**Field Study**

**Distribution of Airborne Bacteria in Railway Stations in Tokyo, Japan**

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**Abstract:** Distribution of Airborne Bacteria in Railway Stations in Tokyo, Japan: Tamami Kawasaki, et al. Biotechnology Laboratory, Railway Technical Research Institute

**Objectives:** We performed the current study to (1) understand the distribution of culturable airborne bacteria over a one-year monitoring period, (2) confirm places in stations where airborne bacteria are highly detected, (3) understand the factors that affect concentrations of airborne bacteria and (4) compare the distributions of airborne bacteria and fungi in railway stations in Japan. **Methods:** Measurements of airborne bacteria were taken at stations A and B located in Tokyo. Station A had under- and above-ground concourses and platforms, whereas station B had spaces only above-ground. Airborne bacteria at each measurement position were collected with an air sampler on plate count agar media. After cultivation of the sampled media, the number of bacteria colonies was counted on each media. **Results:** (1) Airborne bacteria were highly detected in the above-ground concourse in station A. Almost all the indoor-to-outdoor (I/O) ratios of concentrations of airborne bacteria in the above-ground concourse in station A were higher than one throughout the year and were especially high in summer. (2) The factor that affects the concentrations of airborne bacteria seems to be the number of railway customers, not humidity. (3) The characteristics of the distributions of airborne bacteria and fungi were different, even though they were sampled in the same stations on the same days. **Conclusions:** In the case of controlling indoor air quality of stations in the future, the locations in railway stations that would require control of indoor air quality differ between airborne bacteria or fungi, respectively. (J Occup Health 2013; 55: 495–502)

**Key words:** Above-ground concourse, Airborne bacteria, I/O ratio, Railway station

In Japan, railways are the most widely utilized public transportation system. In 2010, more than 62 million people used the railway system each day. Railway customers in Japan are also keenly interested in the hygiene environment of railway facilities. It is therefore very important to understand the microbial environment of railway stations, including both under- and above-ground stations, from the viewpoint of public health.

Kawasaki et al. reported the distribution and identification of airborne fungi in railway stations in Tokyo, Japan. The underground platform was characterized as having a high concentration of airborne fungi. The types of airborne fungi were also different between under- and above-ground stations. However, reports that have compared the distributions of airborne fungi and bacteria in the same stations are very limited, especially in Japan.

Generally, airborne fungi originate from outdoor environments, and airborne bacteria originate from people present in indoor environments. If this is true, the patterns of distribution of airborne bacteria and fungi must be different.

In this study, we measured airborne bacteria on the same days in the same measurement positions as measurements of airborne fungi were performed. The objectives of the current study were to (1) understand the distribution of culturable airborne bacteria on plate count agar (PCA) media over a one-year monitoring period, (2) confirm places in stations where airborne bacteria are highly detected, (3) understand the factors that affect concentrations of airborne bacteria and (4) compare the distributions of airborne fungi and bacteria in railway stations in Japan. The current study could be useful in designing and maintaining railway stations with the emphasis on limiting public exposure to microorganisms.
Materials and Methods

Railway stations

Measurements of airborne bacteria were taken at stations A and B located in Tokyo. Station A had both underground and above-ground concourses and platforms. Station A was chosen as a model station, since data between under- and above-ground spaces within one station were to be compared. There were five measurement positions in station A: an underground platform, underground concourse, above-ground concourse, above-ground platform and a position immediately outside the station, which was used as a control (Fig. 1 (a) to (d)). Located about six km from station A, station B had only above-ground spaces and was selected as a model of an over-track station. There were three measurement positions in station B: an above-ground concourse, above-ground platform and outside the station as a control (similar to that of station A, Fig. 1 (e), (f)). Trains arriving at stations A and B run within a radius of approximately 80 km from station A.

