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Major Article

Impacts of lid closure during toilet flushing and of toilet bowl cleaning on viral contamination of surfaces in United States restrooms



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Key Words:

Aerosol generation
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Environmental surface hygiene intervention
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Background: Viral aerosols generated during toilet flushing represent a potential route of pathogen transmission. The goal of this study was to determine the impact of toilet lid closure prior to flushing on the generation of viral aerosols and cross-contamination of restroom fomites.

Methods: A surrogate for human enteric viruses (bacteriophage MS2) was added to household and public toilet bowls and flushed. The resulting viral contamination of the toilet and other restroom surfaces was then determined.

Results: After flushing the inoculated toilets, toilet seat bottoms averaged $> 10^7$ PFU/100 cm². Viral contamination of restroom surfaces did not depend on toilet lid position (up or down). After toilet bowls were cleaned using a bowl brush with or without a commercial product (hydrochloric acid), a $> 4 \log_{10}$ ($> 99.99\%$) reduction in contamination of the toilet bowl water was observed versus no product. Bowl brush contamination was reduced by $1.6 \log_{10}$ (97.64%) when the product was used versus no product.

Conclusions: These results demonstrate that closing the toilet lid prior to flushing does not mitigate the risk of contaminating bathroom surfaces and that disinfection of all restroom surfaces (ie, toilet rim, floors) may be necessary after flushing or after toilet brush used for the reduction of virus cross-contamination.

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BACKGROUND

The aerosolization of viruses from toilets during flushing has received a lot of attention in recent years, because of concerns with emerging viruses and the potential for viral contamination of restroom fomites following toilet use.^{1–4} Research has demonstrated that people with COVID-19, even those who are asymptomatic, excrete severe acute respiratory syndrome coronavirus (SARS-CoV-2) in fecal matter and other excretions.^{5–9} Viruses contaminating urine and feces can be aerosolized in building restrooms during toilet flushing.^{5–9} Viruses

in droplets and aerosols can contaminate restroom surfaces.^{7–10} Wastewater-based epidemiology has demonstrated that almost any human pathogen can be detected in domestic wastewater including SARS-CoV-2.¹¹ It has been suggested that if aerosols are generated from such wastewater these may contribute to human pathogen exposure and environmental transmission.^{6–8} It has been shown that viral aerosols and droplet nuclei can persist for extended periods of time, can travel on air currents, and deposit on surfaces, contributing to longer-range transport, environmental (indirect) transmission, and dissemination of infectious viruses.^{8,9}

Early studies investigating the aerosolization of microbes during toilet flushing focused on bacteria suspended in fecal matter¹⁰ or simulated fecal matter.¹² In addition, the samples evaluated in previous studies have been limited to toilet surfaces. The Best et al study¹⁰ used fecal matter seeded with *Clostridium difficile* spores to evaluate aerosolization of bacteria during toilet flushing. Those investigators also studied toilet lid position (up and down) in a health care facility, and their results indicated a reduction in large droplet

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aerosolization of *C difficile* spores when the toilet lid was closed prior to flushing.¹⁰ The Barker and Jones study¹² used agar chunks seeded with MS2 (bacteriophage) and *Serratia marcescens* (bacteria) to simulate aerosolization of fecal matter during toilet flushing and disinfecting. A reduction in MS2 and *Serratia* contamination was obtained after each flush (3 times). *Serratia* contamination also was reduced after toilet bowl disinfection; however, MS2 was not assessed following use of a disinfectant.

Aerosol generation during toilet flushing depends on the type of toilet, the toilet bowl radius, and water flow characteristics. Contributors to pathogen concentrations in the generated aerosols include the water supply and the presence of contaminated urine, fecal matter, or vomit. Impacts of the aerosols depend on restroom area and ventilation.¹³

