



Decontamination effect of neutral electrolysed water for spray nozzles of electric warm-water bidet toilet seats in the healthcare setting

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SUMMARY

Background: There has been an increasing demand for more sanitary bidet seats in many healthcare settings because of concerns regarding potential contamination of the spray nozzles of warm-water bidet toilet seats. This contamination is thought to possibly serve as a reservoir for horizontal transmission of drug-resistant bacteria.

Aim: This study was performed to determine the optimal *Pseudomonas aeruginosa* decontamination conditions and verify the effectiveness of these decontamination conditions.

Methods: An *in vitro* test of rinsing with neutral electrolysed water was performed using seven strains of *P. aeruginosa*. The decontamination effect of the neutral electrolysed water was verified by a field test involving an analysis of the number of bacteria isolated from samples collected from the spray nozzles and the sprayed water from 10 toilet seats at the internal medicine ward of Juntendo University Hospital.

Findings: The *in vitro* test results showed that the decontamination effect of neutral electrolysed water tended to be higher with higher free chlorine concentrations in the nozzle-cleaning water and shorter intervals of rinsing. The field test involving the hospital ward toilets showed that routine physical cleaning was satisfactorily effective.

Conclusion: The study results suggest that the risk of horizontal transmission of drug-resistant bacteria via the use of bidet toilet seats in hospitals can be reduced by general cleaning and appropriate control of the free chlorine concentration in the nozzle-cleaning water.

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Introduction

Warm-water bidet toilet seats are common in Japan; their household penetration rate had reached 80.2% by March 2018 [1]. Bidet toilet seats are designed to spray warm water from a nozzle to wash the perianal area after a bowel movement. While they are extremely common in Japan, they have also

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gradually become popular in other Asian countries as well as the rest of the world.

Nearly 100% of large medical institutions founded within the last 10 years in Japan have installed warm-water bidet toilet seats. However, concerns exist that contamination of the spray nozzles of bidet toilet seats might serve as a reservoir for horizontal transmission of multidrug-resistant Gram-negative bacilli and vancomycin-resistant enterococci [2–4]. Conversely, some reports have suggested that bidet toilet seats are unlikely to contribute to an increased risk of horizontal transmission in medical institutions [5–7]. Moreover, one report indicated that anal washing using bidet toilet seats is more hygienic because it can reduce bacterial contamination of the perianal area, thereby reducing bacterial contamination of the fingers after wiping with toilet paper [8]. Overall, this issue remains inconclusive.

These discussions have prompted an increasing demand for more sanitary bidet toilet seats in healthcare settings than in household settings. To meet this demand, some models on the market are designed to electrolyse tap water and produce free chlorine-containing neutral electrolysed water, providing the spray nozzles with a self-cleaning function.

Electrolysed water has long been used to disinfect the environment in medical and nursing care facilities [9]. Its use in the areas of wound care and food hygiene has also been increasing in recent years [10–12]. Free chlorine contained in electrolysed water is a disinfectant with established safety in humans, and it is used for disinfection of drinking water (Japanese water quality standard: residual free chlorine of ≥ 0.1 mg/L), public baths, and swimming pools [13,14].

A study by Kuwahara et al. [15] revealed that the disinfecting effect of neutral electrolysed water was insufficient against *Pseudomonas aeruginosa* contained within the biofilm that had formed on the spray nozzles. However, our previous study showed that *P. aeruginosa* suspended in physiological saline (at approximately 1000-fold dilution of bacterial suspension adjusted to 0.5 McFarland) was adequately disinfected by neutral electrolysed water containing free chlorine at a concentration of 0.5 mg/L [16]. These findings suggest that periodic nozzle cleaning using neutral electrolysed water before the contaminating bacteria on the spray nozzles have formed a biofilm can provide a sufficient decontamination effect. In the present study, we performed an *in vitro* test to determine the optimal decontamination conditions for *P. aeruginosa* followed by a field test at a medical institution to verify the effectiveness of these decontamination conditions [17,18].

