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Original Article

Microbiological analysis concerning the antibacterial effect of atomized Ionless® hypochlorous acid water in a nursery school environment

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ABSTRACT

Introduction: In daycare centers, infants come in close contact with each other, and contact, droplet, and mouth-to-mouth infections may occur owing to sharing of toys. Additional effective disinfection methods should be considered aside from wiping with disinfectants—including alcohol or sodium hypochlorite solution—for environmental disinfection of daycare centers. We aimed to examine the usefulness of hypochlorous acid water atomization in the effective disinfection of the classroom environment and toys at a nursery school.

Methods: Environmental cultures of the nursery and toys were prepared to evaluate the species and bacterial load and to assess the contaminated areas. *Staphylococcus aureus* petri dishes were placed at high-frequency contact sites, and hypochlorous acid water was atomized to achieve a 0.03-ppm atmospheric chlorine concentration. After the atomization, the amount of *S. aureus* bacteria on the Petri dish and the changes in bacterial count isolated from the environment and toys were evaluated.

Results: Hypochlorous acid water atomization was performed for 5 h to avoid condensation. After a 3-h atomization, ≥99.99% of *S. aureus* was eliminated on petri dishes; furthermore, a significant disinfection effect was observed on environmental bacteria at least 1 h after atomization. For rubber and textile toys, the significant disinfection effect was observed 1 h after atomization, and for plastic toys, the effect was observed 3 h after atomization.

Conclusions: Hypochlorous acid water atomization is a useful strategy to disinfect nursery school classrooms.

1. Introduction

In Japan, nursery schools are "institutions where infants requiring daycare commute on a daily basis from their guardians' place to the daycare center." In the group life at nursery schools, there are instances where the infants come in close contact with each other and use shared toys, which causes contact transmission, droplet transmission, and fecal–oral infection [1–3]. Therefore, infection control measures at nursery schools are important for the infants to lead safe and healthy lives. However, in the nursery school playroom, desks, and many toys that are commonly used by the infants, microorganism contamination was reported to be clearly present [4–7]. Therefore, disinfecting the nursery environment requires a considerable amount of time and effort to frequently clean high-frequency contact surfaces using alcohol disinfectants and sodium hypochlorite solutions; thus, the development of more effective and convenient disinfection methods is necessary. In recent years, studies have reported that using atomized hypochlorous acid water—specifically at a concentration lower than that at which inhalation toxicity manifests—might exhibit properties of space purification and extensive environmental disinfection [8,9]. However, the World Health Organization and the Centers for Disease Control and Prevention do not recommend indoor atomization with a disinfectant on

Abbreviations: SCD, Soybean Casein Digest; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt aqueous solution.

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a daily basis [10,11]. However, in Japan, hypochlorous acid water atomization is now used as a means of disinfection after the user verifies the safety and precautions for usage of each product. However, no pharmaceuticals and quasi-pharmaceuticals currently exist with confirmed product quality, efficacy, and safety based on the Japanese Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices, and that have obtained approval as disinfectants for room atomization. If the effectiveness and safety of hypochlorous acid water atomization could be demonstrated as a means of disinfection, other than wipes and cleaning, then it could be added as a new disinfection method for nursery school classrooms and toys, which we believe can provide a safer childcare environment.

Therefore, we performed atomization using Ionless® hypochlorous acid water (Nipro) in a human-free environment and investigated the disinfection effect on *Staphylococcus aureus*, which can become a problem through contact transmission and general environmental bacteria contamination taking into account the daily environmental pollution.

2. Material and methods

Ethical approval

This study was conducted after explaining and obtaining consent from the director of a nursery school and obtaining approval from the Kurume University Ethics Committee (Study No. 21229).

2.1. Study period and venue

The study was conducted from December 2021 to June 2022 with a group of infants in a nursery room of the nursery school that looks after 0–4-year-old kids (nursery room hereafter) having the room volume of approximately 154 m^3. The room was unoccupied during the experiment, and the usage of the room was avoided until the next morning with its air conditioning turned on.

2.2. Target area for sampling

In this study, 6 environmental sites that were considered high-frequency contact surfaces were selected (2 sites of shelves, 3 sites of floors, and 1 site of desk) (Fig. 1). Moreover, five types of toys (rubber, plastic, wood, paper, and textile) were selected.

