concentrations, including temperature, relative humidity, depth, year of construction, number of subway passengers, and ventilation, were investigated. Tryptone soy agar was used as the culture medium. Isolated bacteria were initially characterized according to cell morphology and Gram staining and then further characterized using the VITEK 2 XL microbial identification system. There were significant correlations between CAB concentrations and station temperature, depth, and construction year. *Micrococcus* and *Staphylococcus* species accounted for 66 % of the total number of CABs identified. CAB concentrations in stations with ventilation systems were significantly lower than those in stations without ventilation systems ($p<0.001$). Thus, it is critical to develop techniques to improve indoor air quality in subway stations with no ventilation system.

Keywords Airborne bacteria · Enclosed subway stations · Environmental factors · Air supply · Ventilation

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Introduction

Public indoor environments have sometimes been considered intrinsically different from the outdoors and most of people's time is spent indoors (Smith 2008). Indoor particulate matter is especially linked with detrimental health impacts (Goyal and Kumar 2013; Son et al. 2013). Among the different types of particulate matters, bacteria play an important role in human health. In particular, some types of bacteria are resistant to antibiotics and are likely to spread among crowds of people in public areas, such as subway stations (Zhou and Wang 2013).

Subways are a principal means of public transportation in large cities around the world (Hwang et al. 2010). The confined spaces within subway stations make these locations potential targets for airborne dispersion of biological threat agents, making indoor air quality in subway stations an important consideration for public health (Dybwad et al. 2012). Notably, indoor air quality is worse in subway stations than in aboveground public buildings (Kim et al. 2008). Several recent studies have investigated the concentrations of airborne bacteria in subway environments (Bogomolova and Kirtsideli 2009; Dybwad et al. 2012; Kim et al. 2011; Robertson et al. 2013; Leung et al. 2014). However, the effects of ventilation systems in subway stations on the concentrations of culturable airborne bacteria (CABs) have not been well studied (Zhang et al. 2007).

The purpose of this study was to assess the concentrations of CABs in underground subway stations in Seoul, South Korea. We also evaluated environmental factors affecting the concentrations of CABs, including the temperature, relative humidity, depth, construction year, number of passengers, and presence of ventilation systems in these stations.

Materials and methods

Sampling and analyses

In the spring of 2013, 33 air samples were collected from 16 subway stations in Seoul, South Korea. All of the stations had drainage ditches for underground water under the platform, as well as heating, ventilation, and air conditioning systems. Air samples were taken two or three times at each station at a nominal height of 100 cm (50–150 cm) above the platform floor. Samples were collected through polycarbonate membrane filters to facilitate particle sample collection (filter pore size 0.4 μm, three-piece cassettes measuring 37 mm in diameter; Nuclepore Corp., Cambridge, MA, USA) at a flow rate of 2 L min^{-1} (1.98–2.23) for 60 min, using an air sampler (Escort LC Pump; Mine Safety Appliances Company, Pittsburgh, PA, USA) (NIOSH 1998). The size of bacteria is approximately 1–5 μm in diameter; therefore, the filter pore size used here (0.4 μm) is sufficiently small for collection of significant microbes (ACGIH 1999). During each sampling period, the temperature and relative humidity were recorded three times. The samples were delivered to an analytical laboratory within 24 h after sampling and were analyzed immediately after arrival.

To extract bacteria from the filter samples, 1.5 mL sterile peptone water (0.1 % $w\,v^{-1}$ containing 0.01 % Tween 80) was pipetted onto the support pad through the cassette outlet, after which the connection was plugged. Next, 5 mL of sterile peptone water was transferred through the inlet, and the cassette was capped and vigorously shaken for at least 30 min. After shaking, the suspension was extracted with a syringe.

Tryptone soy agar was used as the culture medium for CABs. Plates were incubated at room temperature (25 °C) for more than 2 days, and the number of colony forming units (CFUs) was determined. The concentration of CABs has been expressed as colony forming units per cubic meter. Values below the detection limit were considered to be 3 CFU m^{-3}. Values below the detection limit were assigned a value of the limit of detection (LOD)/$\sqrt{2}$ (Hornung and Reed 1990).

