

Microorganism levels in spray from warm-water bidet toilet seats: factors affecting total viable and heterotrophic plate counts, and examination of the fluctuations and origins of *Pseudomonas aeruginosa*

Toru Iyo, Keiko Asakura, Makiko Nakano and Kazuyuki Omae

ABSTRACT

The objectives of this study were to conduct an appropriate microbial evaluation of warm-water bidet toilet seats. Health-related advantages and disadvantages have been associated with using warm-water bidet toilet seats, which are classified according to the tank type, including tanks equipped with reservoir water heaters and on-demand tankless systems equipped with an instantaneous water heater. However, related bacterial research is sparse. Here, we performed a long-term survey of the behavior of microorganisms (i.e., the total viable count (TVC), heterotrophic plate counts (HPCs), and *Pseudomonas aeruginosa* count) in a university campus. We also examined the differences between the tank and on-demand types, and the origins of *P. aeruginosa*. A low TVC ($\leq 1/\text{mL}$) in the spray waters from both on-demand and tank-type warm-water bidet toilet seats showed low bacterial contamination, although there was an increase in HPC, i.e., growth of biofilms, inside in the warm-water bidet toilet seats. When *P. aeruginosa* was detected in spray water over an extended duration, the *P. aeruginosa* origin was considered as either from feces or tap water. Collectively our findings demonstrate that hygienic safety of warm-water bidet toilet seats is being maintained overall.

Key words | bidet toilet, heterotrophic plate count, multidrug-resistant *Pseudomonas aeruginosa* (MDRP), PCR-based ORF typing (POT) classification, *Pseudomonas aeruginosa*, residual chlorine

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INTRODUCTION

Warm-water bidet toilet seats are equipped with a device that sprays warm water (spray water) on the external genitalia and anus after urination or defecation (Japan Monthly Web Magazine 2011). A consumer behavior survey conducted by the Japanese Cabinet Office showed that 79.1% of Japanese households have at least one such toilet seat, with every 100 households owning 110.9 units on average (effectively one per household) (Cabinet

Office Government of Japan 2017). Broadly, two types of warm-water bidet toilet seats are available: the tank type and the on-demand type. With the tank type equipped with reservoir water heaters, the spray water is warmed to a suitable temperature in a tank with reservoir water heaters, whereas in the on-demand type tankless systems equipped with an instantaneous water heater, the water is warmed as needed inside a device with tankless, instantaneous water heaters (bidetsPLUS 2017) (Figure 1). Tank-type products are both cheaper and more common than on-demand types in Japan (Ministry of Economy Trade and Industry 2006).

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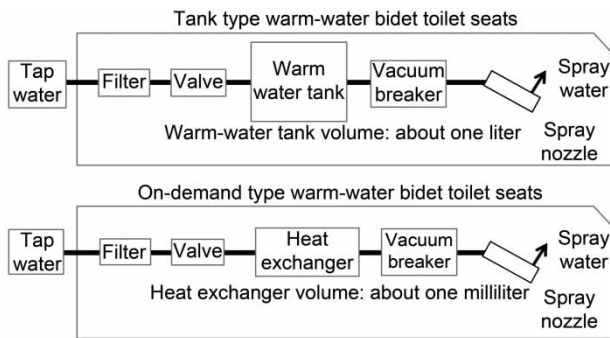


Figure 1 | Schematic diagram of two types of warm-water bidet toilet seats.

Despite the reported health-related advantages (benefit to bowel movement, no clinical health risk for preterm birth and bacterial vaginosis, low incidence rates for bacterial vaginitis) of using warm water in bidets (Uchikawa *et al.* 2007; Asakura *et al.* 2013; Kiuchi *et al.* 2017), there are also health-related disadvantages (aggravation of vaginal microflora, correlation for an itch on the anus) associated with the use of these toilet seats (Ogino *et al.* 2010; Tsunoda *et al.* 2016). Although Katano *et al.* (2014) showed bacterial contamination (i.e., *Pseudomonas aeruginosa* and *Escherichia coli* isolated) of the spray water from the tank-type warm-water bidet toilet seats, research on both the amounts and the types of microorganisms present in the spray water is sparse.

Previously, we conducted a cross-sectional survey on the hygienic conditions of 127 warm-water bidet toilet seats in restrooms on a university campus, revealing relationships between the total viable count (TVC), heterotrophic plate count (HPC), and residual chlorine; the proportion of *Pseudomonas aeruginosa* in the spray water; and the composition of the microbiome in the spray water. We showed that the TVC, fecal indicator bacteria, and *P. aeruginosa* were present in the spray water at very low frequencies and concentrations, indicating that the overall hygienic safety was maintained (Iyo *et al.* 2016).

In this study, we conducted a long-term survey of the hygienic conditions of warm-water bidet toilet seats in a research building in a university campus (12 men's restrooms and one women's restroom), including restrooms in which *P. aeruginosa* was previously detected in the spray water (one men's restroom and one women's restroom). We thus empirically examined TVCs and HPCs, as well as *P. aeruginosa* behavior in the spray water; factors affecting these parameters (residual chlorine and differences between the

tank and on-demand types); and the origins of *P. aeruginosa* from the viewpoint of preventing opportunistic infections due to *P. aeruginosa*. We also studied previously contaminated bidets for *P. aeruginosa* persistence, the age of the systems, variations over time, and the location of *P. aeruginosa* within the bidets. *P. aeruginosa* secretes a biopolymer that enables it to form biofilms and strong resistance to chlorine (Silva *et al.* 2008). The warm-water tanks in the bidets also promote biofilm formation and long-term survival of *P. aeruginosa* on the inner walls of these tanks.

Our aim was to conduct an appropriate microbial evaluation of the on-demand and tank-type warm-water bidet toilet seats in this study.

METHODS

Warm-water bidet toilet seats

In a previous survey of 127 tank-type, warm-water bidet toilet seats, *P. aeruginosa* was detected in the spray water of only two (one men's and one women's restroom) in only one research building on a university campus (Iyo *et al.* 2016). The cause of the *P. aeruginosa* water contamination was not determined. Hence, focusing on the research building containing two warm-water bidet toilet seats where *Pseudomonas aeruginosa* was detected in the spray water, we surveyed warm-water bidet toilet seats in 12 men's and one women's restrooms in the research building (*P. aeruginosa* positive) on the same university campus, in this long-term field study. However, considering the difficulty for male researchers to collect the spray water from bidet toilet seats in women's restrooms, only one female bidet toilet seat in which *P. aeruginosa* was specifically detected in spray water was surveyed. The toilet seats were replaced with either tank-type or on-demand type seats in accordance with the study plan. Breakdown (disassembly) analysis was performed to detect *P. aeruginosa*, *Escherichia coli*, and enterococci in the piping of the toilet seats.

