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PII: S0196-6553(22)00812-4
DOI: <https://doi.org/10.1016/j.ajic.2022.11.011>
Reference: YMIC 6404



To appear in: *AJIC: American Journal of Infection Control*

Please cite this article as: Takayuki Ohishi PhD , Kazuhiro Ootsuki , Sayaka Kanno , Chiharu Ishiyama , Satoshi Kashima , Kimiko Maruyama , Ryo Fushimi M.S. , Adenosine phosphate-based detection of worker exposure to contaminated water during bathroom cleaning, *AJIC: American Journal of Infection Control* (2022), doi: <https://doi.org/10.1016/j.ajic.2022.11.011>

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Highlights

- Cleaners can contract infections when cleaning hospital restrooms
- Workers and surfaces are exposed to contaminated water during bathroom cleaning
- Contaminant exposure increases with the use of a brush as compared to a sponge
- The toilet environment is also contaminated during toilet cleaning
- PPE usage during bathroom cleaning is important for infection control

Journal Pre-proof

**Adenosine phosphate-based detection of worker exposure to contaminated water during bathroom
cleaning**

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Abstract

Background: Workers who clean bathrooms at medical facilities may be regularly exposed to contaminated water harboring pathogenic microbes and should wear personal protective equipment to prevent such exposure at medical facilities, which has not been quantitatively assessed. This study quantified the exposure risk from contaminated water when cleaning restrooms at medical facilities and clarified the importance of wearing personal protective equipment.

Methods: Existing urinals, toilets, and handwashing sinks (16 each) in a hospital environment were coated with a simulated contaminant containing adenosine phosphate and cleaned with a brush or sponge by workers in personal protective equipment. Adenosine phosphate on the personal protective equipment, shelf near the toilet, and toilet paper cover was tested before and after cleaning to compare exposure status.

Results: Adenosine phosphate on the worker's personal protective equipment, shelf near the toilet, and toilet paper cover was significantly higher after cleaning the urinal, toilet, and handwashing sink. More adenosine phosphate was disseminated from urinals and toilets when cleaning with a brush compared with a sponge.

Discussion: Workers and the surrounding environment are exposed to contaminated water during bathroom cleaning. Wearing personal protective equipment while cleaning and wiping down the toilet environment after cleaning deserves further consideration.

Keywords

Adenosine phosphate; tomato juice; contaminated; bathroom; feces

Background

Human feces harbor a massive quantity of microbes¹; in some cases, the human urethra may contain pathogenic microbes.² The feces and urine of infected patients examined at medical facilities are especially likely to contain pathogenic microbes as compared to those of healthy individuals. Thus, fixed toilets and urinals that are constantly contaminated by patient excreta are a potential source of infection. Handwashing sinks in bathrooms harbor Gram-negative bacilli, such as *Pseudomonas aeruginosa*, which thrive in moist environments; other pathogenic microbes that are prone to hand-contact-based transmission have also been implicated in sink-transmitted infections.³

Most medical facilities in Japan outsource the cleaning of toilets, urinals, and sinks to external cleaning companies. According to a 2018 fact-finding survey conducted by the Japan Health Enterprise Foundation, 87.5% of the medical facilities outsourced their cleaning.⁴ Infection can be effectively prevented through the use of personal protective equipment (PPE) when there is a risk of exposure to wet biological materials.⁵ However, cleaning personnel are not always aware of the concept of standard preventive measures and rarely wear gloves, gowns, and face shields, all of which are highly necessary.⁶

Microbes that are found in the feces, urine, and sinks are mostly bacteria that are ordinarily harmless,⁷ and a healthy cleaner is unlikely to develop an infection through exposure alone. However, in case of viruses such as the norovirus and the hepatitis B virus (HBV; found in the feces and urine of infected patients, respectively), infection can occur even with small amounts of the viruses.^{8,9} Moreover, these viruses can remain infectious in the environment for long periods.^{10,11}

Cleaning of toilets, urinals, and sinks requires the use of a brush or a sponge; these tools are likely to disperse contaminated water due to their design. Because cleaners must check for cleanliness during their work, they tend to bring their faces close to the toilets that they are cleaning and risk the exposure of their eyes or mouth to the contaminated water. To prevent a high risk of infection when cleaning toilets, there is a strong need to wear PPE as an infection-control measure. However, no study has quantitatively assessed the risk of exposure to contaminated water when cleaning restrooms at medical facilities. In this study, using adenosine phosphate as an indicator, we quantitatively evaluated the exposure risk that toilets and urinals pose to workers to clarify the importance of preventing pathogenic exposure to microbes when cleaning bathroom fixtures.