Bacteria isolated from the colonies were initially characterized according to cell morphology and Gram staining. Bacteria were further characterized using the VITEK® 2 XL system (bioMerieux, Inc., Marcy l'Etoile, France). Gram-negative (GN) bacteria were identified using the VITEK 2 GN card and subjected to antimicrobial susceptibility testing (AST) using the VITEK 2 AST-P580 and AST-N093 cards. Gram-positive (GP) bacteria were identified using the VITEK 2 GP card. In total, 10 % of the integrated samples were reserved for blank tests, and all of the results were below the detection limit.

Statistical analyses

Nonparametric analysis was used to test for statistically significant differences in CAB concentrations. The Kolmogorov-Smirnov analysis was performed to determine whether the CAB concentrations varied according to the presence or absence of a ventilation system. Spearman correlation analyses were used to assess the relationship between CAB concentrations and the other environmental factors. All statistical analyses were carried out using the SPSS software package (version 17.0).

Results

Table 1 summarizes the concentrations, species, and distributions of CABs in the subway stations. CAB concentrations ranged from 3 to 1952 CFU m^{-3} (average for all stations, 308 CFU m^{-3}). Both GP bacteria (*Micrococcus* spp., *Kocuria rosea*, *Corynebacterium jeikeium*, *Staphylococcus hominis*, *S. hominis* spp., *Staphylococcus saprophyticus*, *Staphylococcus cohnii*, *Bacillus* spp., *Staphylococcus epidermidis*, *Clostridium histolyticum*, and *Diphtheroides*) and GN bacteria (*Sphingomonas paucimobilis*, *Acinetobacter ursingii*, *Acinetobacter lwoffii*, *Moraxella* spp., and *Moraxella catarrhalis*) were identified in the air samples.

Spearman's correlation coefficients were calculated to evaluate whether there were associations between CAB concentrations and station temperature, relative humidity, depth, and year of construction (Fig. 1). We found that there was a significant correlation between CAB concentration and temperature ($r=0.45$, $p<0.01$), construction year ($r=-0.52$, $p<0.01$), and depth of the station ($r=-0.65$, $p<0.01$). However, CAB concentrations were not significantly correlated with relative humidity.

Table 2 shows a comparison of CAB concentrations in subway stations with or without ventilation. CAB concentrations in stations with operating ventilation systems, which served an average of 2856 passengers, were significantly lower than CAB concentrations in stations without ventilation systems, which served an average of 2834 passengers ($p=0.001$). Most of the CAB concentrations were higher in stations without ventilation systems than in those with ventilation systems (Fig. 2).

Discussion

In this study, we measured the concentrations of CABs in subway stations of the Seoul Metro system. Interestingly, our results indicated that the CAB concentrations in subway

Table 1 Concentrations, species, and distributions of culturable airborne bacteria (CABs) and environmental factors in 16 subway stations in Seoul, South Korea