Analytical methods

The levels of residual chlorine (DPD method), TVCs (CompactDry[®] TC; Nissui Pharmaceutical Co., Tokyo, Japan;

growth at 37 °C for 2 days), HPCs (R2A agar culture medium; Eiken Chemical Co., Tokyo, Japan; growth at 20 °C for 7 days), *P. aeruginosa* (USEPA-Approved Defined Substrate Technologies Pseudalert[®] and Quanti-Tray[®] systems, IDEXX Laboratories Inc., Westbrook, ME, USA) and *E. coli* (USEPA-Approved Defined Substrate Technologies Colilert[®] and Quanti-Tray[®] systems, IDEXX Laboratories Inc., Westbrook, ME, USA) were measured in spray water and tap water (control), as described previously (Iyo et al. 2016). Samples of microbes in the internal piping and spray nozzles were collected by scraping the inside with a cotton swab or other similar tools. The collected samples were then inoculated into a nutrient broth (Eiken Chemical Co., Tokyo, Japan) and incubated at 37 °C for 48 h. The incubated solution was then smeared on agar plates. The media used for smear culture were nalidixic acid cetrime (NAC) agar medium (Eiken Chemical Co., Tokyo, Japan), sodium dodecyl sulfate magenta-GAL-X-GLUC agar medium (ELMIEX Limited, Tokyo, Japan), and Chromocult[®] enterococci-agar (Merck KGaA, Darmstadt, Germany) for *P. aeruginosa*, *E. coli*, and enterococci, respectively. The culture temperature and duration used with these media were based on the manufacturers' instructions.

PCR-based open-reading frame (ORF) typing classification of *P. aeruginosa* strains

To investigate the origins of *P. aeruginosa*, the culture medium for *P. aeruginosa* was diluted appropriately and subjected to isolation culture to obtain a few single colonies on NAC agar plates. Subsequently, DNA was extracted from the isolated colonies with the CicaGeneus[™] DNA Extraction Reagent (Kanto Chemical Co., Tokyo, Japan), and PCR-based ORF typing (POT) values (the combination of POT1 value and POT2 value) were measured using the CicaGeneus[™] Pseudo POT (PCR-based ORF Typing) Kit (Kanto Chemical Co., Tokyo, Japan). Using the POT method with two sets of primer pairs, *P. aeruginosa* strains were classified by 10 ORFs from islets, 5 ORFs from genomic islands, and the metallo-beta-lactamases *bla*_{IMP} and *bla*_{VIM} (multidrug-resistant *Pseudomonas aeruginosa* gene) (Suzuki et al. 2015).

Survey methods

Figure 2 shows the timelines for the surveys of the warm-water bidet toilet seats, which were conducted between 2012 and 2016 (Surveys 1–6).

Survey 1: We followed up on the levels of *P. aeruginosa* in the spray water from a women's tank-type warm-water bidet toilet seat (TT-1f) and a men's tank-type warm-water bidet toilet seat (TT-1 m), wherein *P. aeruginosa* was detected in spray water from both in a previous survey. Samples of spray water were collected in the morning.

Survey 2: To eliminate the effects of ageing of the warm-water bidet toilet seats, two toilet seats (TT-1 m and TT-2 m) were replaced with new ones (TT-1 m-2 and TT-2 m-2). After approximately half a year of acclimation to stabilize the state of the biofilm in the toilet seats, spray water was surveyed in each toilet seat. Data from seats TT-1 m–TT-7 m were used as control for those from seats TT-1 m-2–TT-2 m-2. Spray water samples were collected in the morning.

Survey 3: We investigated daily (five times a day at 9:00, 12:00, 15:00, 18:00, and 21:00) and weekly variations (five weekdays from Monday to Friday) of residual chlorine, HPC, and *P. aeruginosa* levels without disinfecting the nozzle surface.

Survey 4: We examined the fluctuations in *P. aeruginosa* levels in spray water from seven men's tank-type warm-water bidet toilet seats (TT-1 m-2, TT-2 m-2, TT-3 m, TT-4 m, TT-5 m, TT-6 m, and TT-7 m) without disinfecting the nozzle surface.

Survey 5: In total, 12 men's tank-type warm-water bidet toilet seats (TT-1 m-2, TT-2 m-2, TT-3 m, TT-4 m, TT-5 m, TT-6 m, TT-7 m, TT-8 m, TT-9 m, TT-10 m, TT-11 m, and TT-12 m) were replaced with new, on-demand-type seats (OD-1 m, OD-2 m, OD-3 m, OD-4 m, OD-5 m, OD-6 m, OD-7 m, OD-8 m, OD-9 m, OD-10 m, OD-11 m, and OD-12 m). We surveyed the quality of spray water once a month between August and December. Spray water samples were collected in the morning. When comparing the spray water quality between the toilet seat types, we used data from November for both the tank-type and on-demand-type toilet seats because data for the tank type were collected in November.

Survey 6: After confirming the *P. aeruginosa* levels and POT values in the spray and tank water of a women's