2.3. Identification of bacteria isolated from the nursery environment and from toys

The entire surface of the SCD agar stamping medium (10 cm^2 of contact area) was forced onto the target flat area with a constant force, and samples were collected before atomizing hypochlorous acid water and 1, 3, and 5 h after the start of atomization. The collected SCD agar stamp medium was incubated at 37 °C for 48 h, and the resulting colonies were subjected to identification and bacterial counts.

Morphological observation and physiological characterization were performed for the observed colonies. Moreover, DNA sequences of the 16S rRNA region were obtained and the sequence was determined using an ABI PRISM 310 GeneTic Analyzer (Life Technologies). Homology searches were conducted using MicroSeq ID Analysis Software (Life Technologies) and the international nucleotide sequence database (DDBJ/EMBL/GeneBank).

2.4. Antibacterial effect of hypochlorous acid water on S. aureus

We referred to European Norm 17272 with slight modifications to evaluate the sterilizing effect on *S. aureus* in this testing. The *S. aureus* petri dishes were placed in the area of interest and collected at each time point, i.e., before hypochlorous acid water atomization, and 1, 3, and 5 h after starting atomization.

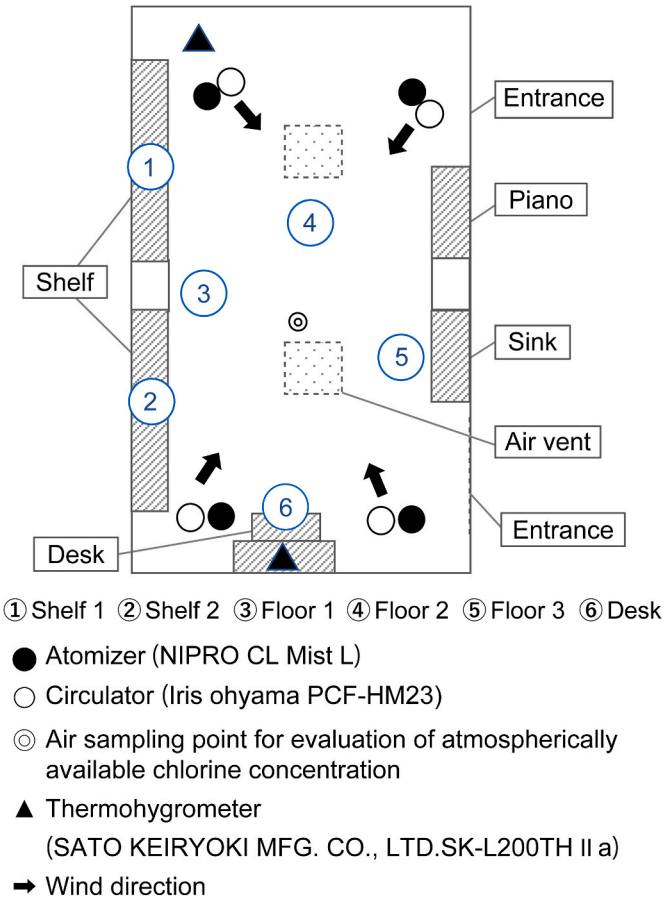

Fig. 1. Sampling and Device allocation.

For the *S. aureus* petri dishes, we used the NBRC strain (NBRC12732). Strain was suspended in saline and inoculated onto SCD agar medium (Nissui Pharmaceutical), followed by incubation for 24 h at 37 °C in aerobic conditions. The resulting colonies were resuspended in saline and adjusted to a 10 MacFarland. 10 μL of the bacterial suspension was dropped on a plastic Petri dish followed by drying, and it was used for further analysis.

Recovery liquid at 2 mL (0.03% sodium thiosulfate aqueous solution) was added to the Petri dish, and the bacteria on it were suspended to calculate the viable bacterial count. Based on the suspension, 1:10 dilution series was prepared with saline, and 0.1 mL of each dilution was smeared onto the SCD agar medium. The number of resulting colonies was counted after incubation for 48 h at 37 °C. Additionally, the sample, which was packaged into an air-tight container during the test period, was prepared and evaluated according to its viable bacterial count in the same manner. The sample was treated as a control, i.e., it was not exposed to hypochlorous acid water. The logarithmic decrease by atomization was determined based on the viable bacterial count of the control sample.