Station	*N*	CFU m^{-3} mean (SD)	Temp. (°C)	R.H. (%)	Construction year	Depth (m)	No. of passengers	Ventilation	Species of CABs identified (%)
a	2	74 (105)	20.7	39.2	1994	37.2	303	Absent	*Micrococcus* spp. (100)
b	2	1952 (293)	26.2	42.0	1983	13.9	5884	Absent	*Acinetobacter* spp. (14), *Bacillus* spp. (4), *Corynebacterium* spp. (23), *Micrococcus* spp. (46), *Staphylococcus* spp. (4), *Sphingomonas* spp. (10)
c	2	9 (0)	25.1	39.5	1984	21.0	4302	Present	*Micrococcus* spp. (100)
d	2	3 (0)	21.2	47.6	1993	26.9	409	Present	ND
e	2	7 (0)	24.7	56.4	1996	21.6	930	Present	*Staphylococcus* spp. (100)
f	2	1051 (662)	26.2	42.0	1985	22.4	1916	Absent	*Kocuria* spp. (67), *Micrococcus* spp. (20), *Sphingomonas* spp. (7), *Staphylococcus* spp. (7)
g	2	333 (88)	29.0	49.4	1985	15.1	1508	Present	*Micrococcus* spp. (76), *Moraxella* spp. (8), *Sphingomonas* spp. (8), *Staphylococcus* spp. (8)
h	2	25 (0)	27.9	61.6	1985	17.6	3832	Absent	*Micrococcus* spp. (67), *Staphylococcus* spp. (33)
i	2	3 (0)	28.1	47.9	1984	16.4	1192	Present	ND
j	2	3 (0)	23.1	39.2	1993	25.1	1138	Present	ND
k	2	3 (0)	24.0	39.7	1993	25.4	841	Present	ND
l	2	17 (0)	25.2	27.8	1985	19.6	4999	Present	*Micrococcus* spp. (40), *Staphylococcus* spp. (60)
m	3	496 (121)	30.5	59.0	1984	11.9	8529	Present	*Clostridium* spp. (11), *Diphtheroides* spp. (11), *Micrococcus* spp.(33), *Moraxella* spp. (11), *Staphylococcus* spp. (33)
n	2	70 (99)	24.4	26.9	1983	15.3	1945	Present	*Sphingomonas* spp. (100)
o	2	3 (0)	28.7	50.9	1985	23.2	5619	Present	ND
p	2	777 (99)	27.3	26.6	1974	12.3	2237	Absent	*Micrococcus* spp. (81), *Corynebacterium* spp. (19)
Total mean	33	308	25.8	43.5	1987	20.3	2849		

Korean guidelines, 800 CFU m^{-3}

CFU colony forming unit, *NA* not applicable, *ND* not detected

stations were lower in 2013 than in previous years (Hwang et al. 2010). In line with this, Seoul Metro has been making an effort to improve the air quality of the underground indoor environment by monitoring air quality, installing highly efficient air conditioners, and improving aged ventilation facilities since 2007 (Seoul Metro 2012). However, CAB

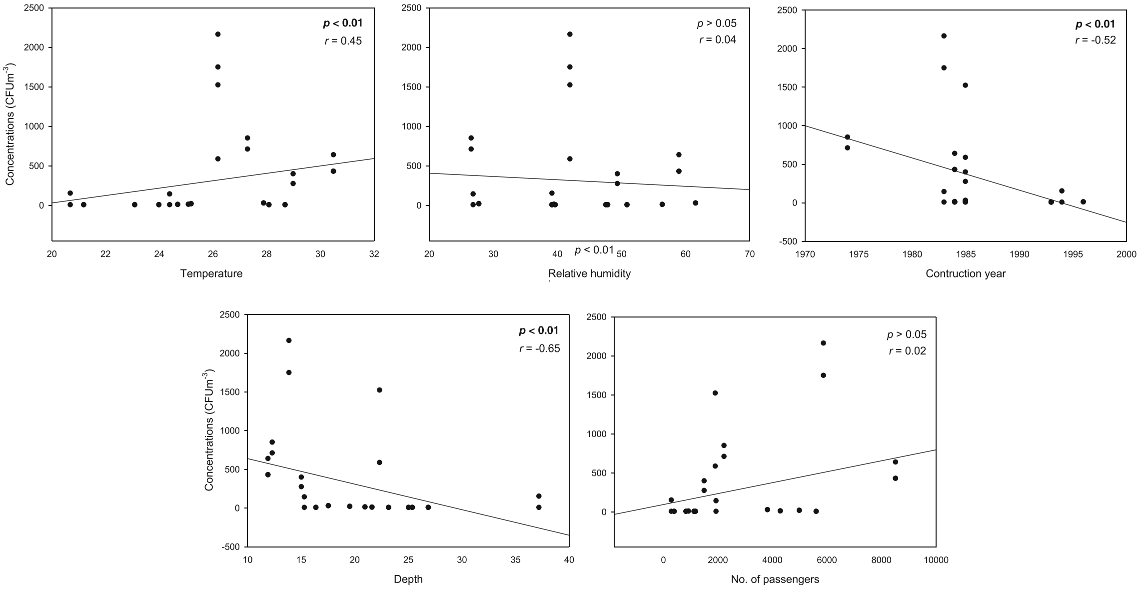

Fig. 1 Correlation analysis between culturable airborne bacteria (*CABs*) and characteristics of 16 subway stations in Seoul, South Korea