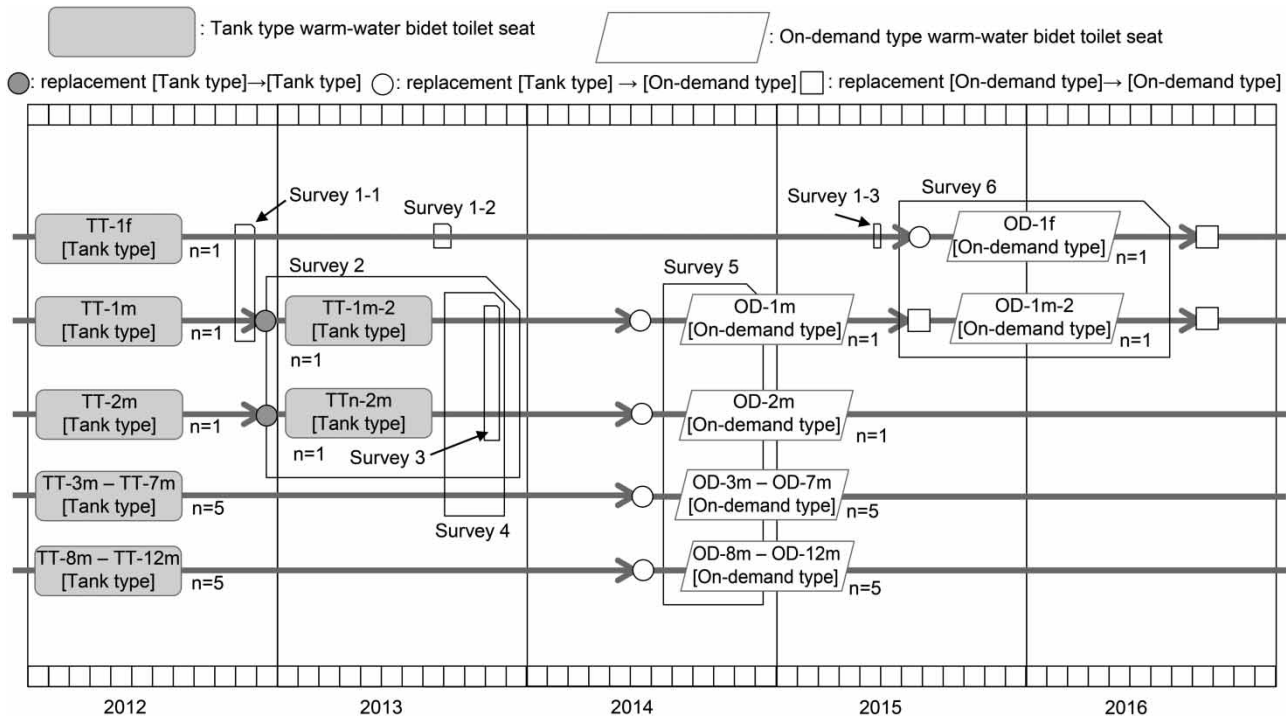


Figure 2 | Experimental timeline showing when samples of spray water were collected from a women's tank-type warm-water bidet toilet seat (TT-1f) and 12 men's tank-type warm-water bidet toilet seats (TT-1 m-2, TT-2 m-2, TT-3 m, TT-4 m, TT-5 m, TT-6 m, and TT-7 m). *P. aeruginosa* levels were determined from all the samples before (Survey 1), after seat replacement from tank type to tank type (Survey 2) or after seat replacement from tank type to on-demand type (Survey 5). In addition, residual chlorine and heterotrophic plate counts were measured during Survey 3 and *P. aeruginosa* levels and PCR-based ORF typing values were measured in the spray and tank water during Survey 6.

tank-type warm-water bidet toilet seat (TT-1f) and in the spray water from a men's on-demand-type warm-water bidet toilet seat (OD-1 m), we examined the internal *P. aeruginosa* levels and POT values after dismantling these toilet seats. Subsequently, we prepared water piping from which tap water-derived *P. aeruginosa* was eliminated through microfiltration and then installed two on-demand-type warm-water bidet toilet seats (OD-1f and OD-1 m-2), using the piping. Spray water samples were collected from these seats and compared with two types of tap water as controls (Figure 3). Spray water and tap water samples were tested approximately once every 2 weeks for *P. aeruginosa*, *E. coli*, and enterococci. We also dismantled seats OD-1f and OD-1 m-2 thereafter to examine the presence and POT values of *P. aeruginosa* inside.

Statistical processing

While calculating the geometric means (GMs) and geometric standard deviations (GSDs) of residual chlorine, TVC, HPC,

and *P. aeruginosa* levels, we also determined the detection limits. Statistical analysis of factors affecting the quality of spray water was conducted by performing analysis of variance (ANOVA). The quality of spray water from tank and on-demand types was compared by performing the Mann-Whitney U test and the non-parametric Kruskal-Wallis test. Statistical analysis was performed using the Statistical Package for the Social Sciences, version 24 (IBM, Armonk, NY, USA). The significance level was set at $P < 5\%$.

RESULTS

Fluctuations in *P. aeruginosa* levels

As indicated in Table 1, *P. aeruginosa* was detected in the same two tank-type warm-water bidet toilet seats (TT-1f and TT-1 m) that showed *P. aeruginosa* in a previous survey. In particular, *P. aeruginosa* was confirmed to have persisted for at least 2 years in the tank type warm-water

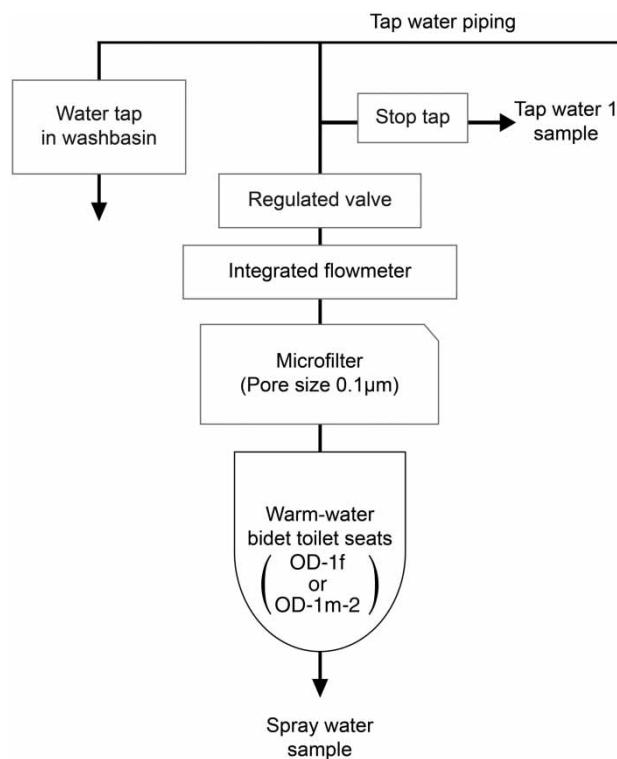


Figure 3 | Schematic representation of the experimental equipment used for investigating the origins of *P. aeruginosa*.