Materials and Methods

Fixtures

This study was conducted from June 20 to June 30, 2022. The authors ensured that the participating cleaners could not be identified from the experimental results and that they were blinded to their own results. The fixtures tested in this study included urinals (n=16 identical urinals; external length: 55 cm, external width: 46 cm, and depth: 28 cm), toilets (n=16 identical toilets; external length: 48 cm, external width: 40 cm, and height: 43 cm), shelves (n=16 existing shelves installed on the toilet reservoir), toilet paper covers (n=16 existing toilet paper covers installed by the side of the toilet), and sinks (n=16 identical hemispherical sinks located in the bathroom; inside diameter: 42 cm).

Materials

The materials used in this study included PPE (gloves [natural rubber], long-sleeved gowns [plastic], face shields [polyethylene terephthalate; PET], and plastic sheets [6×6 cm and 20×20 cm; PET]), cleaning tools (toilet-cleaning brushes and sponges, sink-cleaning sponges, etc.), simulated contaminant (unsalted tomato juice; Nippon Del Monte Corporation, Japan), simulated contaminant applicator (paint brush [for water-based paint, nos. 20 and 50 mm]), adenosine phosphate-testing instrument and cotton swabs (2-in-1 reagent kit: Lumitester Smart LuciPac A3 Surface, Kikkoman Biochemifa Company, Japan), and tap water.

Tomato juice was selected as the simulated contaminant because it contains large amounts of adenosine triphosphate (ATP),¹² has moderate viscosity, is inexpensive to obtain, and poses no risk of infection to the participants.

A3 testing of materials (control)

A new paint brush, toilet-cleaning brush, toilet-cleaning sponge, and sink-cleaning sponge were used in each trial. Before use, each tool was washed with tap water (200 mL, 2 L, 2 L, and 2 L, respectively).

Thereafter, the adenosine phosphate content (adenosine monophosphate, adenosine diphosphate, and ATP;

A3) in the post-cleaning tap water was tested 5 times for each tool.

Verification of the validity of tomato juice as a simulated contaminant

Tomato juice was diluted with tap water to prepare solutions with concentrations of 0.00005%, 0.0005%, 0.005%, 0.05%, and 0.5%. Then, 100 μ L of each solution was collected and applied to the reagent kit's cotton swabs. These swabs were inserted into the reagent liquid and agitated before testing for A3 luminosity (relative light unit [RLU]) using the testing instrument. This procedure was performed 5 times, and the relationship between the tomato juice concentration and A3 was confirmed, thereby verifying the validity of tomato juice as a simulated contaminant.

Method of validating A3 exposure during cleaning

The cleaning involved in this study was performed by professional bathroom cleaners (4 participants in each combination; total: 16 participants) who were employed by the Watakyu Seimoa Corporation and who provided written informed consent to participate in this study. Owing to the varying number of years of experience of the cleaners and to avoid latent bias (i.e., bias arising from increased cleanliness due to pre-experimental training in cleaning), no training in cleaning was provided to the cleaners in advance for any trial.

The cleaners wore PPE, consisting of gloves, a long-sleeved gown, and a face shield. Plastic sheets were affixed to the gloves and gowns worn by the cleaners and to the toilet shelf and toilet paper cover to facilitate the collection of simulated contaminants that were transferred during cleaning. The plastic sheets were affixed with double-sided tape to the glove ($6 \times 6 \text{ cm}^2$) on the back of the hand (which was not cleaned with the brush and other tools), chest of the gown ($20 \times 20 \text{ cm}^2$), toilet shelf ($10 \times 10 \text{ cm}^2$), and

toilet paper cover ($10 \times 10 \text{ cm}^2$). Because face shields are made of plastic, they constitute a naturally easy surface for collecting the simulated contaminant. Thus, instead of attaching a plastic sheet, a $20 \times 20 \text{ cm}^2$ frame was drawn in the center, and the simulated contaminant was collected from that area only.