Table 2 Comparison of culturable airborne bacteria (CABs) in 16 subway stations with and without ventilation in Seoul, South Korea

Type of station	Station	*N*	CFU m^{-3} mean (SD)	CFU m^{-3} median	Average no. of passengers	Species of CABs identified (%)	*p*
No ventilation	a, b, f, h, p	10	776 (787)	645	2834	*Micrococcus* spp. (52), *Corynebacterium jeikeium* (17), *Kocuria rosea* (12), *Sphingomonas paucimobilis* (7), *Acinetobacter ursingii* (5), *Acinetobacter lwoffii* (3), *Staphylococcus hominis* (3), *Bacillus* spp. (2), *Staphylococcus cohnii* spp. (2)	0.001
Ventilation	c, d, e, g, i, j, k, l, m, n, o	23	104 (187)	7	2856	*Micrococcus* spp. (53) *Staphylococcus hominis* (18) *Moraxella* spp. (6), *Staphylococcus epidermidis* (3) *Staphylococcus saprophyticus* (9) *Clostridium histolyticum* (3), *Diphtheroides* spp. (3), *Sphingomonas paucimobilis* (6)	

Kolmogorov-Smirnov analysis

CFU colony forming unit

concentrations in two of the 16 stations (12.5 %) exceeded 800 CFU m^{-3}, the accepted indoor level of airborne bacteria in subway stations, according to Korean guidelines (Ministry of Environment of Korea 2014). Thus, more work is still needed to improve air quality in underground subway stations. A previous study showed that the mean concentration of total airborne bacteria in subway station platforms was 134 CFU m^{-3}, using a one-stage viable particulate cascade impactor for 10 min (Kim et al. 2011). Additionally, the study by Kim et al. (2011) did not detail changes in CABs according to environmental characteristics such as ventilation, depth, and year of construction, which are key determinants of airborne bacteria levels.

The maximum concentration of airborne bacteria in the community is governed by certain guidelines. For example, the guidelines for bioaerosols established by the National Institute of Occupational, Safety, and Health (NIOSH) states that bioaerosol concentrations must not exceed 1000 CFU m^{-3} (NIOSH 1998). Additionally, the Commission of the European Community (CEC) has classified bioaerosol concentrations below 500 CFU m^{-3} as low, those between 500 and 1000 CFU m^{-3} as intermediate, and those above 1000 CFU m^{-3} as high (CEC 1993). According to earlier studies, aggregated bacterial concentrations in 100 US office buildings were not elevated (indoor maximum 281 CFU m^{-3}) (Tsai and Macher 2005). However, elevated bacterial counts, ranging from 600 to 1800 CFU m^{-3}, were found at nine shopping malls in Hong Kong (Lee et al. 2002). Recommendations for improved ventilation are supported in a previous report of mold-damaged buildings, wherein airborne bacteria ranged from 14 to 1550 CFU m^{-3}. In this study, the authors concluded that bacteria levels exceeding 600 CFU m^{-3} could be associated with insufficient ventilation or abnormal sources of microorganisms (Salonen et al. 2007).

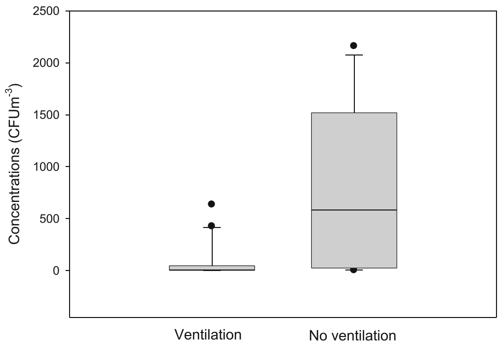

Fig. 2 Box plot showing differences in CAB concentrations in stations with and without ventilation (p=0.001)

The majority of isolated bacterial species belonged to the genus *Micrococcus*, followed by *Staphylococcus*, *Kocuria*, and *Corynebacterium*. Among these, *Micrococcus* and *Staphylococcus* accounted for 66 % of the total number of CABs identified. *Micrococcus*, *Staphylococcus*, and *Corynebacterium*, all of which included several species associated with an anthropogenic origin, were observed in almost all of the stations (Awad 2002; Seino et al. 2005; Kim et al. 2011). Hence, we concluded that anthropogenic sources of bacteria were major contributors to airborne bacteria in subway environments.