Methods

Setting

Juntendo University Hospital is one of the largest teaching hospitals with 1,020 acute-care beds and 3 adult intensive care units, 1 neonatal intensive care unit, and 1 high-dependency unit. In 2018, the average number of outpatients was 4,200 per day and the average length of stay was 10.2 days.

In vitro test

Bacterial strains used for the test

The *in vitro* test involved seven strains of *P. aeruginosa* (four clinical isolates and three environmental isolates) that showed

≥ 10 -fold growth in 12 hours. These seven strains were selected from among 44 clinical isolates of *P. aeruginosa* obtained from blood cultures of patients hospitalized at Juntendo University Hospital from 2015 to 2016 as well as eight environmental isolates of *P. aeruginosa* obtained from hand-washing sinks and around toilet bowls in the hospital [16].

Preparation of neutral electrolysed water

To produce neutral electrolysed water, free residual chlorine was removed from municipal tap water (adjusted pH of 7.5 ± 0.1) supplied by Chigasaki City in Kanagawa Prefecture, and the water was then passed through an electrolyser coated with a platinum/iridium catalyst. The produced neutral electrolysed water had two different free residual chlorine concentrations of 0.5 and 2.5 mg/L. The control water was the tap water before it had passed through the electrolyser.

Test of rinsing with neutral electrolysed water [16,19]

The rinsing test was performed in accordance with ISO 22196 (Measurement of antibacterial activity on plastics and other non-porous surfaces), with changes to the bacterial suspension type and the incubation time and addition of repeated rinsing with neutral electrolysed water. A test bacterial suspension of approximately 10^4 cfu/mL was prepared with nutrient broth diluted to 1/500 (hereinafter referred to as the 'diluted nutrient broth'). A 0.3-mL bacterial suspension was inoculated on a 5-cm² resin plate covered with a 4-cm² Stomacher® film (Seward Ltd., Worthing, England) to spread the bacterial suspension over the entire surface of the plate. The test specimens were incubated at $35^\circ\text{C} \pm 1^\circ\text{C}$ at $\geq 90\%$ relative humidity until rinsing. After incubation for the specified time, the Stomacher® film was removed from the specimen, and the plate was tilted approximately 20° and rinsed for 5 seconds with 8.4 mL of tap water (control) or neutral electrolysed water. A 0.3-mL suspension of the diluted nutrient broth was placed on the rinsed specimen, and the incubation to rinsing processes were repeated. After a total incubation time of 32 hours, a final rinsing was performed, and the total count of viable bacteria on the resin plate was recorded according to the ISO 22196 method. Three different incubation times of 4, 8, and 16 hours were used between rinsing procedures.

Field test

Warm-water bidet toilet seats

A warm-water bidet toilet seat is set on the toilet and has various functions including seat heater, deodoriser, and warm air dryer. The device is widely used in approximately 80% of bathrooms in Japan (Figure 1a). Warm rinse water (30–40°C) (for washing perineal and anal areas) comes out from the nozzle under the toilet seat (Figure 1b). The nozzle has a self-cleaning function for both inside and outside of the nozzle before and after each use (Figure 1c). Even when the toilet is not being used, the nozzle periodically cleans itself under the preset programme.

In the field test, we used 10 warm-water bidet toilet seats (Washlet Apricot P®, TCF5830; TOTO Ltd., Fukuoka, Japan) installed onto the existing ceramic toilets (two male toilets, two female toilets, and one wheelchair toilet in each of two restroom areas) in the internal medicine ward of Juntendo University Hospital. This bidet toilet seat model heats the water on demand (instantaneous heating system); it does not



Figure 1. A warm water bidet toilet attachment. (a) A warm water bidet toilet seat used in this study. (b) Warm-water for cleaning perineum and anal comes out from the nozzle attached under the seat. (c) The nozzle has a self-cleaning function for inside and outside of the nozzle and its around exteriors.

have a tank to keep warm water ready for use. Tap water is used as the spray water for perianal washing. For this test, the product was converted so that either neutral electrolysed water or tap water could be selected for the cleaning water that ran through the water passage and over the spray nozzle surface. The free chlorine concentrations in the nozzle-cleaning water (tap water or neutral electrolysed water) and the spray water were monitored twice a month using the United States Environmental Protection Agency DPD Method 8021 (N,N-diethyl-p-phenylenediamine).