We performed Dunnett's multiple comparison tests for the viable bacterial count on the environmental surfaces and toys in the nursery room. A p-value of ≤ 0.05 was regarded as significant in all tests.

2.5. Hypochlorous acid water atomizing procedure

The Ionless® hypochlorous acid water used for atomization was prepared by electrolysis of sodium chloride aqueous solution using a two-diaphragm three-chamber-type electrolyzed water generator (Nipro) followed by purification with reverse osmosis membrane filtration. The chlorine concentration and pH were set at 30–50 ppm and 5.0–6.5, respectively.

In the nursery room, four ultrasonic atomizers (HM-201, Seiko Giken) filled with hypochlorous acid water, four circulators (PCF-HM23, Iris Ohyama), and two hygrothermographs (SK-L200THIIα, Sato Keiryoki MFG) were set up (Fig. 1). One each of ultrasonic atomizer and circulator were allocated in each corner of the room, and their direction was aimed toward the center of the room. For atomization of the toys, we set up a specific booth (Nipro, approximately 500 L) and installed one ultrasonic atomizer filled with hypochlorous acid water at the bottom, and one humidifier with propeller as a stirring fan (desktop humidifier, Aochy), and one hygrothermograph (Fig. 2). Thereafter, toys were placed with cages and cloth nets in the booth. The atomization rate was adjusted to attain the atmospherically available chlorine concentration of 0.03 ppm with reference to the 0.046 (±0.013) ppm of that at an indoor swimming pool [12].

The available chlorine concentration of hypochlorous acid water was evaluated using a residual chlorine concentration meter (AQ-202, SIBATA SCIENTIFIC TECHNOLOGY LTD.). Furthermore, the impinger method with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt aqueous solution (ABTS) was used to evaluate atmospherically available chlorine concentration. Room air (40 L) was collected into ABTS at the center of the room (Fig. 1) at 5 h after starting atomization, and the available chlorine concentration of the solution was evaluated via colorimetric methods. The atmospherically available chlorine concentration was calculated according to the solution concentration. To verify the accuracy of this evaluation method, we confirmed that the measured value was equal to the theoretical value in terms of the atmospherically available chlorine concentration when 20 L of 1-ppm hypochlorous acid water was left overnight in a 144-L plastic box and the box was filled with gaseous chlorine.

Fig. 2. Materials used for toy atomization.

3. Results

3.1. Bacterial species detected from environment and toys

Micrococcus luteus [ATCC4698] (99.75% Match), *Micrococcus aloeverae* [accession number: KF524364] (99.50% Match), *Micrococcus yunnanensis* [accession number: FJ214355] (99.26% and 99.93% Match), *Bacillus subtilis* [accession number: AB04261] (99.89% and 99.67% Match), *Brevibacterium epidermidis* [ATCC35514] (99.72% Match), and *Brevibacterium luteolum* [DSM15022] (99.76% Match). The bacterial abundance is shown in Table 1 for each sampling site.

3.2. Environmental bacterial load

The environmental bacterial count in the nursery room is presented in Fig. 3. The site with the highest bacterial count before atomization was ⑥ desk at 95 CFU/stamp, followed by ① shelf 1 at 38 CFU/stamp and ⑤ floor 3 at 25 CFU/stamp. Compared to before atomization, sites with a significant decreasing tendency in bacterial count at 1 h after atomization were ① shelf 1 ($p < 0.001$), ② shelf 2 ($p < 0.001$), ④ floor 2 ($p < 0.001$) and ⑥ desk ($p < 0.001$). However, a significant decrease in the viable bacterial count was not confirmed at sites ③ floor 1 and ⑤ floor 3.