The cleaners initiated cleaning after the simulated contaminant had been applied to the toilets, urinals, and handwashing sinks. The designated collection areas on the cleaner's PPE, toilet shelf, and toilet paper cover were then swabbed using cotton swabs from the 2-in-1 reagent kit. Owing to the smaller dimensions of the designated collection areas on the gloves, toilet shelves, and toilet paper covers, each area was swabbed with 1 swab 20 times vertically and horizontally; conversely, the collection areas on the gowns and face shield were swabbed 40 times vertically and horizontally because of their large dimensions. Swabs collected from the designated collection areas were tested for A3 before and after cleaning.

Exposure of the cleaners to the simulated contaminants in each situation

All experiments were conducted after the test surfaces were cleaned normally. For each experiment, the person in charge applied the simulated contaminant (undiluted tomato juice) to each test surface, and the 16 cleaners then cleaned the test surfaces. After the cleaning was completed, the PPE worn by the cleaners and the designated collection areas on the test surfaces were tested for A3.

Urinal

1. Using a paintbrush, the simulated contaminant was applied to the center of the urinal, 3 times on the front and sides.

2. Each cleaner was positioned 60 cm from the urinal.
3. Without using any cleansing agents, each cleaner scrubbed the inside of the bowl (left, right, top, bottom, and front sides) with 15 short strokes applied using identical toilet-cleaning brushes that had been soaked in tap water.
4. The urinal was flushed to rinse and drain, and the brush was rinsed in the tap water filling the bowl.
5. The presence of A3 content on each piece of PPE before and after the cleaning was tested.
6. The simulated contaminant was reapplied to the urinal; each cleaner then cleaned it with a sponge while following the abovementioned 1-5 procedure.

Toilet

1. Using a paintbrush, the simulated contaminant was applied around the inside of the toilet bowl approximately 3 cm above the level of the toilet water.
2. Each cleaner was positioned 80 cm away from the back wall.
3. Without using any cleansing agents, each cleaner scrubbed the inside of the bowl (where the water did not touch the bowl) with 10 short strokes (at the center and on the left, right, back, and front sides) using a toilet-cleaning brush. Finally, the underwater surfaces were scrubbed; toilets have a lesser surface area than urinals, so the number of brush strokes was reduced. Finally, the toilet was flushed, and the brush was rinsed in the tap water that filled the bowl.

4. The A3 content on each piece of PPE after cleaning and that on the toilet shelf and toilet paper cover before and after cleaning were tested.
5. Then, the simulated contaminant was again applied to the toilet; each cleaner cleaned it using a sponge while following the abovementioned procedure.

Sink

1. Using a paintbrush, the simulated contaminant was applied to the inside of the sink near the top.
2. Each cleaner was positioned close to the front of the sink.
3. Without using any cleaning agents, each cleaner cleaned the inside of the sink with 15 short strokes (around the perimeter) applied with a sink-cleaning sponge that had been soaked in tap water.
4. The A3 content on each piece of PPE after cleaning was tested.

Running the faucet

1. The simulated contaminant was applied with a paintbrush around the inside of the sink near the bottom.
2. Each cleaner placed their non-dominant hand, palm down, at a distance of 10 cm from the edge of the sink; they then leaned forward and ran the faucet (water output at each time: 214 mL; distance between the faucet and the base of the sink basin: 23 cm).

3. After the faucet was turned on, the A3 content on each piece of PPE was tested.

Statistical analysis

The Mann–Whitney U test was performed for all A3 readings using JMP[®] (SAS Institute, USA). $P < 0.05$ was considered significant. To detect an effect size (Cohen = 0.5) with 90% power and a 2-sided α of 0.05, 70 cleaners were required for the Mann–Whitney U test of the A3 exposure before and after cleaning.

Ethical considerations

The research protocol for this study was approved by the institutional review board (approval number: 20220027). The authors obtained written informed consent from the participants.

Results

A3 readings of materials

The median A3 value for each material before use in the experiment was low, being 16.0, 6.0, 7.0, and 10.0 RLU for the paint brush used for simulated contaminant application, toilet-cleaning brush, toilet-cleaning sponge, and sink-cleaning sponge, respectively.