Collection of airborne microorganisms

Airborne microorganisms were collected with an air sampler (M Air T, Millipore, Billerica, MA, USA; Fig. 2 (a)). The air sampler was installed at a position about 150 cm above the floor, and was set to take in 200 l (0.2 m³) of air for one and half minutes (Fig. 2 (b)). Airborne microorganisms within 0.2 m³ of air were captured on agar plates that were set in the air sampler. Plate count agar (PCA, Eiken Chemical Co., Ltd., Tokyo, Japan) media was used to collect the airborne bacteria. Dichloran glycerol agar (DG18, Merck KGaA, Darmstadt, Germany) media was used to collect the airborne fungi. Sampling was performed twice at each measurement position. The sample plates were cultivated at 30°C for two days for bacteria and three days for fungi. Then the number of bacteria colonies was counted on each agar plate. All microorganism concentrations were expressed as colony-forming unit, “CFU” per cubic meter of air.

Fig. 1. Illustrations of each measurement position where the samplings were performed in station A and station B.
Temperature and relative humidity were also recorded at each measurement position by a digital temperature-humidity sensor (CTH-170, Custom Co., Tokyo, Japan). Airborne microorganisms in 0.2 m³ of air were also collected immediately outside stations A and B on the same day, serving as controls. The study was conducted from June 2003 to May 2004 over 12 months and collections were performed twice a month.

Calculation of indoor-to-outdoor (I/O) ratio of the concentrations of airborne microorganisms

The I/O ratios of the concentrations of airborne microorganisms for each measurement position at both stations were calculated by the following formula using the concentrations of airborne microorganisms outdoors as the control.

*I/O ratios = concentrations of airborne microorganisms in stations/concentrations of airborne microorganisms of controls.

Identification of airborne bacteria

Representative colonies obtained in the sample plate were tested for Gram stain reaction and examined microscopically; they were then determined to be either Gram positive or negative and cocci or rods. Catalase and oxidase reactions characterized colonies to the presumptive genus level, and the colonies were identified using API Staph, API 20NE or API 20E (bioMérieux, Marcy-l’Etoile, France).

Analysis

Statistical analyses were performed separately for station A and B data. The data were statistically analyzed with nonparametric procedures, the Kruskal-Wallis H test and post hoc with the Mann-Whitney U test by SPSS version 16.0J (SPSS Inc., Chicago, IL, USA). The significance level was \( p < 0.05 \), with the exception of post hoc Bonferroni correction (\( a/n; \alpha = 0.05 \)).

Results

Concentrations of airborne bacteria in the stations

Table 1 shows the concentrations of cultivable airborne bacteria (CFU/m³) at each measurement position in stations A and B throughout the year. There were significant differences among the concentrations of airborne bacteria at the five measurement positions of station A (Kruskal-Wallis H test, \( p < 0.01 \)). The concentration of airborne bacteria in the above-ground concourse of station A was significantly higher than those in the underground platform, underground concourse, above-ground platform and control (Mann-Whitney U test with Bonferroni correction, \( p < 0.005 \)). The concentration of airborne bacteria in the underground concourse was significantly higher than those in the underground platform and the control in station A (Mann-Whitney U test with Bonferroni correction, \( p < 0.001 \), \( p < 0.005 \), Table 1).

At station B, there was no significant difference in concentration of airborne bacteria among the measurement positions (Table 1).

Indoor-to-outdoor (I/O) ratios for concentrations of airborne microorganisms in both stations

Figure 3 (a) shows the I/O ratios of airborne bacteria in station A. All the I/O ratios of airborne bacteria in the above-ground concourse of station A were higher than one with the exception of September, 2003 (Fig. 3 (a)). The I/O ratios of airborne bacteria were especially high about 6–8 in June-August 2003 and April 2004. The I/O ratios of airborne bacteria in the underground platform in station A were 0.41–1.8 throughout the year, and higher than one in June-August, 2003 and March and April 2004. The I/O ratios of airborne bacteria in the underground concourse in station A were 0.72–3.3 throughout year, and higher than one in June-August and October-December, 2003 and March-May 2004. The I/O ratios of airborne bacteria in the underground concourse in station A were 0.72–3.3 throughout year, and higher than one in June-August and October-December, 2003 and March-May 2004.