The environmental bacterial count on the toys is presented in Fig. 4. The material with the highest bacterial count (median) before atomization was rubber at 35 CFU/stamp, followed by plastic at 16 CFU/stamp, and textile at 11 CFU/stamp. Compared to before atomization, materials with a significant decreasing tendency in the bacterial count at 1–3 h after atomization included rubber ($p < 0.01$), plastic ($p < 0.05$), and textile ($p < 0.01$). However, a significant decrease in the viable bacterial count was not confirmed for the materials of wood and paper.

3.3. Disinfection effect on the S. aureus Petri dish set up in the nursery room

A disinfection ratio of ≥99% (bacterial count decrease of ≥2 log CFU/mL) after 1 h of atomization and ≥99.99% (≥4 log CFU/mL) reaching the limit of detection after 3 h of atomization was observed in all sites (Fig. 5).

4. Discussion

In this study, on the environmental surfaces and toys in the nursery room, we detected the *Micrococcus* and *Brevibacterium* groups, which are widely present on humans and in the environment, as well as the *Bacillus* group, which has spores that are resistant to disinfectants. Conversely, *S. aureus*, which is thought to adhere to the fingers, was not detected. The facilities involved in this study were regularly cleaned as per the cleaning guidelines for childcare facilities in Japan, and it was inferred that the cleaning procedure might be related to the reason why *S. aureus* was not detected in this study. However, we observed a higher viable bacterial count of environmental bacteria in the nursery room for the desks and shelves that are particularly frequently touched by the nursery teachers and many infants. While the highest bacterial count was observed in site ⑥ desk, we found a significant decrease after 1-h atomization, and for sites ① shelf 1 and ② shelf 2 also, a significant disinfecting effect was observed. However, regarding the floor, at site ⑤ floor 3, which is used by many infants such as when washing their hands with running water and brushing their teeth, the pre-atomization bacterial count was double that of other floor sites (median 25); however, even 5 h after atomization, no significant disinfection was observed. The concentration of hypochlorous acid water may have decreased in ⑤

Table 1
Colony counts (CFU) in the environment and toys before hypochlorous acid water atomization.

Sampling	Nursery room						Toys				
	Shelf 1	Shelf 2	Floor 1	Floor 2	Floor 3	Desk	Rubber	Plastic	Wood	Paper	Textile
1	38	18	10	13	24	183	9	3	4	4	16
2	36	25	26	7	27	153	23	16	22	7	11
3	60	20	17	16	26	48	59	20	1	7	0
4	99	20	4	11	25	95	59	6	0	20	12
5	24	17	1	7	11	61	35	68	2	93	9
Mean value	51.4	20	11.6	10.8	22.6	108	37	22.6	5.8	26.2	9.6
Median (interquartile range)	38 (36–60)	20 (18–20)	10 (4–17)	11 (7–13)	25 (24–26)	95 (61–153)	35 (23–59)	16 (6–20)	2 (1–4)	7 (7–20)	11 (9–12)

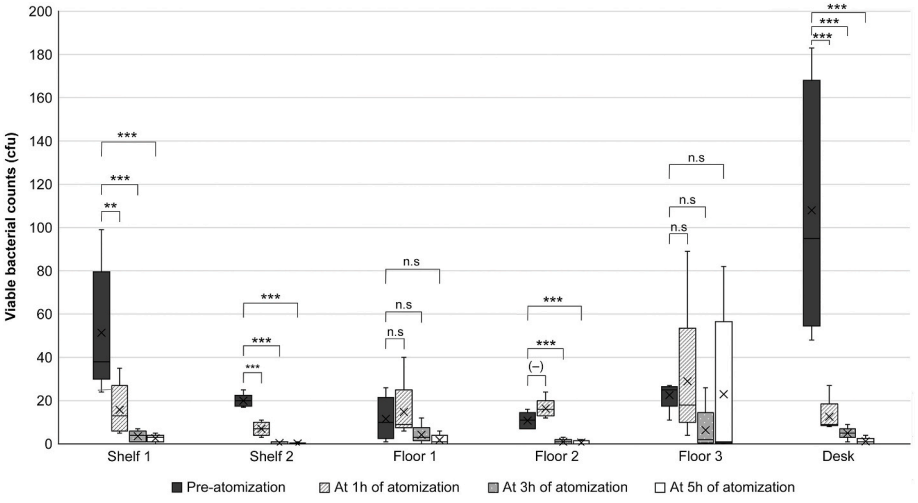

Fig. 3. Environmental bacterial count in the nursery room.

