Quantitative and Qualitative Research of Surface Bacteria on the Floor of Station Restrooms with Two Types of Cleaning Method

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There are two types of method for cleaning station restrooms. One method uses water for cleaning floors, whereas the second does not. This study describes quantitative and qualitative research into the surface bacteria found on station restroom floors where these two cleaning methods were used. Samples for analysis were collected from five positions on the floor of each restroom in each railway station investigated. Samples were treated using a conventional culture method to measure the concentrations of surface bacteria on each restroom floor. Samples were then analyzed with bacterial 16S rRNA genes to analyze the microbiomes on these restroom floors. Results showed that the restrooms cleaned without water had lower concentrations of bacteria, than the microbiomes from restroom floors cleaned with water.

Key words: railway station restroom, cleaning, floor, bacteria, microbiome analysis, 16S rRNA gene

1. Introduction

Railways are a public mode of mass transportation, and stations are a core facility in the railway system. As such, station restrooms are a key passenger facility. Station restrooms receive a high volume of users, therefore, railway companies and cleaning companies invest considerable time and effort every day to maintain and manage them. Nevertheless, surveys conducted by the authors in the past revealed that users are highly sensitive to station restroom odors [1, 2]. Ammonia, which is one of the main components of the unpleasant odor in restrooms, is thought to be generated by the decomposition of urea contained in urine stains by the ammonia-producing bacteria existing on the floor surface [3]. Therefore, the removal of ammonia-producing bacteria, can be considered to be one of countermeasures for reducing amount of ammonia in station restrooms.

Station restrooms are generally cleaned using one of two possible methods: “wet cleaning” or “dry cleaning”, depending on the floor structure. Wet cleaning, which is the most common method, is used in the case of porcelain tile and joint floors. Wet cleaning involves first spreading water on the floor before scrubbing off the dirt with a brush or other similar cleaning tool.

Dry cleaning is used in the case of floor structures consisting of sheets and tiles, such as vinyl chloride. This type of flooring has become increasingly popular in recent years, to allow dry cleaning. In the dry cleaning method, dust and dirt are removed with a machine which scrapes or wipes dirt off the floor with a dry mop or equivalent piece of equipment.

While the number of floor structures suited to dry cleaning is increasing, floor structures requiring wet cleaning are more common in station restrooms in Japan. Wet cleaning is more time and labor intensive than dry cleaning because water on the floor must be wiped and dried after cleaning. In addition, user feedback on both types of cleaning method in station restrooms show that odor tolerance rates are lower in the case of station restrooms where wet cleaning is performed [2].

This suggests that the cleaning method may affect customer experience when using restrooms. However, few studies have examined the difference in the characteristics of bacteria on station restroom floors. Therefore, we carried this study in which we collected bacteria from facilities and the air inside/outside of station restrooms and performed microbiome analysis using 16S rRNA gene [4-8]. This study reports on the quantity and characteristics of bacteria found on railway restroom floors where these two types of cleaning method are used.

2. Materials and methods

This study describes the quantitative and qualitative investigations of bacteria taken from the floors of station restrooms [8] to confirm the difference in quantity and characteristics of bacteria in restrooms where dry and wet cleaning methods are used.

2.1 Subjects of this study

For the purpose of this study one male restroom was selected in four separate stations. In order to ensure comparability, restrooms were selected in stations which had a similar footfalls and been built or renovated all at around the same time. The footfall in each restroom and the main specifications of each are shown in Table 1. In Stations A and B, the selected restrooms had rubber tiled floors and were dry cleaned. In stations C and D, the restrooms had porcelain tiled floors with mortar joints and were wet cleaned.

2.2 Investigation period

Quantitative investigations were conducted in Stations A and C in October 2018, January and July 2019, and in Stations B and D in July 2019. At Stations A and C, a qualitative investigation was conducted during the daytime (during business hours) on January 8, 2019 (Table 2). The temperature and humidity recorded at the time of the investigation are listed in the same table.

2.3 Sampling positions

Each of the 5 sampling positions were investigated qualitatively and quantitatively: under washbasins, under urinals, between urinals, and the central and back corners of the floor, in each station.