bidet toilet seats (TT-1f) installed in a women's restroom (Survey 1). In seven of the tank-type seats (TT-1 m-2, TT-2 m-2, TT-3 m, TT-4 m, TT-5 m, TT-6 m, and TT-7 m), *P. aeruginosa* was detected in the spray water from two toilet seats (TT-1 m-2 and TT-2 m-2), albeit at low concentrations (Survey 4). These findings indicated the possibility of long-term survival of *P. aeruginosa* in warm-water bidet toilet seats (TT-1f), as well as the presence of viable *P. aeruginosa* attributable to use of the toilet seats (TT-1 m and TT-1 m-2). During the long-term survey of six warm-water bidet toilet seats, two (TT-3 m and TT-4 m) occasionally showed *P. aeruginosa* in the spray water. One of the on-demand-type seats (OD-1 m) consistently showed low levels of *P. aeruginosa* in its spray water. As also indicated in Table 1, although one of the tank-type seats (TT-2 m-2) carried *P. aeruginosa* in its spray water before replacement, the bacterium disappeared after the seat was replaced with a new on-demand-type seat (OD-2 m). Thus, the only toilet seats that consistently showed *P. aeruginosa* were TT-1f and TT-1 m through OD-1 m (Survey 5).

Fluctuations in residual chlorine, TVC, and HPC levels

Following acclimation of two tank-type warm-water bidet toilet seats (TT-1 m-2 and TT-2 m-2), the residual chlorine, TVC, and HPC levels in the spray water were measured over approximately a half-year period. The mean values were 0.10–0.15 mg/L for residual chlorine, ≤ 1 CFU/mL for the TVC, and approximately 20,000 CFU/mL for the HPC, which were nearly identical with the mean values obtained with seats TT-1 m–TT-12 m (extracted from our previous data (Iyo et al. 2016); analyzed here as shown in Figure 4(a)). As shown in Figure 4(a), the TVC was nearly undetectable in the spray water from seats TT-1 m-2 and TT-2 m-2, and even when TVC was detected, the value did not exceed 10 CFU/mL. In addition, a weak negative correlation was observed between the residual chlorine and HPC levels (correlation coefficient [R]: 0.457, coefficient of determination [R²]: 0.209).

As shown in Figure 4(b), the TVCs were ≤ 1 /mL in the spray water from the on-demand-type warm-water bidet toilet seats. When the TVCs were detected in the spray water, they tended to decrease as the residual chlorine levels increased. Most toilet seats (9 out of 11; 82%) that showed a TVC exceeding 1 CFU/mL were the on-demand-type seats (OD-1 m and OD-2 m) that replaced the previously *P. aeruginosa*-positive tank-type seats (TT-1 m-2 and TT-2 m-2). A weak negative correlation was also found between the residual chlorine and HPC levels (R: 0.491, R²: 0.241).

Influence of the collection time on the residual chlorine, TVC, and HPC levels

Figure 5 shows the daily and weekly variations of the residual chlorine, *P. aeruginosa*, and HPC levels. The only source of chlorine was the tap water, and the concentration of chlorine decreased due to heating and remaining stagnant for a long period. The graph shows an inverted U-shaped pattern in which the residual chlorine levels tended to be relatively low in the morning and peak around noon before dropping towards the evening. The HPC and *P. aeruginosa* levels, however, tended to be higher in the morning before decreasing in the evening. This result may reflect nocturnal growth of bacteria within the water tank,

Table 1 | Behavior of *Pseudomonas aeruginosa* in spray water from warm-water bidet toilet seats

Survey no.	Spray water	Bidet toilet seat	Year	Sample size	<i>P. aeruginosa</i>			
					GM (MPN/mL)	(GSD)	Positive number	Detection rate (%)
Survey 1-1	TT-1f	Tank type	2012	7 ^a	0.2	(15.1)	5	71.4
Survey 1-2	TT-1f	Tank type	2013	4 ^b	0.5	(1.3)	4	100
Survey 1-3	TT-1f	Tank type	2015	1 ^c	0.02	(-)	1	100
Survey 1-1	TT-1 m	Tank type	2012	7 ^a	2.3	(12.8)	6	85.7
Survey 4	TT-1 m-2	Tank type	2013	19 ^c	0.04	(3.2)	18	94.7
Survey 4	TT-2 m-2	Tank type	2013	19 ^d	0.11	(3.6)	18	94.7
Survey 4	TT-3 m	Tank type	2013	19 ^d	0.02	(2.2)	5	26.3
Survey 4	TT-4 m	Tank type	2013	19 ^d	0.01	(-)	2	10.5
Survey 4	TT-5 m	Tank type	2013	19 ^d	n.d.	(-)	0	0
Survey 4	TT-6 m	Tank type	2013	19 ^d	n.d.	(-)	0	0
Survey 4	TT-7 m	Tank type	2013	19 ^d	n.d.	(-)	0	0
Survey 5	OD-1 m	On-demand type	2014	5 ^e	2.3	(2.2)	5	100
Survey 5	OD-2 m	On-demand type	2014	5 ^e	n.d.	(-)	0	0
Survey 5	OD-3 m–OD-7 m	On-demand type	2014	5 ^e	n.d.	(-)	0	0
Survey 5	OD-8 m–OD-12 m	On-demand type	2014	5 ^e	n.d.	(-)	0	0

GM, geometric mean; GSD, geometric standard deviation; n.d., not determined.

^aSampling date: October 30; November 6, 8, 9, 12, and 19; December 11.

^bSampling date: August 16 and 31; September 1 and 9.

^cSampling date: June 15.

^dSampling date: August 31; September 1 and 9; October 7 and 21; November 11–15, 18, 21, 25, and 28; December 2, 5, 9, 12, and 16.

^eSampling date: August 11, September 18, October 29, November 20, and December 17.

with subsequent washout and dilution of bacteria using spray water tank throughout the day. Alternatively, this result may reflect an inverse relationship between bacterial and chlorine levels. However, no weekly variations were observed. The difference in residual chlorine levels between TT-1m2 and TT-2 m-2 was likely due to the frequency of use.

Comparison between tank-type and on-demand-type toilet seats

After the tank-type toilet seats were replaced with on-demand-type seats, we compared the residual chlorine, TVC, and HPC levels in the spray water. Residual chlorine level increased more in the on-demand type than in the tank type, and HPCs decreased more in the on-demand type than in the tank type ($P < 0.01$; Figure 6). TVCs decreased more in the on-demand type than in the tank type, but the difference was not statistically significant ($P = 0.148$; Figure 6). The on-demand-type of bidet toilet

seat does not have a tank with reservoir water heaters, and the residual chlorine in tap water scarcely decreases because of the low degree of residual chlorine evaporation. Consequently, the residual chlorine level in tap water was highly correlated with that in spray water ($R: 0.963$, $R^2: 0.926$; Figure 7).