Automated self-cleaning and general manual cleaning of the spray nozzle

The device was programmed to perform automated self-cleaning of the spray nozzle to clean the water passage and the spray nozzle surface after each use or at specified intervals. For the cleaning water running through the water passage and over the spray nozzle surface, neutral electrolysed water was used for the first 12 weeks and tap water was used for the next 12 weeks. These 24 weeks were regarded as one cycle, and two cycles were performed for the test. At the end of each 12-week period of cleaning, the warm-water bidet toilet seat was disassembled to the greatest extent possible, cleaned both inside and outside, and decontaminated with disinfectant alcohol.

General manual cleaning of the spray nozzle was performed in accordance with the guideline for toilet cleaning in medical institutions issued by the Japan Sanitary Equipment Industry Association [20]. A trained cleaning vendor decontaminated the spray nozzle with hydrogen peroxide using microfibre cloths between 08:00 and 09:00 daily.

Evaluation of effectiveness of nozzle cleaning

Samples were collected every 7 calendar days (if the sampling day was a holiday, the sampling was performed on the next business day; however, the maximum allowed interval was 10 days), before and after the general manual cleaning, from three sites of the spray nozzle (i.e., spray nozzle opening, spray nozzle surface, and spray water) of the warm-water bidet toilet seats. The sampling was performed on a total of

21 occasions. Subsequently, sample collection only before the general manual cleaning was performed on 27 occasions. To collect samples from the spray nozzle opening and surface, a sterile cotton bud was used to wipe a 1-cm² area, and the sample was suspended in physiological saline containing 0.1% sodium thiosulphate. For sampling of the spray water, approximately 10 mL of the water was collected into a sterile sampling bottle containing 10 mg of sodium thiosulphate. The collected samples were mixed with solid selective media and incubated, and the number of grown colonies was then counted. Common bacteria were incubated on standard agar at 35°C for 48 hours, while heterotrophic bacteria were incubated on R2A agar at 20°C for 7 days. For *P. aeruginosa*, we only counted green colonies on cetrimide agar after incubation at 35°C for 48 hours. For intestinal bacteria, we only counted red to purple colonies on MacConkey agar after incubation at 35°C for 48 hours. The number of bacteria per unit area was calculated from the counted number of colonies.

Statistical analysis

In the statistical analysis, we used the logarithmic value of the number of *P. aeruginosa* in the *in vitro* test and the logarithmic values of the numbers of common bacteria and heterotrophic bacteria in the field test; we used the lower limit of detection if the value was below the lower limit of detection. In the *in vitro* test, the Wilcoxon signed-rank test was used to compare the initial and post-incubation bacterial counts, and the Friedman test and Bonferroni's multiple-comparison test were used to determine the decontamination effects of the various free chlorine concentrations and cleaning intervals. In the field test, the Mann–Whitney U test was used to compare the number of bacteria between different nozzle-cleaning waters. The decontamination effect of cleaning was assessed using the Wilcoxon signed-rank test. The bacterial counts were compared between different intervals of automated cleaning using the Kruskal–Wallis test and Bonferroni's multiple-comparison test. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [21].