Toilet designs differ globally. Public and private toilets in the United States each create turbulent water flow that may propel and aerosolize any viruses present in the bowl water. Such toilet aerosol plumes may reach more than 5 feet from the toilet and may contaminate restroom surfaces (fomites).¹³ Sassi et al³ documented extensive contamination of public restroom surfaces, including toilet seats, floors, and other surfaces, using bacteriophage MS2. Public toilets in the United States use in-line water pressure and larger diameter water supply piping, which result in a higher flow of water into the toilet bowl than observed in household toilets. However, household toilets are siphonic, relying on narrow trapways and channels to produce suction, creating turbulence that can generate significant levels of aerosols.⁷ Thus, both public and household toilets in the United States are assumed to have high-speed airflow generated by flushing that might expel aerosolized pathogens from the toilet bowl water to regions high in the air above the toilet, allowing viruses to spread indoors and potentially disseminate any viruses present in the bowl water.⁷

The potential beneficial impact of closing the toilet lid during flushing has been debated, and lid closure prior to flushing has been a general recommendation for reducing the degree of surface contamination in household restrooms.^{9,12,14,15} Unfortunately, the possible benefit of toilet lid closure during flushing for reducing viral contamination of restroom surfaces has not been demonstrated empirically. In addition, other activities in the restroom, such as cleaning the toilet bowl, may result in the generation of aerosols.¹⁵ The goal of this study was to determine if closing the toilet lid prior to flushing actually reduces the viral contamination of household restrooms, as has been suggested, and if cleaning of the toilet bowl has an impact. This information will inform the potential benefit of these risk mitigation strategies for use as environmental surface hygiene interventions during future viral outbreaks, including those associated with enteric viruses such as noroviruses.

METHODS

Escherichia coli (ATCC 15597) and coliphage MS2 (*Emesvirus zinderi*, ATCC 15597-B1) were obtained from the American Type Culture Collection (ATCC). The bacteriophage was selected as a model for human pathogenic enteric viruses. MS2 was prepared as previously described with minor modifications.¹⁶ Since it is not a human or animal pathogen no protective clothing is required. Briefly, 0.1 mL of MS2 suspension and 0.3 mL of a log-phase *E coli* (host bacterium) culture were added to top agar, and the agar was melted and maintained at 55 °C. The inoculated top agar was mixed and poured over Tryptic Soy Agar (Difco). The solidified agar overlay plates were then inverted and incubated at 37 °C for 24 hours. Tryptic Soy Broth (TSB) (Difco) was then added to each plate and maintained at room temperature for 2 hours. Phosphate-buffered saline (PBS) or TSB (MP Biomedicals) eluates were aspirated and centrifuged to remove bacterial debris, after which the supernatants were filtered through

0.22- μ m-pore-size Steriflip filters (Millipore Sigma). The MS2 stock was stored at 4 °C until needed.

Two types of toilets of United States design were used. The public toilet was located in a public restroom serving an office building. The public toilet was tankless, using water line pressure for flushing, as is typical of United States public toilets. The seats were U-shaped with a gap in the front, as required by uniform plumbing codes. The toilet was contained in a stall with a 0.61 × 1.52 m door. The home toilet was a typical siphonic toilet with a tank and no gap in the center of the seat. It was located in a residential home with a restroom measuring 2.4 × 3 m.

Before seeding of the United States toilet bowls with MS2, a 5-mL sample of the toilet bowl water was collected and placed in Lethen broth (3M Corporation) to neutralize any residual disinfectant present in the tap water that might have been present. This sample was tested to determine if any bacteriophage might have been present that could infect the *E coli* host cells used in this study. No bacteriophage were detected in any of the toilet bowl water samples taken prior to addition of the MS2. A solution of 100 mL of sterile TSB was then added to stimulate organic matter during a bowel movement and 10 mL of MS2 was added and mixed with a 5-mL pipette. The toilet was then flushed and, after waiting one minute, 100-cm² areas of the restroom surfaces were sampled with a sponge stick containing Lethen broth to neutralize any residual disinfectant. The liquid was squeezed from the sponge and the volume was recorded. Samples were diluted in PBS, if needed, and assayed by the double agar overlay method. The numbers of phage plaques were counted after 24 hours.