Figure 3 (b) shows the I/O ratios of airborne bacteria in station B. The I/O ratios of airborne bacteria in the above-ground concourse in station B were higher than one throughout the year except for December, 2003 (Fig. 3 (b)), showing highest, 4.7 in August in 2003. The I/O ratios of airborne bacteria in the above-ground platform in station B were 0.6–1.6 throughout the year.
Table 1. Concentrations of airborne bacteria and environmental conditions in the two stations

<table>
<thead>
<tr>
<th>Measurement position</th>
<th>Concentration of bacteria (CFU/m³)</th>
<th>Humidity %</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underground platform</td>
<td>195 (38−403)</td>
<td>44−90</td>
<td>16−25</td>
</tr>
<tr>
<td>Underground concourse</td>
<td>370 (123−658)</td>
<td>32−81</td>
<td>16−27</td>
</tr>
<tr>
<td>Above-ground concourse</td>
<td>665 (120−1,300)</td>
<td>29−81</td>
<td>15−29</td>
</tr>
<tr>
<td>Above-ground platform</td>
<td>245 (115−585)</td>
<td>28−70</td>
<td>12−33</td>
</tr>
<tr>
<td>Control</td>
<td>208 (63−735)</td>
<td>28−67</td>
<td>10−35</td>
</tr>
<tr>
<td>Station B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above-ground concourse</td>
<td>80 (20−795)</td>
<td>26−68</td>
<td>13−33</td>
</tr>
<tr>
<td>Above-ground platform</td>
<td>98 (0−580)</td>
<td>26−66</td>
<td>10−34</td>
</tr>
<tr>
<td>Control</td>
<td>120 (10−670)</td>
<td>26−65</td>
<td>11−36</td>
</tr>
</tbody>
</table>

*Concentrations of airborne bacteria are shown as medians (min−max).  
*Significantly higher than those of the underground platform and control in station A, Mann-Whitney U test with Bonferroni correction (p<0.001).
*Significantly higher than those of the underground platform, underground concourse, above-ground platform, and control in station A, Mann-Whitney U test with Bonferroni correction (p<0.005).
*No significant differences in concentrations of airborne bacteria among above-ground concourse, above-ground platform and control in station B.

Fig. 3. Seasonal change in the I/O ratio of airborne microorganisms in stations. The I/O ratio of (a) airborne bacteria in station A, (b) airborne bacteria in station B, (c) airborne fungi in station A and (d) airborne fungi in station B.
Figure 3 (c) shows the I/O ratios of airborne fungi in station A, and Fig. 3 (d) shows the I/O ratios of airborne fungi in stations B. The I/O ratios of airborne fungi in the underground platform in station A throughout the year were high and were the highest 17 in March in 2004. The next highest I/O ratios of airborne fungi in station A in order were in the underground concourse, above-ground concourse and above-ground platform. The average I/O ratios of airborne fungi in the underground platform were 7.4 in winter and 8.3 in spring. The I/O ratios of airborne fungi in the above-ground concourse and above-ground platform in station B throughout the year were both generally one but were 2.8 and 2.6 in June 2003, respectively.

Identification of airborne bacteria

Table 2 shows the results of identification of representative colonies of airborne bacteria collected in both stations. In station A, *Staphylococcus* spp. were dominantly detected in the underground platform, underground concourse, and above-ground concourse. *Pseudomonas* spp. were dominantly detected in the underground concourse. *Brevundimonas* spp. were dominantly detected in the above-ground concourse. *Micrococcus* spp. and *Burkholderia* spp. were dominantly detected in the above-ground platform. *Aerococcus* spp. and *Bacillus* spp. were dominantly detected in the control. In station B, *Staphylococcus* spp. were dominantly detected in all measurement positions.