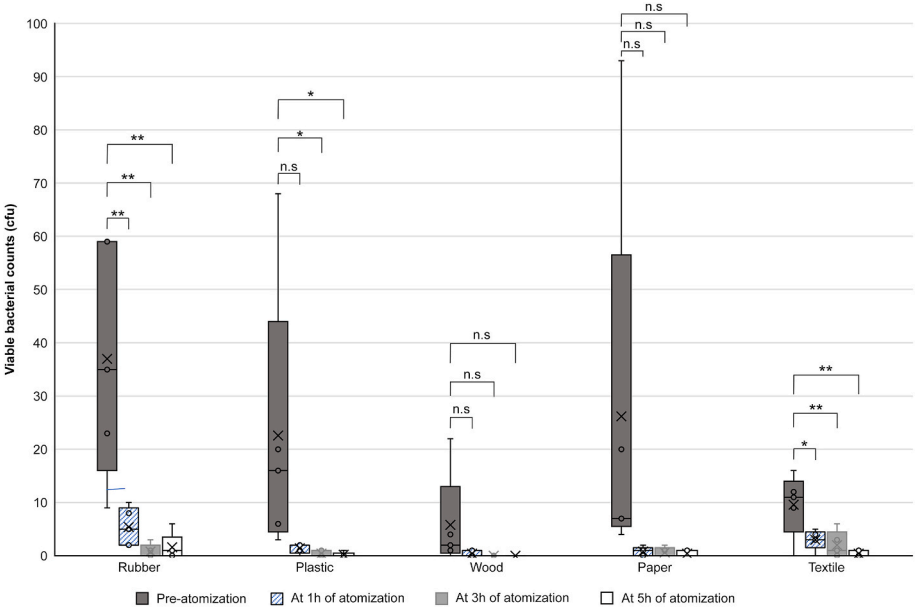

Fig. 4. The environmental bacterial count on the toys.

floor 3 because it is located in front of a sink and is a wet environment due to splashing, etc., and also because it is under conditions where environmental bacteria easily adhere [13]. We believe that in the present experiment, significant disinfection could not be achieved with the atomization conditions that were set. It is conceivable that the infants include some individuals who pass their time in the nursery room barefoot, and because the *Bacillus* group with spore-forming ability was detected, when a sporicidal effect is expected with hypochlorous acid water atomization, we believe that the use of hypochlorous acid water at a higher concentration should be considered. However, at the present

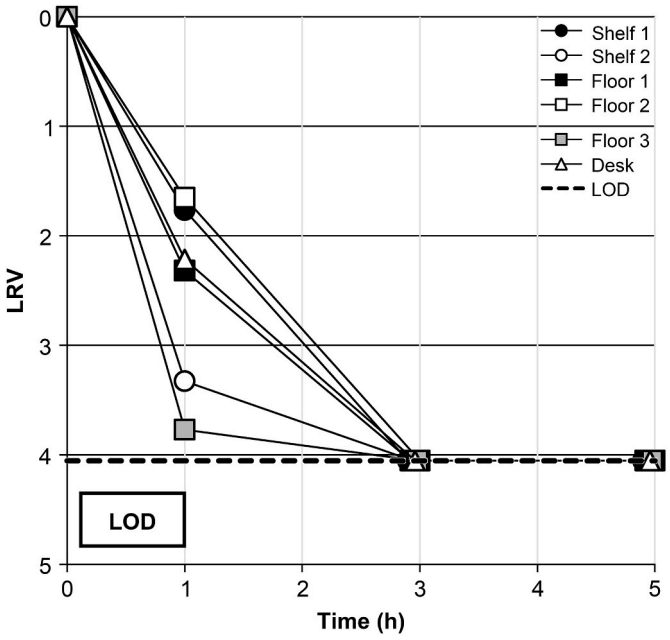

Fig. 5. Disinfection rate of *S. aureus* in the nursery room.

stage, wiping the floor with wet wipes is a convenient method to physically remove dirt. Furthermore, to manage spores with resistance to alcohol disinfection, cleaning the floor with disinfectants, including diluted sodium hypochlorite, after wiping with a wet wipe is recommended.