The United States restroom sites sampled during the evaluation of toilet aerosols and lid closure included the toilet bowl water, top and bottom of the toilet seat and lid, 3 locations on the floor in front and right and left sides of the toilet, and walls on the right and left sides (Fig 1). Each site on the floor was 30 cm distant from the rim of the toilet bowl. One site was situated directly in front of the toilet, and the others to the right and left of the toilet (Fig 1). The wall samples were 60 cm to the right (location 1) and left (location 2) of the toilet bowl rim and 95 cm from the floor. All samples were returned to the laboratory in an ice chest containing ice packs. Between sampling events, the restroom surfaces and the toilet bowl

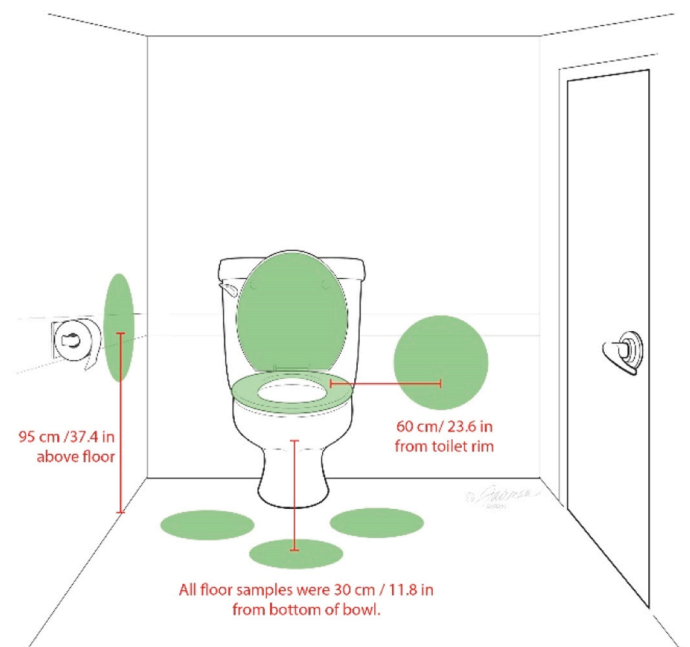


Fig. 1. Schematic diagram of restroom sampling sites for toilet flushing aerosol and lid closure experiment.

Table 1
Impact of toilet flushing and lid closure on restroom contamination by bacteriophage MS2

Test condition (MS2 dose)	MS2 contamination of toilet surfaces (\log_{10} PFU; mean \pm standard deviation, n = 3–13 replicates)				
	Toilet bowl water*	Toilet lid top**	Toilet lid bottom**	Toilet seat top**	Toilet seat bottom**
Home toilet lid down (10^{10} PFU MS2)	10.57 \pm 10.4	1.66 \pm 1.18	1.70 \pm 0.57	5.39 \pm 5.75	4.22 \pm 4.61
Home toilet lid up (10^{14} PFU MS2)	14.12 \pm 14.06	1.70 \pm 0.40	1.70 \pm 0.70	6.80 \pm 8.00	7.85 \pm 8.00
Home toilet lid down (10^{14} PFU MS2)	14.41 \pm 13.94	1.72 \pm 0.70	1.65 \pm 1.01	5.62 \pm 5.77	7.26 \pm 7.48
Public toilet—no lid (10^{11} PFU)	11.16 \pm 11.34	NA	NA	7.51 \pm 7.72	7.26 \pm 7.65

NA, not applicable, PFU, plaque forming units.

* MS2 in the 2.8-L volume of water in the bowl.

** MS2 per 100 cm² sampled surface.

were disinfected either with a commercially available toilet bowl cleaner or hydrogen peroxide wipes.

A one-tailed t test was used to analyze the resulting data. Samples for the cleaning and toilet brush evaluation were collected in the restroom from the same sites used for the toilet aerosol and lid closure study, except that no wall or front toilet floor samples were collected in the cleaning experiment. Toilet lid samples in the cleaning study were modified to include a 100-cm² area located on the bottom center of the toilet lid, due to low levels of contamination observed in the toilet aerosol and lid closure study.