<table>
<thead>
<tr>
<th>Measurement position</th>
<th>Number of colonies per plate (n=2)</th>
<th>Most detected genus of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Station A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underground platform</td>
<td>4</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td><em>Pseudomonas</em> spp.</td>
</tr>
<tr>
<td>Underground concourse</td>
<td>19</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td>Above-ground concourse</td>
<td>46</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td><em>Brevundimonas</em> spp.</td>
</tr>
<tr>
<td>Above-ground concourse</td>
<td>17</td>
<td><em>Micrococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td><em>Burkholderia</em> spp.</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td><em>Aerococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td><em>Bacillus</em> spp.</td>
</tr>
<tr>
<td><strong>Station B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above-ground concourse</td>
<td>22</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td>Above-ground platform</td>
<td>25</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
</tbody>
</table>

Relationship between concentration of airborne bacteria and humidity

Figure 4 shows the relationship between relative humidity (%) and the concentrations of airborne bacteria during each season at the two stations. For station A, the relationships during summer (June-September) and winter (December-February) are shown in Fig. 4 (a), and the relationships during autumn (October-November) and spring (March-May) are shown in Fig. 4 (b). At station A, there were negative correlations between the concentration of airborne bacteria and the humidity in summer ($r=-0.39$), autumn ($r=-0.36$), spring ($r=-0.27$) and winter ($r=-0.39$), but there was no correlation throughout the year ($r=0.10$). Figure 4 (c) shows the relationships between relative humidity and the concentration of airborne bacteria during summer and winter for station B, and Fig. 4 (d) shows the relationships during autumn and spring. At station B, there were negative correlations between the concentration of airborne bacteria and the humidity in winter ($r=-0.61$) and summer ($r=-0.22$), however, there was no correlation throughout the year ($r=0.00$).

Discussion

High bacteria concentrations in the above-ground concourses

Currently, there is no standard concerning airborne bacteria in indoor environments released by the Japanese government. The Architectural Institute of Japan (AIJ)\(^9\) has a recommended standard of airborne bacteria in typical offices as 500 CFU/m\(^3\). In this study, the range of concentrations in the above-ground
concourse where bacteria were most highly detected in station A was 120–1,300 CFU/m$^3$. Ikeda et al.\textsuperscript{10} reported that the range of concentrations of airborne bacteria in typical Japanese offices was approximately 100–1,300 CFU/m$^3$. Therefore, the range of concentrations of airborne bacteria collected in both railway stations was almost the same as that in conventional offices in Japan. Usually, railway customers do not stay inside railway stations for a long time. Railway workers work in stations according to a daily shift schedule, which means they do not stay in the same position all day long and do not stay in one position throughout the week. Thus, the amounts of airborne bacteria exposed to them seem not to be high.

On the other hand, in Korea, the Ministry of Environment released an indoor bio-aerosol guideline of 800 CFU/m$^3$ as the total concentration for airborne bacteria\textsuperscript{11}. Hwang et al.\textsuperscript{12} reported that the concentration of airborne bacteria was 4,997 CFU/m$^3$ in a subway station in Korea, suggesting that much ventilation and trapping of bacteria by filters with HVAC (Heating, Ventilation, and Air Conditioning) are one of efficient majors\textsuperscript{12}.

The reason for high I/O ratios of airborne bacteria in the above-ground concourse in station A

The I/O ratio is used for considering possible sources of airborne microorganisms in indoor environments\textsuperscript{13}. When the I/O ratio is higher than one, there are some sources of airborne microorganism in the indoor environment\textsuperscript{8}.

The concentrations of airborne bacteria in indoor environments have been reported to be affected by activities caused by people in the indoor environments\textsuperscript{9}. The concentrations of airborne bacteria in indoor environments, however, would be expected to be lower with high ventilation rates\textsuperscript{14}.