Origins of *P. aeruginosa*

We dismantled tank-type toilet seat TT-1f and on-demand-type toilet OD-1 m, both of which were consistently *P. aeruginosa*-positive, and analyzed the spray water (for both) and the tank water (for TT-1f). *P. aeruginosa* was detected near the filter in the piping and in the spray water in seat TT-1f, as well as in the piping within the nozzle and on the nozzle surface in seat OD-1 m (Figure 8). The *P. aeruginosa* POT values (i.e., POT1 and POT2) differed between TT-1f and OD-1 m.

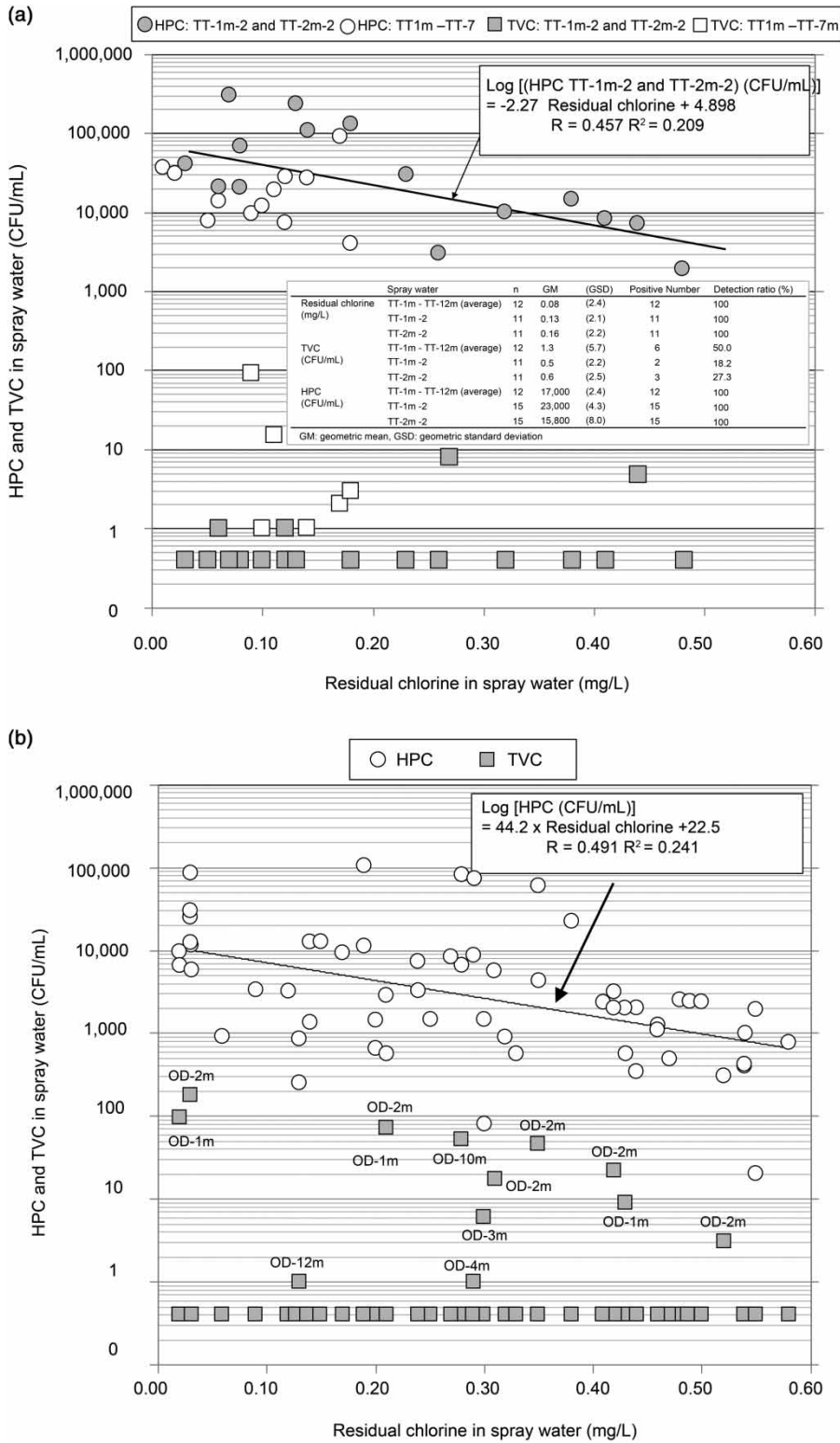


Figure 4 | Relationships between the residual chlorine, TVC, and HPC levels in (a) tank-type (Survey 2) and (b) on-demand-type (Survey 5) toilet seats.