Additional samples collected during the cleaning study included the toilet rim, rug in front of the toilet, toilet bowl water after cleaning with brush, toilet bowl brush, and toilet bowl brush caddy. The caddy and toilet rim samples were collected using D/E sponge sample sticks (3M Corporation) and the procedure described above. The rug samples were collected by rolling a lint roller over the entire rug. Rug samples were collected by placing the lint roller sheet into a 250-mL sterile bottle, adding 10 mL of PBS, and vortexing for 1 minute. The toilet bowl brush samples were collected by submerging the entire brush bristle top into a sterile 500-mL bottle. The handle was then removed from the brush and 20 mL of PBS was added to the bottle containing the intact toilet brush head. The bottle with the towel brush was then vortexed for 1 minute, and the resulting liquid was removed, neutralized with 5 mL of D/E Neutralizing Broth (EMD), and assayed per the MS2 detection procedure described above. Toilet cleaning was performed, after the addition of the MS2 phage suspended in 100 mL of TSB and mixing with a pipette, by the addition of 5 mL of Lysol Power Toilet Bowl Cleaner (Reckitt) which contains 12% hydrochloric acid as the active microbicidal ingredient, following the manufacturer's instructions and using a contact time of 10 minutes.

RESULTS

Contamination of public and household toilet surfaces after flushing

Two dose levels (10^{10} – 10^{14} PFU) of MS2 (used as a surrogate for nonenveloped enteric viruses such as noroviruses) were added to United States public and private (household) toilet bowl water. This

resulted in different phage concentrations per mL, depending on the water volumes in the toilet bowls seeded. United States household toilets have less water volume in the bowls (2.82 L) than public toilets (3.04 L). Household toilet bowls received 10^{10} or 10^{14} PFU of MS2 phage, and public toilet bowls received 10^{11} PFU. United States public toilets do not have lids, so lid closure could not be assessed in that case. However, both the tops and bottoms of the public toilet seats exhibited a greater degree of MS2 contamination compared to household toilet seats, despite lower initial phage concentration in the bowl water in the former case (Table 1).

Contamination of household restroom surfaces following toilet flushing

Phage contamination of the United States household toilet surfaces evaluated was not statistically different after flushing, regardless of toilet lid position (up or down) (Table 1). There also was no statistical difference found between toilet seat bottom or top contamination, regardless of toilet lid position prior to flushing (up or down) (Table 1). However, when the toilet lid was closed (down), contamination of the toilet seat bottom in toilets receiving the high-dose MS2 (10^{14} PFU) was 3 \log_{10} (99.9%) higher than in toilets receiving low-dose MS2 (10^{10} PFU), reflecting the increase in the dose of MS2 added in the former case. The increase in toilet seat bottom contamination observed was not detected on the top of the toilet seat or the lid. Surprisingly, MS2 contamination of the bottom or top of the toilet lid was consistently low, regardless of lid position prior to flushing.

Contamination of household restroom surfaces by MS2 was also evaluated after flushing with the toilet lid up and down (Table 2). The floor and the walls of the restroom were contaminated after toilet flushing, but no significant differences were observed between the contamination occurring with lid position up or down. Wall contamination was minimal, regardless of lid position, and there was no significant difference in contamination level between the surfaces assessed, but data indicated that the trajectory of the aerosol plume contamination may have changed (Table 2). Floor contamination was not found to be reduced consistently by toilet lid closure prior to flushing. However, averages for the left side and front floor contamination levels were higher if the toilet lid was

Table 2
Impact of home toilet lid position prior to flushing on bacteriophage MS2 contamination of floor and restroom walls

Test condition (MS2 dose)	Contamination of restroom surfaces by MS2 (\log_{10} PFU; mean \pm standard deviation, n = 3 replicates)					
	Toilet bowl water*	Floor to left of toilet**	Floor to right of toilet**	Floor in front of toilet**	Wall to right of toilet**	Wall to left of toilet**
Lid up (10^{14} PFU MS2)	11.02 \pm 10.77	3.64 \pm 3.19	6.23 \pm 6.47	4.25 \pm 4.48	1.77 \pm 1.97	2.31 \pm 2.54
Lid down (10^{14} PFU MS2)	11.23 \pm 10.79	5.11 \pm 5.28	4.81 \pm 4.95	5.17 \pm 5.40	1.86 \pm 1.78	1.61 \pm 1.81

PFU, plaque forming units

* MS2 in the 2.8-L volume of water in the bowl.

