We carried out a basic study in order to evaluate the bacterial contamination in water dispensers. Water samples were obtained from water dispensers from October 2012 to November 2013, and standard plate counts (at 36°C, 24 h) of the samples, as well as heterotrophic plate counts (at 25°C, 7 d), were estimated with the standard methods for the examination of drinking water in Japan. Standard plate counts exceeding the water-quality standard (1.0 × 10^2 CFU/ml) were observed in 42 of the 140 samples (30.0%), with a maximum detected bacterial count of 2.1 × 10^5 CFU/ml. The rate of the standard plate counts exceeding the water quality standard tended to be higher when using a one-way type method or water dispensers with natural water. Ralstonia spp. was most commonly isolated, and Pseudomonas aeruginosa was isolated in a few cases. Some opportunistic pathogens were also isolated, suggesting that we should be more concerned about bacterial contamination in cold water supplied from water dispensers.

Key words : Water dispenser / Bacterial contamination / Cold water.
dispensers, it is necessary to address this issue by assessing the true status of bacterial contamination and to develop countermeasures taking into consideration the growing dissemination of water dispensers in Japan. This study was performed to clarify the status of bacterial contamination in cold water obtained from water dispensers, and against this background, to provide a basic review of possible measures to prevent adverse health effects resulting from the consumption of water from water dispensers.

Approximately 30 mL of cold water was collected from water dispensers widely installed in various buildings primarily in the Tokyo Metropolitan area and Kanagawa Prefecture in the Kanto Region during the period from October 2012 to November 2013. The samples were refrigerated and promptly analyzed.

Culture tests were carried out using standard plate counts and heterotrophic plate counts in compliance with waterworks laws. A standard plate count is defined as the number of bacteria derived from colonies after testing at 36 °C for 24 h using a standard plate count agar, and serves as a significant indicator of hygiene. Heterotrophic bacteria are bacterial groups, including standard plate counts, whose numbers reflect the abundance of bacteria in water samples. We also evaluated the number of colonies after 7 d at 25 °C by smearing 0.1 mL of the sample onto R2A agar (Becton, Dickinson and Company).

Testing of the isolates was conducted according to a genetic method based on the base sequence of the 16S rRNA (approx. 500 bp) in order to identify the contaminant species (Furuhata, 2007).

The status of bacterial detection in the cold water obtained from the water dispensers indicated that, as shown in TABLE 1, standard plate counts exceeding the water quality standard of $1.0 \times 10^2$ CFU/mL were detected in 42 of the 140 (30.0%) specimens. TABLE 1 shows the detected bacterial count distributions according to the location of the water dispensers. Healthcare-associated facilities, such as hospitals, clinics, dental offices and pharmacies, showed the highest contamination rates (37.5%), followed by facilities for reflexology (34.5%) and offices (29.2%). The highest detected standard plate count was $2.1 \times 10^3$ CFU/mL in cold water obtained from a water dispenser in a healthcare-associated facility. No specimens exceeding the count of $10^5$ CFU/mL were found in the facilities for reflexology or offices. Furthermore, with respect to heterotrophic bacteria, 72.9% of the total facilities had specimens exceeding the desired maximum control value of $2.0 \times 10^2$ CFU/mL.

The pattern of detection of bacteria according to the supply system of the water dispensers is shown in TABLE 2. While the rate of samples exceeding the standard value for the standard plate count was 24.2% for the conventional returnable system, the one-way system (next-generation type) exhibited a frequency as high as 46.9%, displaying a significant difference, with a

### TABLE 1. The detection of bacteria from the cold water samples obtained from water dispensers.

| Location                | No. of samples examined | Standard plate counts (CFU/mL) | Heterotrophic plate counts (CFU/mL) |
|-------------------------|-------------------------|--------------------------------|------------------------------------|
|                         |                         | $\times 10^2$ | $\times 10^3$ | $\times 10^4$ | Total (%) | $>2.0 \times 10^2$ (%) |
| Home                    | 33                      | 0*            | 3             | 2             | 1         | 6 (18.2) | 25* (75.8) |
| Reflexology facility    | 29                      | 5              | 5             | 0             | 0         | 10 (34.5) | 24 (82.8)  |
| Office                  | 24                      | 4              | 3             | 0             | 0         | 7 (29.2)  | 22 (91.7)  |
| Medical facility        | 24                      | 4              | 4             | 0             | 1         | 9 (37.5)  | 11 (45.8)  |
| Amusement complex       | 6                       | 0              | 0             | 1             | 0         | 1 (16.7)  | 5 (83.3)   |
| Others**                | 24                      | 4              | 4             | 1             | 0         | 9 (37.5)  | 15 (62.5)  |
| Total                   | 140                     | 17             | 19            | 4             | 2         | 42 (30.0) | 102 (72.9) |

*: No. of samples

| Supply type             | No. of samples examined | Standard plate counts (CFU/mL) | Heterotrophic plate counts (CFU/mL) |
|-------------------------|-------------------------|--------------------------------|------------------------------------|
|                         |                         | $>1.0 \times 10^2$ (%) | $>2.0 \times 10^2$ (%) |
| Returnable              | 99                      | 24*                           | 69*                               |
| One-way                 | 32                      | 15                            | 28                                |
| Bag-in-the-box          | 9                       | 3                             | 5                                 |
| Total                   | 140                     | 42 (30.0)                     | 102 (72.9)                        |

*: No. of samples
1% level of significance (t test of the percentages). Although the bag-in-the-box system was represented by only nine specimens, three of these specimens (33.3%) exceeded the standard value for the standard plate count.