2.4 Bacterial quantity investigation method

Bacteria was collected from floors using one of two possible methods. The first method used a 10-cm square frame, which was
laid on the floor and the surface within the square was then wiped with a cotton swab (Eiken Chemical) (Fig. 1). Cotton swabs were then suspended in 10 ml of phosphate buffered saline, and a certain amount of suspension was applied on soybean casein digest medium containing lecithin and polysorbate 80 (SCDLP), which inactivates the effects of detergents, inter alia. The colonies appearing on the SCDLP plates after culturing at 32°C for 48 hours were then counted.

The second method collected bacteria adhering to the surface of the target floor using a circular stamp-type agar medium with an area of 25 cm² using the same soybean casein digest medium as described above (SCDLP, Eiken Chemical, Fig. 2, left). Two samples were collected from each sampling site, and after culturing at 32°C for 48 hours, the number of bacterial colonies (Fig. 2, right) that appeared on the agar medium was measured. Since the survey in July 2019 was conducted with the monitor survey [2] on the same day, the easier sampling method with stamp-type agar media was performed.

2.5 Qualitative method used for investigating bacteria

Microbiome analysis using the 16S rRNA gene, inherent to bacteria, was used to investigate the bacteria qualitatively.

2.5.1 Microbiome analysis

The microbiome analysis flow chart is shown in Fig. 3. Details are described below.
(1) Sampling method

A 10-cm square frame was placed near the position where the quantitative survey was conducted (Fig. 1, left), and the inside of the frame was wiped off with a dry cotton swab (4N6FLOQSwabs, Copan Flock Technologies). One sample was collected at each location, and the cotton swab after wiping was sealed in a 1.5 ml container by cutting off only the cotton part of the cotton swab, and used for the subsequent DNA extraction procedure.

(2) DNA extraction

The PowerLyzer PowerSoil DNA Isolation Kit (QIAGEN) was used for DNA extraction. Qubit 3.0 (Thermo Fisher Scientific) was used to measure the concentration of the extracted DNA.

(3) Library preparation

Using the extracted DNA as a template, PCR (polymerase chain reaction) was performed using primers targeting the 16S rRNA gene V3-V4 region of bacteria. The number of PCR amplifications was set to 35 cycles, the same as in the previous study [8, 9]. The obtained PCR products were indexed and purified after confirming the peak size by LabChip GX Touch HT (PerkinElmer), ready for the library preparation.

(4) Bacterial flora analysis

The gene sequence was determined by reading 300 bases using the next-generation sequencer MiSeq (Illumina). The obtained gene sequence information was analyzed by QIIME (ver1.9.0). A group having 97% or more target sequence similarity was defined as an OTU (operational taxonomic unit). OTUs were identified by matching the sequences with data in the SILVA 132 database. In the process of calculating the Shannon index, each OTU defined in this study was treated as individual type of bacteria.

3. Results

3.1 Result of the quantitative investigation

The results of quantification of adherent bacteria collected by wiping men’s restroom floors in October 2018 and January 2019 in Stations A and C, are shown in Fig. 4. Figure 4 does not show the amount of adherent bacteria under the wash basin at Stations A and C in October 2018, because the number of colonies appearing on the agar mediums was very large and it was difficult to measure them. Comparing the amounts of bacteria in October and January at all collection points, the amount of bacteria found in October was larger than in January. In addition, the largest amounts of bacteria tended to be detected under the wash basins in both stations. Comparing the average number of bacteria in October and January from all collection points, the amount of bacteria found in October was larger than in January. In addition, the largest amounts of bacteria tended to be detected under the wash basins in both stations. Comparing the average number of bacteria in October and January from four sampling points, excluding the point beneath the washbasins, in Station A (dry cleaning) and Station C (wet cleaning), showed that the number of colonies/25 cm² ranged between 663 and 3,831 for Station A where dry cleaning was used, and between 14,550 to 27,169 in Station C where wet cleaning was used. The number of bacteria was statistically significantly higher in Station C than in Station A (p <0.05, t-test).

The results of collecting floor bacteria using a stamp-type agar medium in stations A, B, C, and D in July 2019, are shown in Fig. 5. In all sampling positions, the attached bacteria detected in order of quantity in each station was: Station C> Station D> Station B> Station A. In addition, looking at each sampling position, a large amount of average adherent bacteria was detected under the urinal and in the back corners of the floor.