** MS2 per 100 cm² sampled surface.

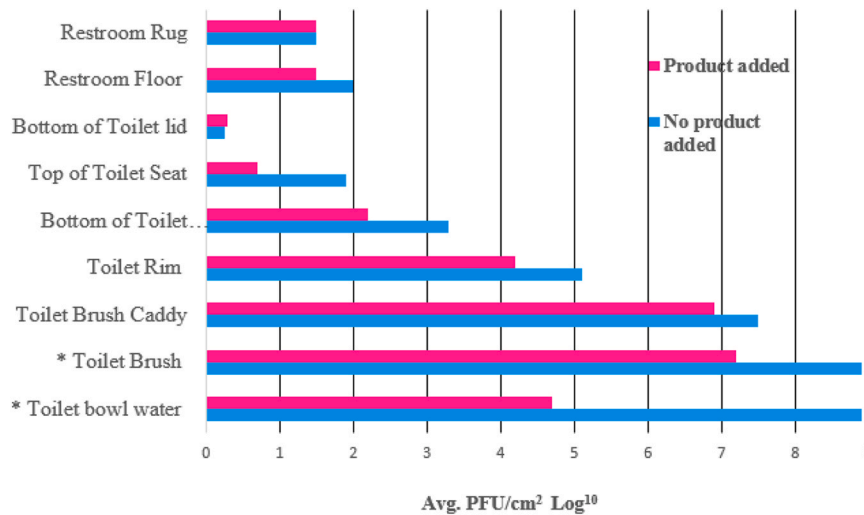


Fig. 2. MS2 restroom fomite/surface contamination before (no product added) and after (product added) toilet bowl cleaning. Cleaning intervention included using a disinfectant (hydrochloric acid) and a towel bowl brush. *Reductions in MS2 contamination after cleaning with the disinfectant product were statistically significant for toilet bowl water ($P = .00079$) and toilet bowl brush ($P = .0013$).

closed prior to flushing, while right floor contamination levels were lower in that case, see [Table 2](#).

Contamination of toilet surfaces during toilet cleaning

Additional investigation of phage contamination occurring during cleaning of household toilets with a toilet brush, prior to and following use of a disinfectant product, was conducted with the lid kept up. The results revealed similar patterns of surface contamination before or after the cleaning product was used ([Fig 2](#)).

Toilet lid contamination was minimal in both experimental conditions, while the toilet seat was highly contaminated with phage. Relatively high levels of viral contamination were found on the toilet brush, brush caddy, and toilet bowl rim after cleaning the toilet bowl with a brush and disinfectant ([Fig 2](#)). There was a statistically significant ($P = .00079$) difference in the level of MS2 recovered from the toilet bowl water, a $> 4\text{-log}_{10}$ ($> 99.99\%$) reduction, after cleaning with the product. A statistically significant ($P = .0013$) 1.6 log_{10} reduction (97.64%) in MS2 contamination of the toilet bowl brush itself was observed when a cleaning product was used versus no product was used to clean the toilet bowl ([Fig 2](#)). Phage contamination of the restroom floor, rug, toilet lid bottom, and toilet seat top was found to be $\leq 2\text{ log}_{10}$ PFU/cm², while contamination of the toilet seat bottom, toilet rim, and brush caddy was $\geq 2\text{ log}_{10}$ PFU/cm². There were no statistically significant differences found in MS2 contamination levels on other bathroom surfaces (toilet brush caddy, toilet bowl rim, toilet seat, toilet lid, or floor) assessed before versus after the use of cleaning product.

DISCUSSION

Outbreaks of viruses have been associated with toilet use¹⁵ and contamination of restroom fomites might play a role in infection transmission. Contamination of restroom surfaces is of particular concern in health care settings where both infected individuals and immunocompromised individuals may be present. Several studies have reported the contamination of hospital patient restrooms shared by patients with 53% to 63% of the surfaces contaminated by SARS-Cov-2.¹⁵ In a study of hospital restrooms adenoviruses were detected on ~70% of the surfaces. Understanding the sources and control are important in health care settings.¹⁷ It has been proposed

that closing the toilet lid prior to flushing might reduce the degree of surface contamination after flushing a toilet.^{5,8,9,14,15} Previous studies by Best et al¹⁰ and Barker and Jones¹² used bacterial spores, bacteria, or MS2 mixed with fecal matter, to evaluate the potential for aerosol generation during toilet flushing and surface contamination. These studies demonstrated microbial contamination of toilet surfaces after the aerosolization of large droplets from fecal matter.