Results

In vitro test

Figure 2 shows the relationship between the logarithmic count of bacteria and the rinsing interval at a total incubation time of 32 hours. The decontamination effect tended to be stronger with higher free chlorine concentrations and shorter intervals of rinsing. The number of remaining bacteria was significantly smaller with rinsing at 4-hour intervals than with 8- and 16-hour intervals, regardless of the free chlorine concentration. When rinsing at 8-hour intervals using different concentrations of free chlorine (i.e., 0.0, 0.5, and 2.5 mg/L), the number of remaining bacteria was significantly smaller for the higher free chlorine concentration. When rinsing at 16-hour intervals, the number of remaining bacteria was significantly

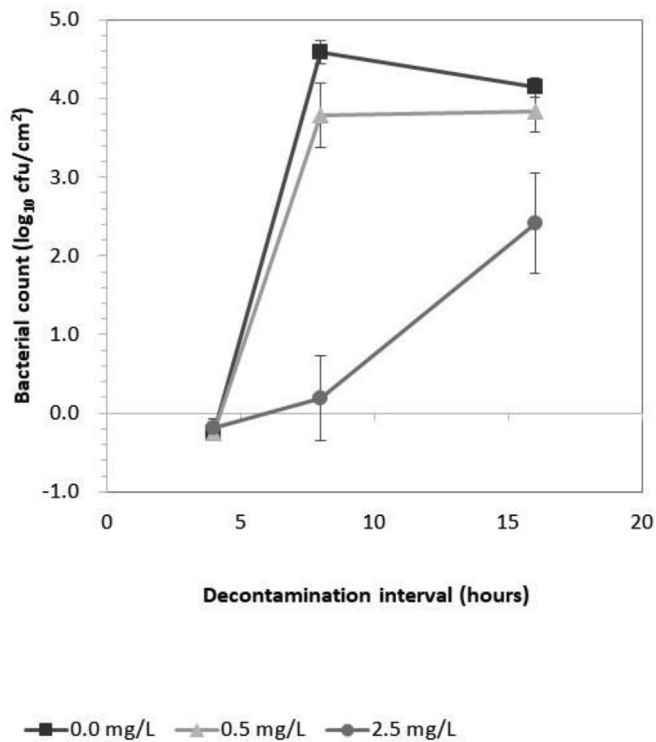


Figure 2. *In vitro* test results. The incubation and decontamination processes were repeated, and the relationship between the decontamination interval and the bacterial count (\log_{10} cfu/cm²) (mean from seven strains \pm 2 standard error) at 32 hours is shown.

smaller for the free chlorine concentration of 2.5 mg/L than 0.0 and 0.5 mg/L.

Field test

Validation of effectiveness of nozzle cleaning

To evaluate the effectiveness of general manual cleaning of the spray nozzle in accordance with the guideline, samples were collected from three sites of the spray nozzle (i.e., spray water, spray nozzle opening, and spray nozzle surface) before and after the general manual cleaning, and the number of detected bacteria was compared. In total, 210 samples were collected. Pre- and post-cleaning samples were compared, and the findings were grouped into three categories: 'bacteria increased', 'bacteria decreased', and 'no bacteria'. These findings accounted for 4%, 5%, and 91%, respectively, of the spray water samples; 16%, 24%, and 60%, respectively, of the spray nozzle opening samples; and 19%, 27%, and 54%, respectively, of the spray nozzle surface samples. The number of remaining bacteria was significantly smaller after than before the general manual cleaning for the spray nozzle opening samples only where automatic nozzle cleaning was difficult.

Free chlorine concentrations and bacterial counts

The free chlorine concentration in the tap water used for the warm-water bidet toilet seats ranged from 0.4 to 0.8 mg/L, while the free chlorine concentration after electrolysis ranged from 1.7 to 2.0 mg/L. Figure 3 shows the distribution of the number of detected bacteria from the three sites of the spray

nozzles according to the type of nozzle-cleaning water; i.e., tap water or neutral electrolysed water. The median number of common bacteria was the lower limit of detection for all sites and cleaning conditions. The Mann–Whitney test results showed that the number of common bacteria on the spray nozzle surface and the number of heterotrophic bacteria at all sites were significantly lower for the neutral electrolysed water than for the tap water.