From the point of view of ventilation, the ventila-

Fig. 4. Seasonal relationship between relative humidity and concentrations of airborne bacteria. The relative humidity (%) and the concentration of airborne bacteria (CFU/m$^3$) were plotted on the horizontal axis and vertical axis, respectively. The correlations between relative humidity and bacteria concentration at station A during (a) summer and winter and (b) autumn and spring and at station B during (c) summer and winter and (d) autumn and spring. Data for summer, winter, autumn and spring are colored in blue, red, brown, and green, respectively.
tion ratio in the above- and underground concourses in station A seemed not to be bad. Because the above-ground concourse is located next to the biggest ticket gate in station A, and both the ends of the concourse in front of the ticket gate are open to the outdoor air with no-roof. The underground concourse in station A does not directly connect to the outdoor air, however, the underground concourse is a big space and connects with a few concourses. Thus, the ventilation rates in the above- and underground concourses seemed to be good. Before starting this study, we expected that the concentration of airborne bacteria in the above-ground concourse would be low because of its good ventilation rate. But, contrary to our expectations, almost all the I/O ratios in the above-ground concourse were higher than one, except for September 2003 in station A. The I/O ratios in the underground concourse were higher than one, except for September 2003 and January and February 2004 (Fig. 3 (a)). Since the I/O ratios in the above- and underground concourses were higher than one, this result suggests that a source of airborne bacteria must exist in above- and underground concourses. When railway customers go into or out from railway stations and take trains, most of them walk in concourses in stations. But customers do not go to all platforms in a station, and instead only go to the platform where the train they intend to board stops. The total number of railway customers walking in concourses seems to be higher than the numbers walking on each platform in a station. Increased numbers of railway customers coming from many places might make the I/O ratios high in this season.

Therefore, it is reasonable to conclude that the activity of a large number of railway customers caused the I/O ratios to be high in the above- and underground concourses.

The results of this study showed that in the case of controlling of indoor air quality of railway stations, the positions to target in stations are different for airborne bacteria or fungi, respectively. When a new railway station is designed, understanding the places where the concentrations of airborne bacteria are high

Different characteristics of distribution of airborne bacteria and fungi in railway stations

The I/O ratios of airborne bacteria in station A were high in the above- and underground concourses, suggesting that a possible source of airborne bacteria exists in the above- and underground concourses. The large number of railway customers is a possible source. On the other hand, the I/O ratios of airborne fungi in station A were high in the underground platform, suggesting that there is a possible source of airborne fungi in underground spaces. Since each possible source of airborne bacteria and fungi is different, the characteristics of the distributions of airborne bacteria and fungi were different, even though airborne bacteria and fungi were measured in the same measurement positions on the same days.

Characteristics of the genera of airborne bacteria in railway stations

The dominant bacterial genera sampled in station A and station B were Staphylococcus spp., which is consistent with previous studies in the subway environment. Staphylococcus spp. were also detected from air in restaurants, public buildings, gymnasiums, and a high school boy’s locker room. Humans, especially men, have been reported to release much Staphylococcus spp. present on the surfaces of their skin. In the above-ground concourse in station A, Staphylococcus spp. and Brevundimonas spp. were detected. Brevundimonas spp. have been isolated from various environmental sources, such as air, a river, toilets, and a paper mill, with very rare infection to humans. Pseudomonas spp. detected in the underground platform in station A have been isolated in air and restaurants. In the above-ground platform in station A, Micrococcus spp. and Burkholderia spp. were detected. Micrococcus spp. have been reported to originate from humans. Burkholderia spp. have been also isolated from various environmental sources, such as air, soil, and underground water. Although most of the species in the genus Burkholderia are not pathologic, a few species are reported to be capable of causing infections. To better understand the significance of airborne bacteria, whether they are pathogenic to humans or not, more data for identification to the species level is needed in the future.

Implications

The results of this study showed that in the case of controlling of indoor air quality of railway stations, the positions to target in stations are different for airborne bacteria or fungi, respectively. When a new railway station is designed, understanding the places where the concentrations of airborne bacteria are high
according to the state of congestion might be helpful for limiting exposure of people to airborne bacteria.

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References