When cleaning, sodium hypochlorite is diluted to a concentration of 100–1,000 ppm, and to avoid inhalation toxicity caused by chlorine gas, ventilation of the room needs to be adequately performed [10]. It has been pointed out that both sodium hypochlorite and hypochlorous acid water produce chlorine, which, at particularly high concentrations, can induce localized toxicity in the skin, ocular-mucous membrane, and lung function [14]. With regard to the safety of hypochlorous acid water, it has been found that in the event of hypochlorous acid water atomization at a liquid concentration of below 1,000 ppm, there is little possibility of inducing localized toxicity [8]. In this study, we conducted our experiment in a human-free environment with 30–50 ppm of the hypochlorous acid water, and the atmospherically available chlorine concentration was approximately 0.03 ppm, thereby ensuring safety. Furthermore, in Japan, it has been reported that the corrosive properties of Ionless® hypochlorous acid water atomization is equal to or less than that of tap water, which we believe has little impact of corrosion and damage on the nursery room environment.

Furthermore, although *S. aureus* was not detected from the nursery room environment, *S. aureus* is found in the nasal cavity of approximately 30% of healthy adults, and it is the most commonly detected pathogenic microorganism in the nose and throat of healthy children [15]. *S. aureus* has been found to be highly pathogenic, cause food poisoning, and relatively serious infections such as bacteremia and skin infection. In particular, incidences of skin infection caused by community-acquired Methicillin-resistant *S. aureus* in healthy children has been reported [16]. *S. aureus* survives for long periods on dry surfaces once it adheres to the environment; thus, the routine cleaning of floors and toy surfaces frequently touched and/or licked by infants is considered an essential countermeasure [17]. In this study, significant disinfecting effects were confirmed for environmental bacteria and *S. aureus* on shelves, floors, and desks in the nursery room within 1 h after atomization.

Previous studies have demonstrated that toys made of rubber, plastic, and wood have a high level of contamination [4–7], and in this study also, the bacterial count prior to atomization was the highest (median 35) for rubber toys, and bacterial contamination was observed on toys made of other materials. Therefore, it is difficult to remove toys of any material from the nursery room. Reportedly, wooden toys have a high level of contamination before washing, which persists after cleaning by wiping with a damp cloth [6]. Therefore, for hygiene control of toys, in addition to washing and wiping, the introduction of a more effective disinfection method is needed. With the present atomization test on toys, significant disinfecting effects were observed on rubber, plastic, and textile, suggesting that the use of hypochlorous acid water atomization as a substitute for the long hours of washing and wiping toys by daycare center staff will enable more efficient disinfection of toys. In this study, no significant differences were observed between wood and paper; however, a clear downward trend was observed in terms of the number of colonies, which was presumably due to the small sample size. Furthermore, as seen in our experiment, it was thought that maintaining atomization operations in the specific chamber can prevent the impact of localized toxicity on infants and staff.

In the present experiment, we performed atomization for frequently touched surfaces and toys in the nursery room with atmospherically available chlorine concentration maintained at 0.03 ppm and confirmed its effectiveness; however, we believe that further examination is needed in the future in terms of atomization concentration, duration, frequency, and placement of atomizers to manage spores in order to demonstrate the criteria for greater safety and higher effectiveness.

Reportedly, hypochlorous acid is easily disrupted when it comes in contact with organic matter [18]. Therefore, the indicator bacterium (*S. aureus*) suspension did not contain any organic matter other than *S. aureus* itself to evaluate the maximum disinfection potency of hypochlorous acid water atomization. Further studies are required to clarify which interference materials, including BSA or artificial saliva, are included into indicator bacterium suspension and examine the disinfection potency under harsher conditions.

5. Conclusions

We detected many microorganisms from the environment and toys of a nursery room. In this study, we performed hypochlorous acid water atomization for a nursery room and toys to achieve more effective environmental disinfection, and as a result, it was suggested that hypochlorous acid water atomization is widely effective for the disinfection of *S. aureus* and environmental bacteria.