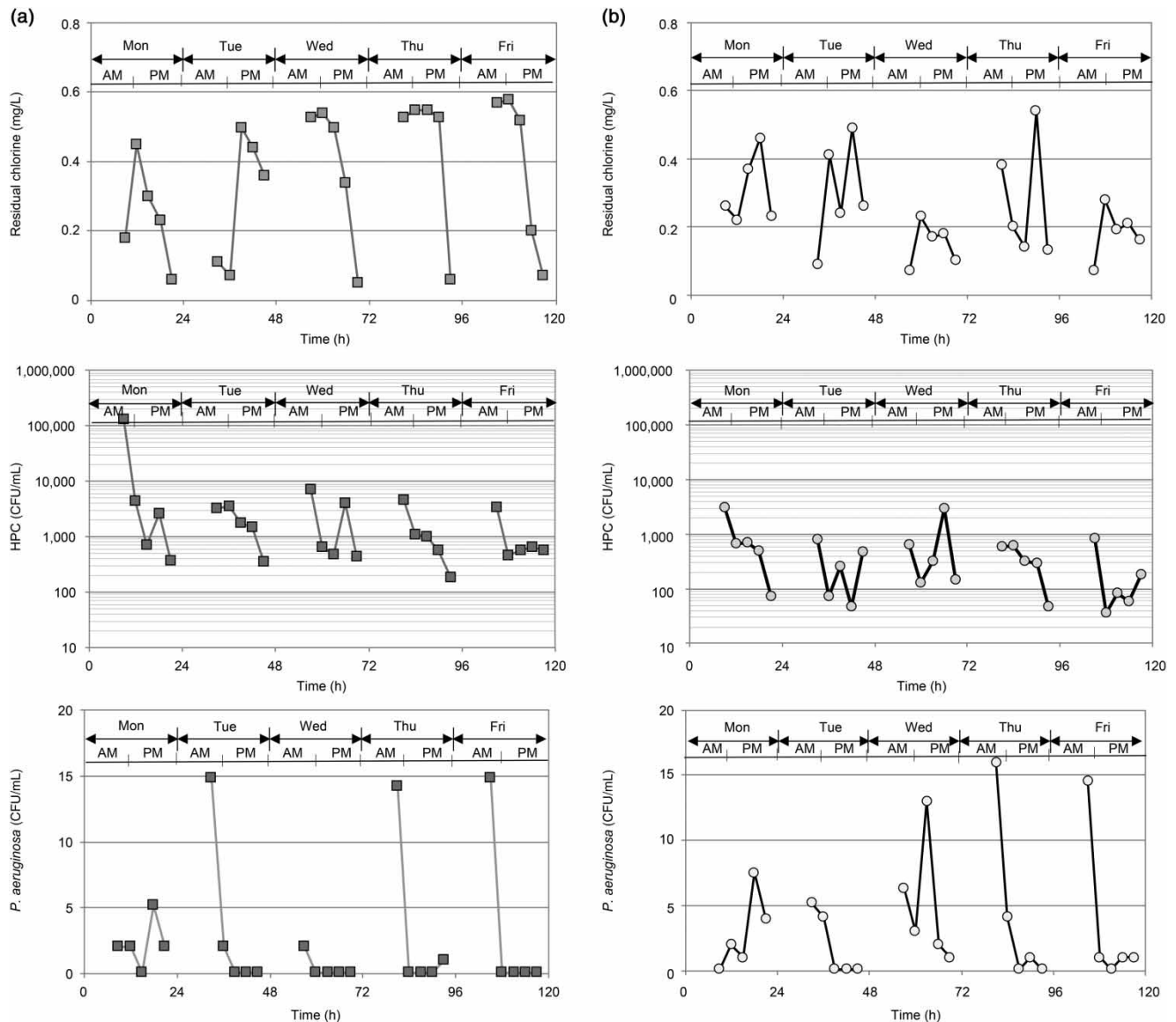


Figure 5 | Temporal variations in the residual chlorine, HPC, and *P. aeruginosa* levels in spray water from tank-type toilet seats (a) TT-1 m-2 and (b) TT-2 m-2.

Table 2 shows the results of an experiment performed to determine the origins of *P. aeruginosa*, as described in Figure 3. With seat OD-1f, only the tap water sample upstream of the microfilter (tap water 1) consistently showed high *P. aeruginosa* levels. In contrast, seat OD-1 m-2 occasionally showed *P. aeruginosa* in the spray water. The POT values in TT-1f were identical with those in OD-1f, but the POT values in OD-1 m and OD-1 m-2 were similar but not identical to each other. *P. aeruginosa* was not detected in the water from the washbasins of OD-1f and OD-1 m-2 in the restroom where

these seats were installed (tap water 2). When OD-1f and OD-1 m-2 were dismantled for further investigation, *P. aeruginosa* was detected in the inlet of the microfilter for OD-1f and in the spray nozzle for OD-1 m-2 (Figure 8). The *P. aeruginosa* POT values differed between OD-1f and OD-1 m-2; in the case of the former, the POT values at the inlet of the microfilter were identical to those in tap water 1 (Table 2). With OD-1 m-2, *P. aeruginosa* was detected at the tip of the spray nozzle, but the POT values differed from those in the OD-1 m-2 spray water and tap water 1 (Figure 8 and Table 2).

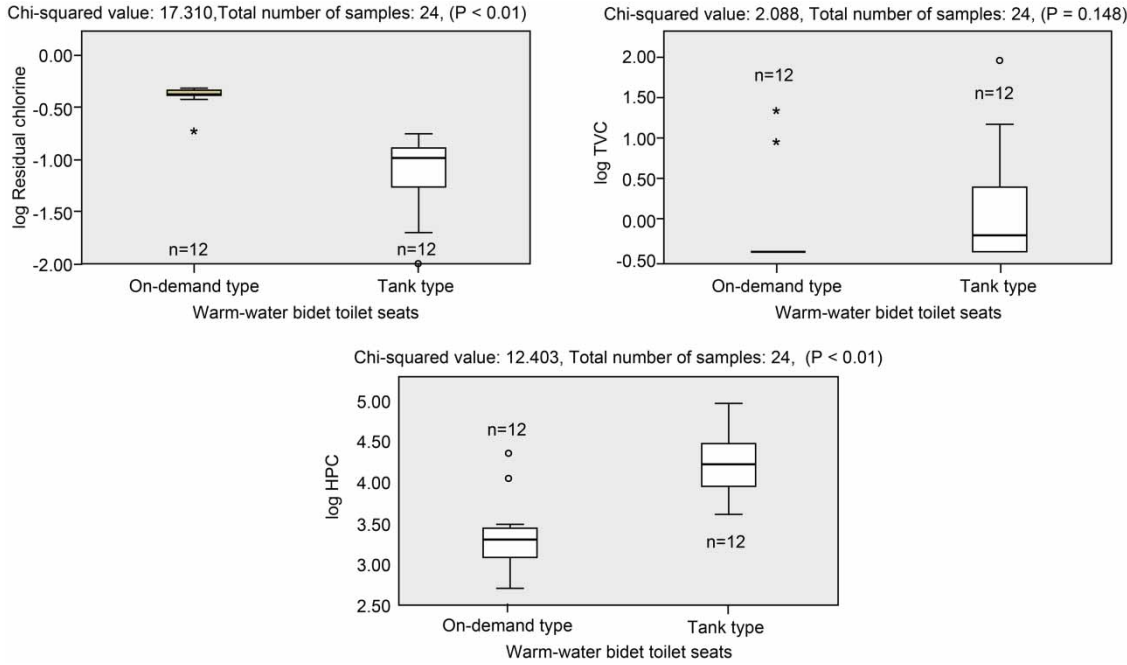


Figure 6 | Comparison of the residual chlorine, TVC, and HPC levels in spray water between tank-type and on-demand-type toilet seats.