Results of the validity of tomato juice as a simulated contaminant

The relationship between the dilution concentration and A3 is shown in Figure 1. The median A3 values

were 18, 331, 2,148, 10,741, and 108,328 RLU at concentrations of 0.00005%, 0.0005%, 0.005%, 0.05%, and 0.5%, respectively. The coefficient of determination (R^2) was 0.9896, indicating a high level of regression.

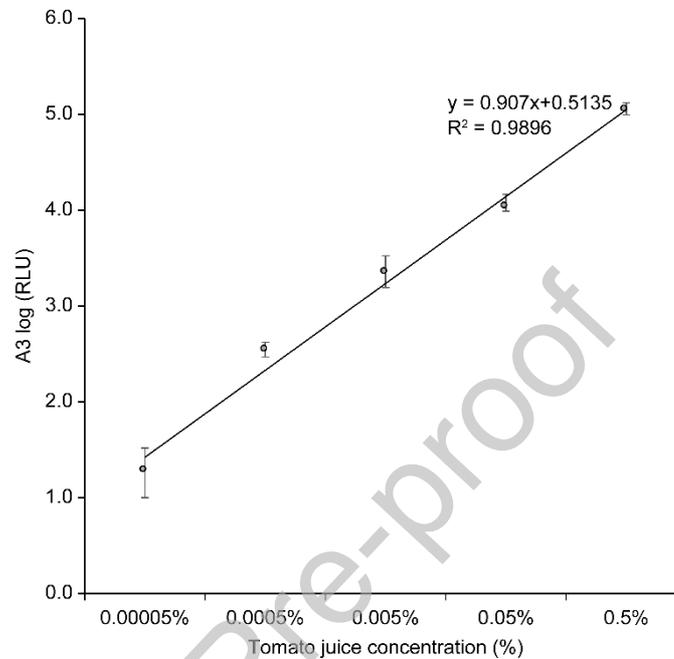


Figure 1. Relationship between tomato juice concentration and A3. Circles represent the median.

Straight lines passing near each point approximate a straight line. R^2 is the coefficient of determination.

Error bars indicate the minimum and maximum values. A3; adenosine phosphate. RLU; relative light unit (two-column image).

Exposure of each target to the simulated contaminant

Table 1 and Figure 2 show the A3 content of each test area before and after cleaning. For urinals and toilets, the A3 concentration on the cleaning gloves, gowns, and face shields increased after cleaning regardless of whether a brush or sponge was used (P for all <0.05). The A3 content on the toilet shelves and toilet paper

covers increased significantly after the sinks were cleaned and the faucets were run (P for all <0.05). A3 exposure tended to be greater in all situations wherein a brush was used than in those wherein a sponge was used.

Table 1. A3 content of each target before and after cleaning

PPE	Target	Mean	Range			95% CI			P value
Gloves									
	Before	9.3	4	–	14	7.7	–	10.9	No data
	Urinal brush	556.9	52	–	2,713	168.0	–	945.7	<0.05
	Urinal sponge	109.9	10	–	580	20.7	–	199.2	<0.05
	Toilet brush	188.1	23	–	1,676	-26.0	–	402.2	<0.05
	Toilet sponge	82.8	11	–	741	-12.9	–	178.6	<0.05
	Sink	366.1	18	–	2,008	32.0	–	700.1	<0.05
Faucet	74.6	8	–	288	26.3	–	123.0	<0.05	
Gown									
	Before	26.1	8	–	52	19.7	–	32.5	No data
	Urinal brush	149.4	57	–	335	107.3	–	191.5	<0.05
	Urinal sponge	103.8	15	–	236	62.8	–	144.8	<0.05
	Toilet brush	366.7	87	–	1,983	86.6	–	646.8	<0.05
Toilet sponge	94.1	17	–	224	69.9	–	118.2	<0.05	