The detection status according to the type of supply water indicated, as shown in TABLE 3, that 19.0% of conventional RO water samples exceeded the standard value for the standard plate count, while natural water, which has become very popular, had bacteria exceeding the standard limit in 40.0% of the samples, indicating a significant difference compared to the RO water, with a 1% level of significance (t test of the percentages). Although there were only six specimens of distilled water, five of these specimens (83.3%) exceeded the standard limit for the standard plate count.

The identification of the 43 strains derived from the standard plate counts isolated from the cold water obtained from the water dispensers is shown in TABLE 4. Thirteen strains (30.2%) belonged to Ralstonia spp., the most frequently isolated bacteria, which were cultured from five types of water dispensers, followed by six strains (14.0%) of Acidovorax spp., five strains (11.6%) of Acinetobacter spp., four strains (9.3%) of Herbaspirillum spp. and three strains (7.0%) of Sphingomonas spp. and Novosphingobium aromaticivorans. Although the rate of isolation was low, it should be noted that three strains of Pseudomonas aeruginosa were isolated (7.0% of samples). In addition, one strain each (2.3%) of Aquabacterium fontiphilum, Delftia acidovorans, Mitsuaria chitosanitabida, Pseudacidovorax intermedius, Caulobacter sp. and Chryseobacterium sp. were found. All of these species are widely distributed in the natural world, including aquatic environments, and are generally non-pathogenic. Furthermore, there was a tendency for the same species to be isolated from the water obtained from water dispensers provided by the same manufacturer. The analyses identified some heterotrophic bacteria, including Sphingomonas spp. with yellow-pigmented colonies, Methylobacterium spp. with pink-pigmented colonies and Pelomonas spp. with white-pigmented colonies.

The water obtained from water dispensers is treated as a food until the container is opened, making it subject to the Food Sanitation Act. On the other hand, water dispensers are defined as home appliances, meaning that the water that passes through them is not considered a food. Importantly, this water is not tap water either, which requires chlorination. This means that no legislation currently exists concerning the sanitation of water obtained from water dispensers in Japan.

With respect to the level of microbial contamination in water derived from water dispensers, Hosaka et al. (2001) reported a case involving a musty odor resulting from Actinomycetes contamination (Sadamasu et al., 2001).

### TABLE 3. The detection of bacteria based on the supply water.

| Supply type   | No. of samples examined | Standard plate counts (CFU/mL) >1.0×10<sup>2</sup> (%) | Heterotrophic plate counts (CFU/mL) >2.0×10<sup>2</sup> (%) |
|---------------|-------------------------|--------------------------------------------------------|----------------------------------------------------------|
| RO water      | 79                      | 15<sup>*</sup>                                          | (19.0)                                                   |
| Natural water | 55                      | 22                                                     | (40.0)                                                   |
| Distilled water | 6                      | 5                                                      | (83.3)                                                   |
| Total         | 140                     | 42                                                     | 102                                                      |

*: No. of samples.

| Species                          | No. of strains (%) | Subtotal (%) |
|----------------------------------|--------------------|--------------|
| Ralstonia pickettii              | 6                  | 14.0         |
| R. insidiosa                    | 1                  | (2.3)        |
| Ralstonia sp.                   | 6                  | (14.0)       |
| Acidovorax temperans            | 2                  | (4.7)        |
| A. delafieldii                  | 1                  | (2.3)        |
| Acidovorax sp.                  | 3                  | (7.0)        |
| Acinetobacter junii             | 1                  | (2.3)        |
| A. radioreisistens              | 1                  | (2.3)        |
| A. septicus                     | 1                  | (2.3)        |
| Acinetobacter sp.               | 2                  | (4.7)        |
| Herbaspirillum huttieense        | 2                  | (4.7)        |
| Herbaspirillum sp.              | 2                  | (4.7)        |
| Sphingomonas pauchimobilis      | 2                  | (4.7)        |
| Sphingomonas sp.                | 1                  | (2.3)        |
| Novosphingobium aromaticivorans | 3                  | (7.0)        |
| Pseudomonas aeruginosa          | 3                  | (7.0)        |
| Aquabacterium fontiphilum       | 1                  | (2.3)        |
| Delftia acidovorans             | 1                  | (2.3)        |
| Mitsuaria chitosanitabida       | 1                  | (2.3)        |
| Pseudacidovorax intermedius     | 1                  | (2.3)        |
| Caulobacter sp.                 | 1                  | (2.3)        |
| Chryseobacterium sp.            | 1                  | (2.3)        |
| Total                           | 43 (100.0)         | 43 (100.0)   |
Uehara et al. (2013) considered that the source of contamination was most likely the water, rather than the air. Fortunately, however, no episodes of health hazards related to consuming water from water dispensers have been reported in Japan to date.