To clarify the role of viruses in restroom surface contamination and pathogen spread, and to focus on small droplet dispersion, this study investigated the aerosolization of bacteriophage MS2 during toilet flushing using no fecal material. The Best et al¹⁰ study found that lid closure reduced the aerosolization of bacterial spores and the contamination of nearby surfaces during toilet flushing. In contrast, our study demonstrated that lid position (up or down) prior to toilet flushing had no significant effect on the MS2 contamination of household restroom surfaces. MS2 was recovered from all restroom surfaces tested, and lid closure had no impact on the results. Low (10^{11} PFU/mL) and high (10^{14} PFU/mL) MS2 titers were used to challenge the impact of toilet lid closure. When flushing a toilet with the lid closed (down), MS2 contamination increased on the bottom of toilet seat when the dose added to the bowl water was higher (final concentration of 10^{14} PFU/mL). Flushing of public toilets (with no lids) led to consistently high levels of MS2 contamination on the toilet seat, despite the lower inoculum of 10^{11} PFU/mL. Aerosolization of viruses via toilet flushing may be more impactful, compared with aerosolization of bacteria, given differences in response to humidity and in droplet size. Various studies have indicated that viruses retain viability at both low or high relative humidities^{18,19} and, therefore can remain infectious in droplet nuclei and other aerosols, which may stay suspended in the air for hours to days.²⁰ Also, viruses are likely to aerosolize more easily than bacteria and 94% of virus has been shown to partition into liquid phase rather than solid phase.²¹ Droplet nuclei and aerosols can enter the upper respiratory tract or the lower respiratory tract and the lungs, which are susceptible tissues for initiation of infection with respiratory viruses.²²

In the present study, changes in the MS2 deposition pattern after flushing with the toilet lid down were observed ([Fig 3](#)), although the pattern changes were not consistent. The toilet lid (bottom and top) and wall contamination were relatively low after flushing with the

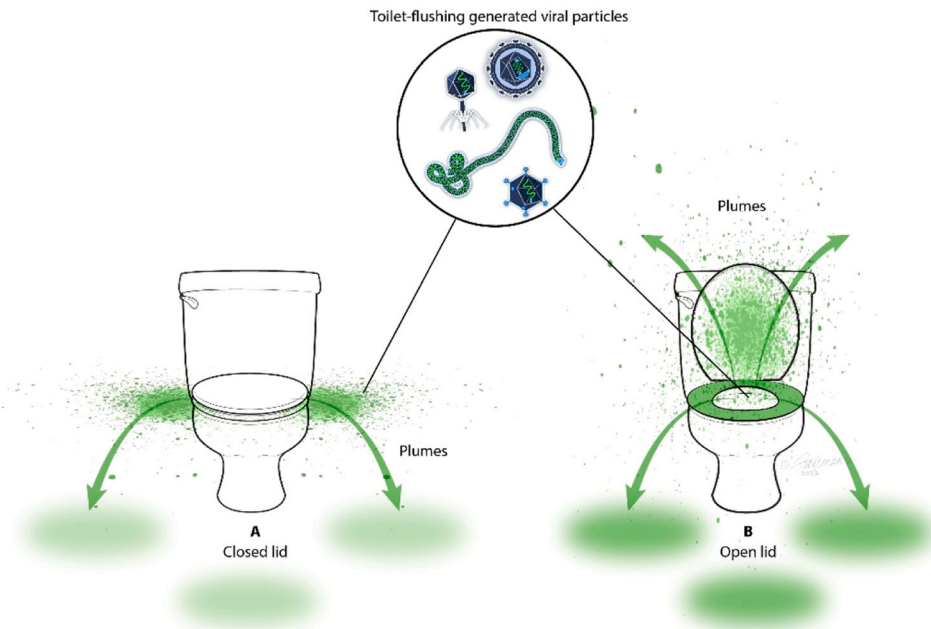


Fig. 3. Schematic depiction of MS2 aerosolization and spread to adjacent areas after flushing a home toilet of U.S. design.