	Sink	409.9	49	–	3,111	-52.5	–	872.2	<0.05
	Faucet	458.9	34	–	5,655	-281.8	–	1,199.7	<0.05
Face shield									
	Before	24.9	10	–	47	18.6	–	31.3	No data
	Urinal brush	209.1	56	–	507	133.8	–	284.5	<0.05
	Urinal sponge	83.3	6	–	259	37.5	–	129.0	<0.05
	Toilet brush	187.9	56	–	508	111.7	–	264.0	<0.05
	Toilet sponge	161.7	21	–	673	71.8	–	251.5	<0.05
	Sink	166.3	8	–	376	100.8	–	231.7	<0.05
	Faucet	154.2	10	–	580	77.9	–	230.5	<0.05
Shelf									
	Before	17.1	9	–	28	14.4	–	19.8	No data
	Toilet brush	73.2	29	–	163	53.4	–	93.0	<0.05
	Toilet sponge	79.4	5	–	337	33.9	–	125.0	<0.05
Toilet paper cover									
	Before	15.4	8	–	26	12.2	–	18.6	No data
	Toilet brush	69.6	27	–	267	36.3	–	102.8	<0.05
	Toilet sponge	58.9	10	–	150	35.8	–	82.0	<0.05

P value: based on the A3 value before cleaning. Before: Before cleaning. Urinal brush: after cleaning the urinal using a brush. Urinal sponge: after cleaning the urinal with a sponge. Toilet

brush: after cleaning the toilet with a brush. Toilet sponge: after cleaning the toilet with a sponge. Sink: after cleaning the sink with a sponge. Faucet: after running the faucet in the sink.

PPE, personal protective equipment; 95% CI, 95% confidence interval.

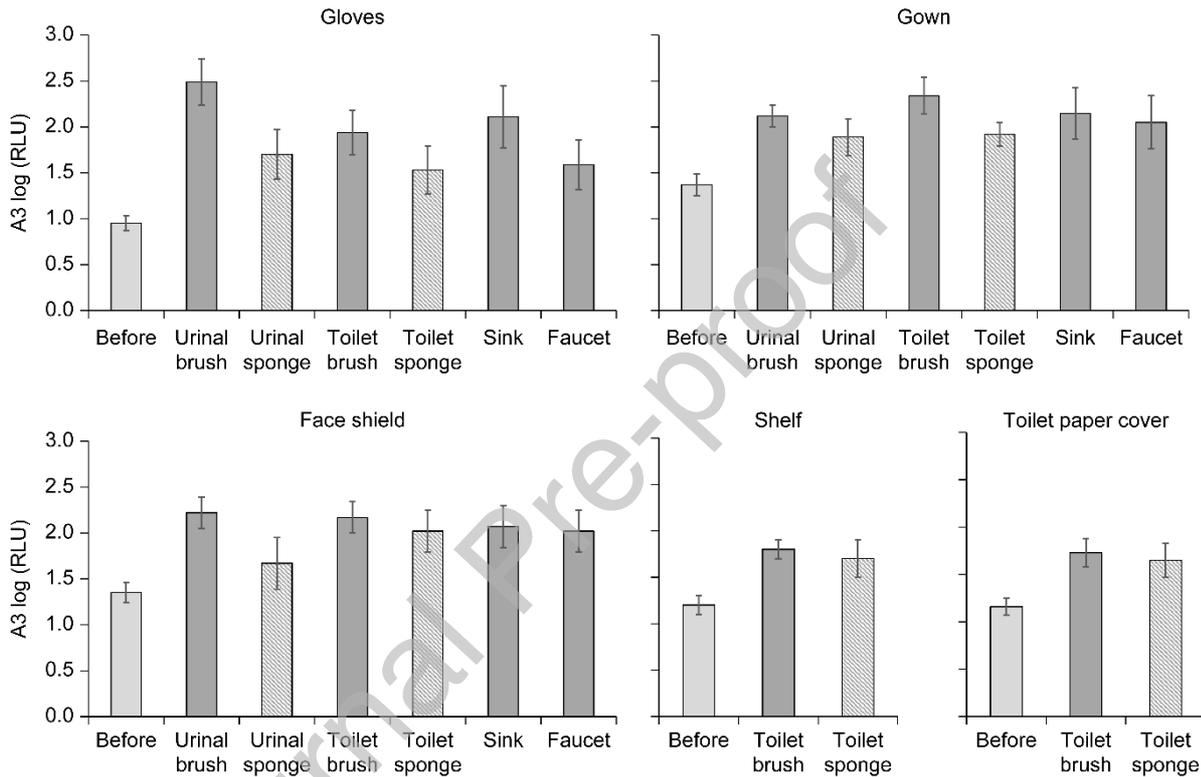


Figure 2. A3 exposure to personal protective equipment (gloves, gowns, and face shields), shelves (for small items), and toilet paper covers before and after the cleaning of urinals, toilets, and sinks (all n=16).