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Authorship statement

Miura M, Gotoh K, Kawamura N and Mihashi M designed this study. Miura M, Fuketa H, Tomoike H and Kawamura N analyzed the data. Miura M, Tanamachi C and Gotoh K drafted the manuscript. All other authors collected the data and reviewed the manuscript. All authors have approved the submission of this version of the manuscript.

All authors meet the ICMJE authorship criteria.

Declaration of competing interest

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References

[1] Kokuryo T, Kinoshita K, Yoshio K, Fukumoto C, Nanbu T. Effect of intervention by the infection control team on an outbreak of epidemic keratoconjunctivitis in a hospital day-care center. Jpn J Infect Prev Control 2014;29:293–8. https://doi.org/10.4058/jsei.29.293.

[2] Stéphanie Wesley M, Sophie Q, Chloé W, Charlotte M, Dimitri V, et al. Outbreak of invasive meningococcal disease caused by a meningococcus serogroup B in a nursery school, Wallonia, Belgium. Euro Surveill 2022 2018;27:8–15. https://pubmed.ncbi.nlm.nih.gov/35241214/.

[3] Higuchi Y, Meno Y. An approach to infection-control measures at nursery school A: effect and issues. Bull Seinan Jo Gakuin Univ 2015;24:1–9.

[4] Ibfelt T, Engelund EH, Permin A, Madsen JS, Schultz AC, Andersen LP. Presence of pathogenic bacteria and viruses in the daycare environment. J Environ Health 2015;78:24–9.

[5] Hohashi N, Ikehara E, Omori K. A survey on general bacterial contamination of infant toys covering pediatric outpatient facilities, nurseries and homes. Jpn J Infect Prev Control 2005;20:105–9.

[6] Yoshikawa H, Wakimoto H, Yano H. Current status and issues in the hygienic management of toys for infants at nurseries. Jpn J Infect Prev Control 2022;37:41–7. https://doi.org/10.4058/jsei.37.41.

[7] McKay I, Gillespie TA. Bacterial contamination of children's toys used in a general practitioner's surgery. Scot Med J 2000;45(1):12–3.

[8] Fuketa H, Shinkai N, Takada N, Saitoh T, Kawamura N. Systemic and local toxicities of inhaled administered hypochlorous acid water in rats. Jpn Pharmacol Ther 2021;49:41–52.

[9] Kawamura N, Fuketa H, Saitoh T, shinkai N, Mitsuhashi M, Shoyama T, et al. Characterization of a new type of hypochlorous acid solution: effect on aerially sprayed bacteria and phage, in vitro SARS-CoV-2, and aerial-spraying safety. The J Funct Water 2020;15:1–4.

[10] The World Health Organization. COVID-19: infection prevention and control/WASH. https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-covid-19/;. [Accessed 24 October 2022].

[11] Centers for Disease Control and Prevention. Guideline for disinfection and sterilization in healthcare facilities, vols. 87–8; 2008. Update. May 2019, https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines-H.pdf. [Accessed 9 March 2023].

[12] Patel K, Hon C-Y. Evaluating if airborne chemical levels in indoor swimming pools are influenced by type of water treatment. Environ Health Rev [review 2020;63:48–53. https://doi.org/10.5864/d2020-014.

[13] Uchida M. Effects of drying on the amount of contaminated bacteria of the hand-washing sink. J Healthc-Assoc Infect 2019;12:15–24.

[14] European Union risk assessment report chlorine (CAS no: 7782-50-5): HUMAN HEALTH. Available from: https://docslib.org/doc/5677502/european-union-risk-assessment-report.

[15] Oishi T, Muratani T, Tanaka T, Sato M, Urara K, Ouchi K, et al. Study of normal flora in the pharynx of healthy children. Jpn J Infect Dis 2021;74:450–7. https://doi.org/10.7883/yoken.JJID.2020.824.

[16] Yamamoto T, Nishiyama A, Takano T, Yabe S, Higuchi W, Razvina O, et al. Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance. J Infect Chemother 2010;16:225–54. https://doi.org/10.1007/s10156-010-0045-9.

[17] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:130. https://doi.org/10.1186/1471-2334-6-130.

[18] Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 2006;11:147–57.