lid either up or down. The toilet seat was the most contaminated surface after flushing. Greater contamination of the toilet seat probably reflects the airflow that occurs during toilet flushing (ie, largely around the top and bottom of the toilet seat). Flow over the toilet bowl rim and under the toilet seat in household toilets of United States design is enabled since the seat is raised from the rim ~0.5 in. This directed flow also contributed to the MS2 contamination of the restroom floors and walls. Thus, it appears that closure of the toilet lid is not effective in reducing the contamination of the toilet seat area or contamination of other areas (fomites, floors, walls) within the restroom.

Elimination or inactivation of viruses in the bowl water is necessary to prevent continued contamination of the restroom since not all of the viruses in the bowl are removed to the drain after a flush.^{5,6} Cleaning of the toilet bowl with a disinfectant (formulated hydrochloric acid) and bowl brush in this study significantly ($P = .00079$) reduced MS2 contamination of toilet bowl water, with $> 4 \log_{10}$ ($> 99.99\%$) reduction, compared to cleaning with a brush only. In addition, it was found that the toilet bowl brush itself was significantly ($P = .0013$) less contaminated when a disinfectant product was used during cleaning. In this case, a $1.6 \log_{10}$ (97.64%) reduction was observed. Reductions in the levels of MS2 contamination in the toilet bowl and brush can be attributed to contact with disinfectant, in addition to vigorous cleaning with the brush. Removal of phage likely occurred through the generation of aerosols as well as drops from the brush as it was removed from the toilet and placed in its caddy.

While the levels of MS2 contamination were relatively low in this experiment (toilet lid, floor, wall, etc.), the fact that infectious virus was observed at all implies that aerosols generated during toilet flushing are produced and these subsequently contaminate restroom surfaces. This aerosol generation and contamination of surfaces represent potential routes of infection transmission (ie, through direct exposure to the aerosolized virus or indirectly through via fomites to hands).^{18,23}

Patients with infections with viruses transmitted via the fecal-oral route often eliminate large amounts of viruses into the toilet

during use, and this should be regarded as a possible source of the spread of the virus.⁵ Virus levels in stool samples will vary during the course of an infection and can range as high as 10^{10} to 10^{12} PFU/g, and may approach 10^{14} PFU/g after a typical 150-g bowel movement.¹⁵ Depending on the specific virus disseminated (eg, rotaviruses, noroviruses, or the Ebola virus), it may only take a few infectious virus particles (1–100 infectious units) to initiate an infection a new human host.^{24,25} While our studies did not specifically model the involvement of fecal material, we feel that this does not represent a major limitation. Most studies on aerosol generation do not use actual or other fecal material.¹⁵ Liquid stool (diarrhea) would represent the worst-case with respect to the generation of aerosols, and that is why a liquid proxy (TSB) for fecal material was used in this study. In addition, a previous study¹⁶ did not reveal a significant difference in aerosolization whether solid or homogenized stool was used in flushing experiments.

CONCLUSIONS

Our study demonstrated that lid position (up or down) prior to flushing of household or public toilets of United States design seeded with MS2 bacteriophage had no significant effect on the MS2 cross contamination of household restroom surfaces. MS2 was recovered from all restroom surfaces tested, and lid closure had no impact on the results. The most effective strategies for reducing restroom cross-contamination associated with toilet flushing include the addition of a disinfectant to the toilet bowl before flushing³ and the use of disinfectant/detergent dispensers in the toilet tank.^{4,26,27} To reduce the risk associated with exposure to contaminated fomites in the restroom, regular disinfection of all restroom surfaces following toilet brushing, and/or use of a disinfectant that leaves residual microbicidal activity²⁸ is suggested particularly when the household is occupied by an individual with an active infection with a virus, such as norovirus, causing acute gastroenteritis. Because many viral infections may be asymptomatic, this is even more important in health care facilities where immunocompromised individuals are often present.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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