There was a significant correlation between CAB concentrations and station temperature, construction year, and depth. Temperature is a typical environmental factor influencing pathogenic agents (Heseltine and Rosen 2009), and our results were consistent with a previous study showing that higher CAB concentrations were significantly correlated with higher temperatures (Hwang et al. 2011). Moreover, we found a significant negative correlation between CAB concentrations and station construction year, which could be explained by the fact that older stations, built in the 1980s, were shallower than stations built in the 1990s. Similarly, the depth of the stations was significantly negatively correlated with CAB concentration, which may be related to the improvement of aged ventilation facilities since 2007 (Seoul Metro 2012).

The concentrations of CABs in stations with operating ventilation systems were significantly lower than those in stations without ventilation. This result is consistent with a previous study of university laboratories, which showed that the concentrations of microbes in laboratories with ventilation systems were significantly higher than those in laboratories without ventilation systems (Hwang et al. 2011). Ventilation is intended to remove or dilute the concentration of airborne microbes that can cause infectious diseases and to control the temperature and humidity. However, some poor ventilation systems have been associated with the prevalence of sick building syndrome (Seppanen and Fisk 2002). Better hygiene and the commissioning, operation, and maintenance of air-handling systems are particularly important in reducing the negative effects of ventilation systems (Mendell et al. 2008).

Other studies have found that the number of passengers 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 (Bogomolova and Kirtsideli 2009; Boudia et al. 2006; Cho et al. 2006). High concentrations of CABs in the morning and evening may be due to rush-hour commuter traffic in Korea. A similar study showed that the daytime levels of airborne bacteria at subway stations are higher than those at night (Dybwad et al. 2012). Moreover, changes in microbial communities between peak and nonpeak commuting hours can be largely attributed to increases in skin-associated genera (Leung et al. 2014) such as *S. epidermidis*, *S. hominis*, *S. cohnii*, *Staphylococcus caprae*, and *Staphylococcus haemolyticus*, which are present on human skin (Robertson et al. 2013). Our study suggested that supply of clean air via operational ventilation systems might reduce the concentration of CABs associated with high passenger load; indeed, the concentrations of CABs were lower in stations with ventilation, which had a higher average number of passengers than stations without ventilation. Another interesting finding of this study was that *Acinetobacter* spp. was only found in stations without ventilation. *Acinetobacter* is an important causative pathogen in nosocomial infections in healthcare settings (Horii et al. 2011). Hence, the presence of an operating ventilation system may help to reduce the presence of this GN bacterial species in enclosed subway stations.

One limitation of this study was that we were unable to measure the effectiveness of ventilation systems at various stations. Additionally, we sampled a relatively small number of sites, which may not be representative of all underground subway stations. Nevertheless, study showed that the overall concentrations of CABs were associated with environmental factors, such as station indoor temperature, relative humidity, depth, and construction year. We also found that an operating air supply system was critical for reducing the concentrations of CABs in the enclosed indoor environments of subway stations.

Conclusion

We measured the concentrations of CABs in 16 subway stations of the Seoul Metro system. The results showed that the concentrations of CABs in stations with ventilation systems were significantly lower than those in stations without ventilation systems. Thus, we suggest that all underground subway stations should have an adequate ventilation system. In particular, stations with a high level of passenger flow (over 2000 passengers per hour), such as stations b and p, could greatly benefit from a proper ventilation system, especially during commuting times (8–10 a.m. and 5–7 p.m.) and during the summer, when the ventilation system should be open and running at maximum power. It is important to check the supply of clean air regularly with a reliable device in order to monitor the concentrations of CABs and improve the air quality in underground subway stations.

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