The results of collecting floor bacteria using a stamp-type agar medium in stations A, B, C, and D in July 2019, are shown in Fig. 5. In all sampling positions, the attached bacteria detected in order of quantity in each station was: Station C> Station D> Station B> Station A. In addition, looking at each sampling position, a large amount of average adherent bacteria was detected under the urinal and in the back corners of the floor.
3.2 Result of qualitative investigation

3.2.1 Bacterial genus

Bacteria are classified by the hierarchy of kingdom, phylum, net, order, family, genus, and species [9]. In this study, the number of bacterial genera identified by microbiome analysis are shown in Table 3. As a result, more genera were detected from the restroom in Station A than from the restroom in Station C, in all of the five sampling points except the central part of the floor.

The characteristics of the genus of bacteria detected from each collection point at Stations A and C, are shown in Table 4. The genus *Kocuria* and the genus *Deinococcus* were commonly detected in all the samples in the men’s restrooms of both Stations A and C. It has been reported that the genus *Kocuria* includes ammonia-producing bacteria in the environment such as water and soil and is also indigenous to human skin [10, 11]. The genus *Kocuria* has also been detected in the air inside the men’s restrooms and inside stations at both Stations A and C [6]. The genus *Deinococcus* has been reported to be detected in activated sludge [12].

On the other hand, the genus *Streptococcus*, containing ammonia-producing bacteria, was detected only from samples of the floor in the men’s restroom at Station A. The genus *Streptococcus* has been detected in household toilets [13] and has been reported to exist as a human intestinal bacterium [14]. The genus *Pseudomonas* and *Acinetobacter*, containing ammonia-producing bacteria, were detected only in samples from the floor in the men’s restroom in Station C. These genera are also reported to have been detected in households [15, 16] and in soil [17, 18].

3.2.2 Microbiome analysis

The Shannon index is an index that indicates the number of types of bacteria detected in a sample and the uniformity of the composition ratio of the types [19]. The results of the Shannon index value obtained by microbiome analysis in this study are shown in Fig. 6. The higher the Shannon index, the higher the number of bacteria types and uniformity of the composition of bacterial types. From Fig. 6, the Shannon index was higher on average for Station A, suggesting that the number of bacterial types and the type composition ratio on the floor of the men’s restroom were highly uniform, compared to Station C. The Shannon index was the highest for the floor (between urinals) in the restroom in Station A and was the lowest for the floor (under urinals) of the restroom in Station C. In other words, the number of bacterial types present on the floor (between urinals) in Station A was higher and the types composition ratio was more uniform. There are fewer bacterial types and the type composition ratio was lower, and uniformity was also low on the floor (under the urinals) of the restroom in Station C. This section considers the characteristics of dry and wet cleaning, based on the results of the above quantitative and qualitative investigation of collected bacteria.

4. Discussion

This section considers the characteristics of dry and wet cleaning, based on the results of the above quantitative and qualitative investigation of collected bacteria.

4.1 From the results of the quantitative investigation

The time and place where many bacteria are detected in this study are considered as follows from the bacterial growth conditions, such as amount of water, temperature, and nutrients (urine...
bacteria, is not fixed throughout the year. From this, it would be interesting to see whether the ratio of bacterial type composition remains stable while the amount of bacteria fluctuates, or whether the quantity of bacteria fluctuates, as the bacterial type composition ratio varies. Therefore, further investigations carried out in different seasons to confirm the presence or absence of microbiome fluctuations at the time of the survey will be needed to clarify this point.

5. Conclusions

This study investigated the bacteria found on the surface of men’s restroom floors in railway stations which employed either wet or dry cleaning methods, quantitatively by culture method and qualitatively by genetic analysis. The results showed that the floors in men’s restrooms where wet cleaning was used in Station C, produced a larger amount of bacteria than in Station A, and that the microbiome tended to differ from point to point on the floor.

Future work will seek to confirm the reduction of ammonia-producing bacteria after cleaning, in order to more effectively keep unpleasant odors in railway restrooms to a minimum, by characterizing the distribution of ammonia-producing bacteria on floors with wet and dry cleaning.

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