Figure 4 shows the distribution of the number of detected bacteria from the three sites of the spray nozzles according to the self-cleaning condition; i.e., 4-hour intervals, 8-hour intervals, and no self-cleaning. The results of the Kruskal–Wallis test and Bonferroni's multiple-comparison test showed no significant difference in the number of common bacteria among the different conditions of self-cleaning for any site. The number of remaining heterotrophic bacteria in spray water was significantly smaller with self-cleaning at 4-hour intervals than with no self-cleaning, and that at the spray nozzle surface was significantly smaller with self-cleaning at 4- than 8-hour intervals. Common bacteria are used in water quality and food management, and heterotrophic bacteria are used in evaluation of the water purification process.

The detection rates of intestinal bacteria and *P. aeruginosa* in the samples were 0.00% and 0.00%, respectively, for spray water; 0.00% and 0.68%, respectively, for the spray nozzle opening; and 0.00% and 1.60%, respectively, for the spray nozzle surface.

Discussion

The free chlorine concentration in neutral electrolysed water after electrolysis is affected by the chloride ion concentration in the tap water, applied electric current level, and flow volume. The free chlorine concentration in the nozzle-cleaning water used in currently available warm-water bidet toilet seats with a built-in electrolyser is around 0.5 to 5.0 mg/L (mean concentration, 2.5 mg/L).

The *in vitro* test showed that the decontamination effect was stronger with higher free chlorine concentrations in the nozzle-cleaning water and shorter intervals of rinsing. The decontamination effect was stronger with rinsing at 4-hour intervals than 8- and 16-hour intervals, regardless of the free chlorine concentration. This indicates that bacterial growth and metabolite production at 4 hours of incubation were limited and that mechanical rinsing alone therefore provided a sufficient decontamination effect of the dispersed bacterial solution.

Conversely, with 8- and 16-hour rinsing intervals, the decontamination effect was stronger at higher free chlorine concentrations. This indicates that bacterial growth and metabolite production were already so substantial that rinsing with tap water alone was no longer sufficient for decontamination, and the decontamination effect was therefore dependent upon the free chlorine concentration in the neutral electrolysed water.

A similar tendency was shown in the field test, where various pollutions were present, but the impact of shortened intervals of rinsing/cleaning on the decontamination effect was smaller in the field test than in the *in vitro* test. Furthermore, in the field test, regardless of whether the nozzle-cleaning water was tap water or neutral electrolysed water, the median number of detected common bacteria from spray