This investigation of contamination found standard plate counts exceeding the water quality standard set in the waterworks laws in 30.0% of the cold water samples obtained from the water dispensers, with the highest counts reaching $2.1 \times 10^5$ CFU/mL (TABLE 1). Furthermore, heterotrophic bacteria, exceeding the provisionally designated count of 2.0 $\times 10^5$ CFU/mL, were found in as many as 72.9% of the specimens, with the highest bacterial count being $2.2 \times 10^5$ CFU/mL (TABLE 1). The regulation of water obtained from water dispensers, which is not applicable to waterworks laws under the legislation of water quality standards, remains a matter of debate. However, water quality standards were applied in this study because they have previously been demonstrated to correlate with effects on human health.

When the bacterial detection status was compared based on the type of water supply used in the water dispensers, the proportion of specimens exceeding the standards for the standard and heterotrophic bacteria plate counts in the one-way system was 46.9% and 87.5%, respectively, which was higher than the values of the returnable system, at 24.2% and 69.7% (TABLE 2). This observation indicates that, although the newer systems were developed after taking bacterial contamination into consideration, the outcomes were actually worse than those of the conventional system. Even though the one-way system was designed after taking into consideration the possibility of bacterial contamination from the air, high standard plate counts were detected in the residual water inside the container in some cases, indicating that this system does not prevent bacterial contamination in the cold water in the water dispensers. Furthermore, standard plate counts exceeding the quality standard values were detected in some of the high-performance dispensers equipped with an auto-clean function, in which warm water automatically circulates within the dispenser for a one-way system. However, no bacterial contamination was found in the cold water obtained from the water of one type of dispenser. Of note, natural water was used as the water supply for this dispenser. The dispenser used a two-bag system, with separate chilled and hot water systems, which allowed the cold water to be kept cold in the container (bag). As described above, temperature control is believed to be important for preventing microbial contamination in cold water, even before considering the introduction of a sterilizer. In other words, it is advisable to consider how to prevent bacterial growth in cold water obtained from water dispensers in which sterilization is not a prerequisite. Further improvements in dispensers are needed in the future from the standpoint of bacterial contamination in order to provide a safe source of water from water dispensers.

The analysis of the bacterial detection status according to the supply of water revealed that the rate of standard plate counts exceeding the quality standards was higher in natural water. There has been an increase in the use of “natural water” in recent years based on the pursuit of better taste; however, this type of water showed more than twice as much contamination as RO water, the conventional type of water used in water dispensers, thus indicating the need to raise awareness regarding the use of natural water.

Bacterial contamination in water obtained from water dispensers has been reported in other countries (Lévesque et al., 1994). For example, according to a report by Baumgartner and Grand (2006), $10^5$ CFU/mL of bacteria were detected. Furthermore, Zanetti et al. (2009) and Sacchetti et al. (2009) proposed using acetic acid or hydrogen peroxide to reduce contamination.

Some of the strains isolated from the cold water obtained from the water dispensers may be harmful to human health. For example, P. aeruginosa was isolated from more than two specimens. Because over-the-counter mineral water is subject to the Food Sanitation Act as a food, it is required that it be tested negative for P. aeruginosa under the ingredient standards included within soft drink standards in Japan. In contrast, because the supply water (unopened water) for water dispensers is also treated as a food, it is often believed that this water has passed the ingredient standards. However, even if a large number of P. aeruginosa are detected in the cold water passing through water dispensers, no regulatory standards for addressing this issue have been established. P. aeruginosa is widely distributed in the natural environment (Aoi et al., 2000), which increases the risk of it contaminating water dispensers. However, this bacterium is a pathogen associated with opportunistic infections (Edamoto et al., 2009), requiring special attention with respect to the potential for healthcare-associated infections (Spencer et al., 2000).

Furthermore, Ralstonia pickettii (Woo et al., 2002) and R. insidiosa (Van der beek et al., 2005), which exhibited the highest isolation rates observed in this study, also include species that may influence health. These bacteria are widely distributed in the natural world, including in water, soil and plants. These bacteria are also present in healthy human bodies and are
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primarily indigenous to the upper respiratory tract, such as the oral cavity, pharynx, trachea, etc. Therefore, while it is very rare for a healthy person to develop an infection caused by these bacteria, those with an immune deficiency or reduced resistance may develop respiratory infections or blood poisoning due to these organisms (Woo et al., 2002; Van der beek et al., 2005). It has also been reported that these bacteria function as pathogens of healthcare-associated infections (Ryan et al., 2006). Furthermore, due to their ability to pass through membrane filters, cases of infection spread by the consumption of “purified” water contaminated with these bacteria have been documented (Kendirli et al., 2004). In recent years, water dispensers have been rapidly disseminated, and it is therefore necessary to raise awareness regarding the potential for such water sources to affect both healthy individuals and groups at high risk for opportunistic infections, such as senior citizens or patients with chronic diseases.

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