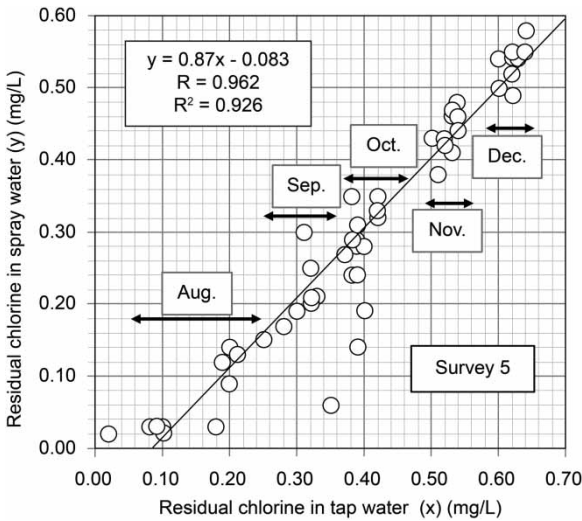


Figure 7 | Correlation between residual chlorine levels in tap water and spray water in on-demand-type toilet seats.

DISCUSSION

The ability to maintain and regulate residual chlorine is an important built-in factor for maintaining the hygiene and disinfection of spray water. Since there was no obvious

change in the toilet seats surveyed over time, the observed decrease in residual chlorine was not due to deterioration of the toilet seats themselves. In this study, we only surveyed warm-water bidet toilet seats in 12 men’s and one women’s restrooms in the research building in our university. Further studies are needed on warm-water bidet toilet seats by age, location, and time, increasing the number of samples.

When *P. aeruginosa* was not detected in the spray water over an extended duration, the probability of a measurable TVC was low in both the tank and on-demand types (TT-1 m-2, TT-2 m-2, OD-3 m, OD-4 m, OD-5 m, OD-6 m, OD-7 m, OD-8 m, OD-9 m, OD-10 m, OD-11 m, and OD-12 m). The overall HPC levels tended to be lower in the on-demand type (3 log₁₀) than in the tank type (4 log₁₀). The lack of internal parts, such as a storage tank to heat the tap water in the on-demand type, may account for this tendency. The results of our survey clearly differed from those reported by Katano et al. (2014), who showed bacterial contamination in the spray water from tank-type warm-water bidet toilet seats.

Seasonal temperature fluctuations in tap water cause the residual chlorine levels to fluctuate as well (Futigami 2011; Fisher et al. 2012). As mentioned above, the structure of

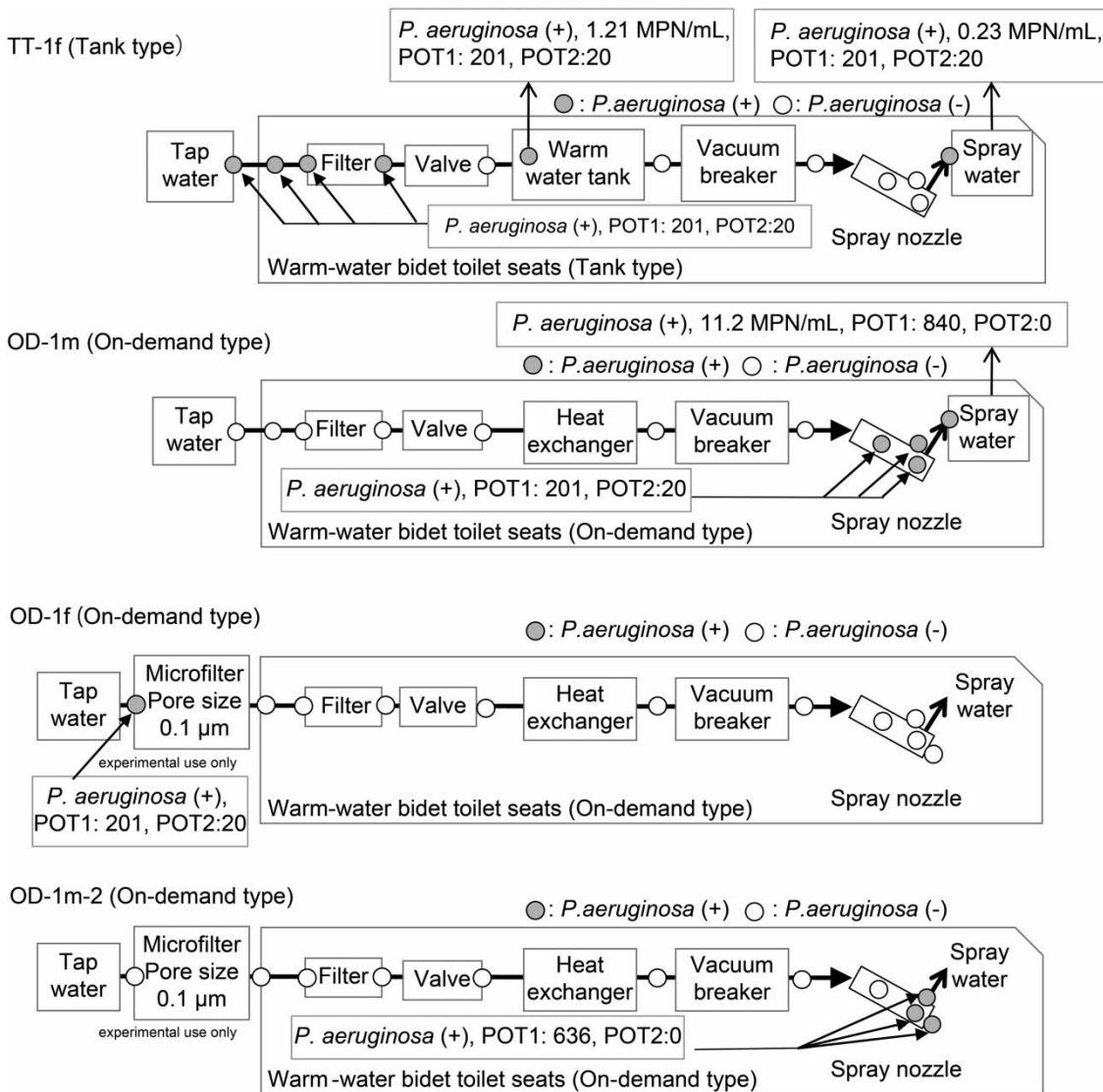


Figure 8 | Localization and POT values of *P. aeruginosa* in tank-type (TT-1f) and on-demand-type (OD-1f, OD-1 m, and OD-1 m-2) toilet seats in the origin test, using on-demand-type toilet seats.