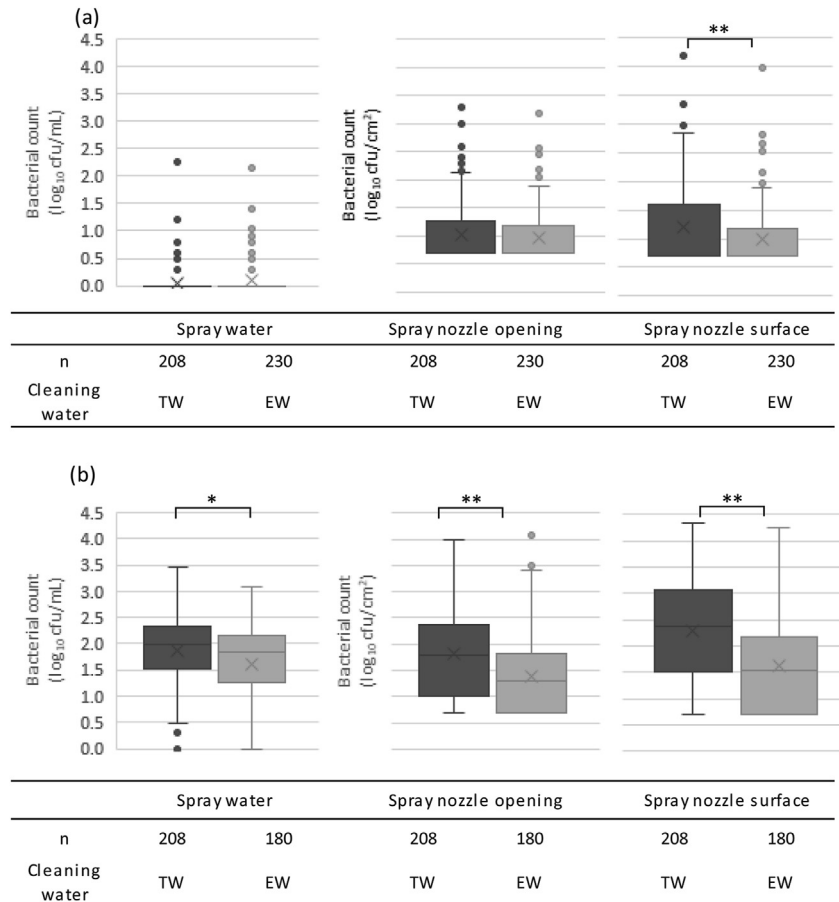


Figure 3. Field test results: Distribution of detected bacterial counts by sample site for cleaning with tap water versus neutral electrolysed water. (a) General bacteria. (b) Heterotrophic bacteria. TW, tap water; EW, electrolysed water. Mann–Whitney test, * $P < 0.05$; ** $P < 0.01$.

water was at the lower limit of detection, and most samples were below the quality standards for Japanese tap water, with no detection of *P. aeruginosa* or intestinal bacteria in particular. This can be explained by the effect of mechanical removal of microbial contamination by routine daily cleaning performed by the cleaning vendor. In addition, the free chlorine concentration in the tap water in this medical institution was higher than that in the tap water (0 mg/L) used in the *in vitro* test, which might have led to chemical decontamination of a portion of the bacteria and thus a relative decrease in the decontamination effect of automated nozzle self-cleaning. The median number of heterotrophic bacteria was smaller in the 4-hour electrolysed water than in the 8-hour electrolysed water (data not shown).

Many published reports have described detection of *P. aeruginosa* and intestinal bacteria from spray water or nozzles [2–4], but the detection rates of these bacteria in our field test were still low. In previously published reports, proper toilet-cleaning management was performed in limited cases, and the spray water or nozzle contamination rates could have been reduced by proper routine toilet cleaning. Although Tsunoda *et al.* [22] reported that faecal indicator bacteria were still recovered from nozzle surfaces and spray water despite daily manual cleaning, their cleaning method, disinfectant, and analysis method were completely different from those in our study.

The risk of bacterial contamination can be further reduced by the use of high free chlorine concentrations in tap water as well as instantaneous water-heating bidet toilet seats, allowing limited reduction of free chlorine concentrations and thereby reducing bacterial contamination of spray water [7]. The field test evaluated product use up to 1 year. Given that the mean duration of product use is 10 years, and considering the potential influence of ageing of the product, a long-term survey on bacterial counts is needed.

The results of this study suggest that the risk of pathogenic bacterial transmission via the use of warm-water bidet toilet seats in medical institutions can be reduced by performing periodic general manual cleaning to mechanically decontaminate the spray nozzle, maintaining the free chlorine concentration in the nozzle-cleaning water at ≥ 0.5 mg/L, and ensuring nozzle self-cleaning intervals of ≤ 4 hours.