Error bars indicate 95% confidence intervals. Before: before cleaning. Urinal brush: after cleaning the urinal with a brush. Urinal sponge: after cleaning the urinal with a sponge. Toilet brush: after cleaning the toilet with a brush. Toilet sponge: after cleaning the toilet with a sponge. Sink: after cleaning the sink with a sponge. Faucet: after running the faucet in the sink. A3; adenosine phosphate, RLU; relative light unit

(2-column image).

Discussion

Using A3 as an indicator, this study demonstrated that cleaning hospital urinals, toilets, and sinks exposes the cleaner and the environment to contaminated water. Our findings suggest the importance of wearing PPE when cleaning and wiping down environmental surfaces.

Several outbreaks and cases of patient infection with various pathogenic microbes have been caused by hospital environments and via healthcare professionals.¹³⁻¹⁵ The workers who clean hospital bathrooms are outsourced from external companies, and as such, do not come into direct contact with the patients. However, these workers are responsible for not only cleaning the bathrooms, but for also cleaning the floors in the hospital rooms and for collecting garbage. The inconsistent use of PPE while cleaning bathrooms may predispose the patient's surroundings to contamination by pathogenic microbes.

Furthermore, urinals and toilets are contaminated by urine and feces; this poses a high risk of infection to cleaners who are likely to be exposed to the pathogenic microbes that these fixtures harbor. HBV has been detected in urine,¹⁶ and infection develops through contact with mucous membranes.¹⁷ *Clostridioides difficile* and viruses that are found in the intestines spread to the environment through bathroom cleaning with a brush,^{18,19} and it is vital to take precautions against exposure when using a brushing technique.

This study demonstrated that the A3 content on PPE and the environment tended to be high after the cleaning of toilets, urinals, or sinks. According to a 2013 study by the authors, 10,000 RLU is equivalent to approximately 10^6 colony-forming units (CFUs) of *Staphylococcus aureus* and *Pseudomonas*

aeruginosa.²⁰ Therefore, the fact that ATP was quantified to approximately 100 RLU after toilet cleaning in this study suggests that toilet cleaning exposes bacteria to the toilet environment in real-world settings.

These findings confirm that cleaners are exposed to contaminated water after cleaning bathrooms and reaffirm the necessity of PPE. The findings further suggest that the toilet environment is also contaminated during toilet cleaning, which necessitates cleaning of the surrounding environment after toilet cleaning. A comparison of different cleaning tools showed that exposure to contaminants tends to increase with the use of a brush rather than a sponge; thus, more caution is necessary after cleaning with a brush.

Splashes from the sink when running the faucet warrant attention, because antibiotic-resistant *Enterobacteriaceae* bacteria have been detected in sinks at hospitals, and these can potentially cause an outbreak.²¹ Because cleaners are exposed to water contaminated by microbes every day through bathroom cleaning, PPE must be used appropriately not only to prevent an outbreak caused by bathroom cleaning, but also to protect the cleaners themselves from infections.

Cleaners should be thoroughly trained by both the company responsible for cleaning and the medical facilities to which they are dispatched. Recent studies have recommended the use of a bundle approach (which includes a documented set of products, tools, and procedures) for environmental maintenance²²; introducing a bundle approach when cleaning is highly useful.

This study had some limitations. Tomato juice was used as the simulated contaminant; however, this is not a common practice. Nevertheless, in a pre-experimental evaluation, dilutions of tomato juice were confirmed to have a high regression with A3; thus, tomato juice was judged to be appropriate for use as a