the on-demand-type warm-water bidet toilet seat makes it susceptible to the residual chlorine levels in tap water (Figure 7). Therefore, as shown in Figures 4(b) and 7, the residual chlorine levels also fluctuated seasonally in the spray water from the on-demand-type toilet seat. An inverse relationship was observed between the residual chlorine levels and the HPCs (Figure 4(a) and 4(b)). The HPC is a widely used indicator of the bacterial count and biofilm growth inside pipes and tanks, which represent an aquatic environment with low nutrition (Ndiongue et al. 2005; Berry et al. 2006). The residual chlorine levels tended to

be relatively low in the morning and peaked around noon before dropping towards the evening. This was probably due to heating of the tank water, coupled with a prolongation of the water-retention time from evening to dawn, which decreased the residual chlorine level in the tank. When variance analysis of the HPCs was performed with nozzle disinfection and temporal variation in sample collection studied as experimental parameters, the HPC values were significantly associated with the temporal variation. This result suggested that the HPCs of the spray water were more likely to be affected by the time of

Table 2 | Genotyping and molecular epidemiological analysis of *Pseudomonas aeruginosa* from warm-water bidet toilet seats (Survey 6)

Survey date	OD-1f (On-demand type)					OD-1 m-2 (On-demand type)				
	Classification	Number of isolates	<i>P. aeruginosa</i>			Classification	Number of isolates	<i>P. aeruginosa</i>		
			MPN/mL	POT1	POT2			MPN/mL	POT1	POT2
08/28/2015	Tap water 1	4	2,400	201	20	Spray water	1	0.05	876	0
09/11/2015	Tap water 1	5	1,600	201	20	Spray water	–	n.d.	–	–
09/25/2015	Tap water 1	4	910	201	20	Spray water ^a	3	0.01	876	0
							4		620	16
10/09/2015	Tap water 1	8	5,800	201	20	Spray water	1	0.01	876	0
							2		620	16
10/30/2015	Tap water 1	2	6,100	201	20	Spray water	–	n.d.	–	–
11/09/2015	Tap water 1	6	450	201	20	Spray water	–	n.d.	–	–
11/25/2015	Tap water 1	4	21	201	20	Spray water	1	0.02	876	0
12/07/2015	Tap water 1	4	790	201	20	Spray water ^b	–	n.d.	–	–
12/25/2015	Tap water 1	7	540	201	20	Spray water	–	n.d.	–	–
	Detection rate		100 (%)			Detection rate		44.4 (%)		

MPN, most probable number; POT, PCR-based open reading frame typing.

^aColiforms-positive.

^b*E. coli*-positive.

collection than by contamination on the nozzle surface (Iyo et al. 2015).

Furthermore, *P. aeruginosa* can remain inside biofilms on pipes for long periods (Mena & Gerba 2009). *P. aeruginosa* is not pathogenic in immunocompetent, healthy individuals; therefore, there is no need to worry about the risk of infection from this organism from warm-water bidet toilet seats in regular households. However, it can cause opportunistic infections in individuals with reduced immunity (Reuter et al. 2002; Aumeran et al. 2007; Trautmann et al. 2008). *P. aeruginosa* can easily develop resistance to antibiotics (Bert et al. 1998; Ferroni et al. 1998; Durojaiye et al. 2011). Therefore, when warm-water bidet toilet seats are placed in hospital wards, sufficient attention needs to be paid to the possibility of *P. aeruginosa* contamination and propagation, which should include regular testing for *P. aeruginosa*.

Although tank-type toilet seats have lower concentrations of residual chlorine in the tank compared with on-demand-type toilet seats, the former has a longer hydraulic retention time, which can influence the residual chlorine values (Zamyadi et al. 2012).

Empirical investigation into the origins of *P. aeruginosa* revealed the presence of both feces-derived and tap water-

derived strains. The genotypes obtained using POT are described with two types of POT values (POT1 and POT2). The POT1 and POT2 values for a presumed tap water-derived strain (detected in TT-1f and OD-1f, and compared with detection point of both TT-1f and OD-1f in Figure 8) were only 201 and 20, respectively, whereas those values for presumed feces-derived strains (detected in OD-1 m and OD-1 m-2) were [POT1: 840 and POT2: 0], [POT1: 876 and POT2: 0], [POT1: 620 and POT2: 1] and [POT1: 636 and POT2: 0], respectively. These variations in POT values probably reflect the fact that contamination of the nozzle was caused by feces from different users. As none of the POT2 values of the detected strains exceeded 64 (metallo beta-lactamase-negative) (Suzuki et al. 2015), these were most unlikely to be multidrug-resistant *P. aeruginosa* (MDRP) strains. From the viewpoint of preventing opportunistic infections due to *P. aeruginosa*, reducing the presence of MDRP to undetectable levels should reduce the infection risk.

Thus, here, we reported a long-term survey of the hygienic conditions of warm-water bidet toilet seats in a university research building. Microorganism levels in spray water from warm-water bidet toilet seats showed that the

overall hygienic safety was maintained. In a future study, an increased number of samples from warm-water bidet toilet seats is needed, especially for bidet toilet seats installed in female restrooms. In addition, to improve the hygienic safety of spray water from warm-water bidet toilet seats in their many different locations, self-cleaning spray nozzle mechanisms should be improved and structural changes should be made to prevent the growth of biofilms inside both warm-water tanks and pipes.

CONCLUSIONS

We performed a long-term survey of the behavior of microorganisms within warm-water bidet toilet seats. The TVCs were $\leq 1/\text{mL}$ in spray water samples from both on-demand and tank-type warm-water bidet toilet seats. The HPCs in the spray water were $4 \log_{10}$ in the tank type and $3 \log_{10}$ in the on-demand type. However, the residual chlorine and HPC levels in the spray water were only weakly correlated. When *Pseudomonas aeruginosa* was detected in spray water over an extended duration, the bacterial origin was likely feces or tap water. The POT values of presumably tap water-derived *P. aeruginosa* were uniform, whereas those of presumably feces-derived *P. aeruginosa* varied. The isolated *P. aeruginosa* strain was unlikely to possess enzymes conferring multidrug resistance. Microorganism levels in spray water from warm-water bidet toilet seats showed that the overall hygienic safety was maintained in these facilities.

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