This study has several limitations. The *in vitro* test focused on *P. aeruginosa* and did not examine the decontamination effect against other species of multidrug-resistant microorganisms associated with other problematic nosocomial infections. In addition, the test conditions were in line with ISO 22196, and the nutrient status and inoculum size of the bacteria in the test might have differed from those in the real-world environment. In the field test, the frequency of bacterial detection and the number of detected bacteria may have varied depending on the structure of the bidet toilet seats and the free chlorine concentrations in the

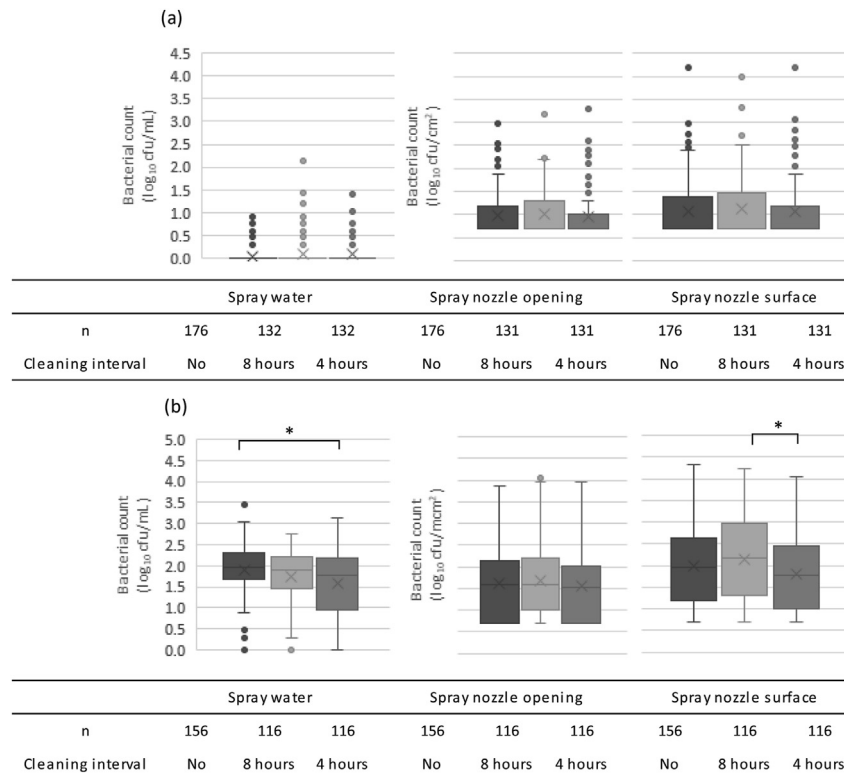


Figure 4. Field test results: Distribution of detected bacterial counts (\log_{10} cfu/mL) by sample site for no self-cleaning and self-cleaning intervals of 4 and 8 hours while not in use. (a) General bacteria. (b) Heterotrophic bacteria. X indicates the mean value. Kruskal–Wallis test, Bonferroni’s multiple-comparison test, $*P < 0.05$.

supplied tap water and spray water. Thus, the study results cannot be applied to areas where tap water is disinfected using methods other than chlorine. The bacterial count and residual chlorine concentration in tap water and spray water can greatly vary among different countries or regions. Warm-water bidet toilet seats are becoming increasingly popular, mainly in Asia; however, the quality of tap water may affect the hygiene of warm-water bidet toilet seats.

Conclusions

The *in vitro* and field tests demonstrated that proper cleaning of the spray nozzles of warm-water bidet toilet seats using neutral electrolysed water can prevent contamination of the spray nozzles and spray water. Nozzle self-cleaning at 4-hour intervals showed the strongest decontamination effect. The field test conditions that included once-daily general manual cleaning revealed that the frequency of bacterial detection and the number of detected bacteria from the spray nozzles were maintained at low levels. These results suggest that the risk of horizontal transmission of drug-resistant bacteria via the use of warm-water bidet toilet seats in hospitals can be reduced by general cleaning and appropriate control of the free chlorine concentration in the nozzle-cleaning water.

CRedit author statement

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Satoshi HORI: Supervision, Project administration, Funding acquisition, Writing-Review & Editing.
Shigeki MISAWA: Resources, Writing-Review & Editing.

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Conflict of interest statement

Aiko Itami is an employee of TOTO Ltd. Satoshi Hori has received research funding from TOTO Ltd. Shigeki Misawa has no conflict of interests to declare.

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