simulated contaminant (considering its appropriate viscosity and safety). Furthermore, although it has been suggested that ATP readings are a useful tool for objectively evaluating environmental cleanliness,²³ they have not been standardized and must be used with care.²⁴ However, in the present study, ATP readings were not used as an absolute scale (e.g., to evaluate cleanliness), but as a relative scale to demonstrate the status of A3 exposure before and after cleaning. ATP readings were found to be highly useful as an objective evaluation tool. Hereafter, it is necessary to use other major indicators (such as living microbes for validating exposure) to provide a more faithful replication of exposure to microbes in bathrooms. Moreover, the procedure was not strictly standardized among the cleaners, and their knowledge that this was an experiment on exposure in bathrooms may have led them to clean with a greater intention to avoid exposure than normal. To avoid this bias, re-training on procedures was not provided; however, we cannot rule out the possibility that exposure to contaminated water would be greater in real-world settings. To assess the statistical validity of the results of this study, 70 cleaners were needed for each experiment; however, this could not be achieved owing to cost and time constraints. Although the statistical validity could not be assessed, statistical significance was detected even with fewer than 70 participants, and the exposure after cleaning tended to be higher than that in the pre-cleaning stage; this suggested that toilet cleaners and their surrounding environment are generally contaminated after cleaning the toilets.

Conclusions

Using A3 as an indicator, the exposure of cleaners to contaminated water when cleaning bathrooms was demonstrated objectively. This study is significant because its findings indicate that cleaners are likely to be easily exposed to contaminated water while cleaning restrooms, which proves the importance of using PPE during bathroom cleaning and the need to wipe down the bathroom environment after cleaning.

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Authors' contributions

Takayuki Ohishi is the chief investigator and is responsible for data analysis, organization, and writing.

Ootsuki, Kanno, Ishiyama, Kashima, Maruyama, and Fushimi provided practical guidance to cleaners. Ryo

Fushimi coordinated the study conduct, confirmed the authenticity of the raw data, and contributed to the

writing, reading, and approval of the final manuscript. Only the authors were involved in the creation of

this manuscript.

Informed consent and patient details

Cleaners who participated in this study were orally informed about the study and provided consent to

participate.

Data Statement

Data from this study is available from the corresponding author upon reasonable request.

Funding

Funding for this study was provided by Watakyu Seimoa Corporation, and the authors were responsible for the costs of writing the paper. The sponsors had no part in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Acknowledgements

We would like to thank the cleaners who are employees of WATAKYU SEIMOA corporation for their cooperation in this study.

Declaration of Competing Interest

Takayuki Ohishi has an unpaid leadership or fiduciary role in Japanese society for infection prevention and control. The co-authors declare no competing interests. None of the authors have commercial or financial interests related to the study, product, or medical devices.

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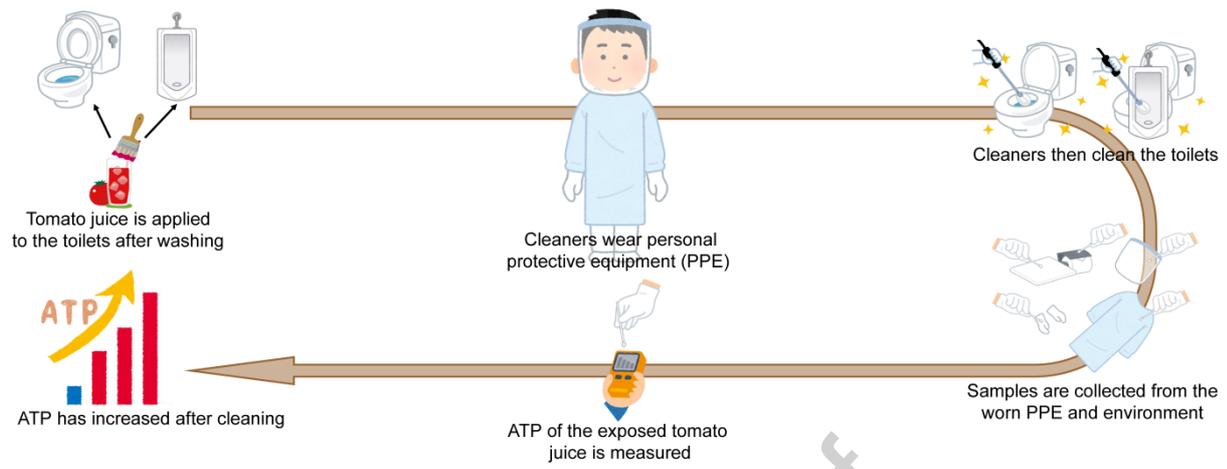
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