**Evaluation of Hospital-grade Disinfectants on Viral Deposition on Surfaces after Toilet
Flushing**

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23 **Highlights**

- 24 • Droplet deposition of virus occurred on surfaces around the toilet when untreated,
25 infectious material was flushed.
- 26 • The highest concentration and incidence of surface contamination occurred nearest to the
27 toilet bowl on the toilet seat and the toilet bowl rim.
- 28 • The deposition of droplets was significantly reduced ($p<0.05$) when the waste was treated
29 using a peracetic acid, quaternary ammonium, and chlorine-based disinfectant prior to
30 flushing.

Abstract

Background: Past studies have shown that infectious aerosols created during toilet flushing results in surface contamination of the restroom. The goals of this study were to quantify viral contamination of surfaces in restrooms after flushing and the impact of disinfectants added to the toilet bowl prior to flushing on reducing surface contamination.

Methods: The degree of contamination of surfaces in the restroom was assessed with and without the addition of coliphage MS-2 to the toilet bowl before flushing. The bowl water and various surfaces in the restroom were subsequently tested for the presence of the virus.

Results: The toilet bowl rim, toilet seat top, and toilet seat underside were contaminated in all trials without a disinfectant added to the bowl water before flushing. All disinfectants significantly reduced concentrations on surfaces when the contact time was 15 min or greater. Hydrogen peroxide resulted in very little reduction of virus in the toilet bowl ($<1 \log_{10}$). Peracetic acid and quaternary ammonium had the greatest log-reductions on virus in the organic matter in the toilet.

Conclusions: Toilet flushing resulted in extensive contamination of surfaces within the restroom. Addition of disinfectant to the toilet bowl prior to flushing reduced the level of contamination in the bowl and fomites after flushing.

Keywords: Healthcare Associated Infections; Ebola virus; Aerosols; Fomite Transmission; Disinfection

Introduction

With the Ebola virus (EBV) outbreak in West Africa and the introduction of the disease into the United States for the first time (in humans) in 2014, safe handling and effective disinfection practices of potentially infectious waste have become especially important in the healthcare setting^{1,2}. It has been widely recognized for some time that infectious disease transmission in healthcare environments can occur among patients and healthcare workers (HCWs)³. The transmission dynamics and highly infectious nature of EBV are extremely important factors to consider, in terms of protecting HCWs in all settings including outbreak control centers and hospitals. It is well-established that the primary mode of transmission for EBV is through direct contact with infected bodily fluids. The levels of virus in bodily fluids can range from $10^{5.5}$ - 10^8 EBV genome copies per mL⁴⁻⁶. This is assumed to be well over the suspected median infectious dose of <10 viral particles. EBV is excreted not only in blood but also in feces, urine, and vomit. When a patient is infected, they can release up to nine liters of stool per day, discharging copious amounts of virus into the environment⁴.

Human pathogenic viruses shed in bodily fluids, such as norovirus, adenovirus, and Torque teno virus are known to be aerosolized and deposited on hospital surfaces^{7,8}. EBV surrogates have recently been studied for aerosolization in waste disposal systems, specifically toilets, aeration basins and sewer pipe convergences⁹. This exposure route of virus could result in a heightened risk of environmental contact and transmission for HCWs. In 1979, a Sudanese outbreak of EBV reported that HCWs were up to five times more likely to contract the virus than those who did not practice patient care¹⁰. Fifteen years later, during the 1995 outbreak of EBV in the Democratic Republic of the Congo, at least 32% of the infected individuals (N=296) were healthcare workers¹⁰. Since these outbreaks, the Centers for Disease Control and Prevention

(CDC) has released multiple guidance documents for hospitals for managing EBV patients and suspected patients. In the most recent document, measures to control environmental spread were provided and outlined¹¹.

Use of an Environmental Protection Agency (EPA) registered disinfectant with claims against non-enveloped viruses (noroviruses, enteroviruses, adenoviruses) was specified in recommendations by the CDC as a method to reduce environmental fomite transmission of EBV¹¹. Fomite transmission of diseases has become one of the most recognized routes of transmission in healthcare settings¹². Because of this, environmental disinfection could be one of the most important steps to containing an EBV outbreak in a hospital or healthcare setting. The current standard for disposing of human waste materials is flushing into a sanitary sewer without prior disinfection¹³. It is likely that EBV, like other viruses, is being aerosolized during flushing and subsequently settling onto surfaces. The resulting deposition of infectious droplets could present an environmental transmission route for HCWs. Depending on the conditions, the virus may be able to survive on surfaces between disinfections. EBV-Zaire was dried onto glass and plastic surfaces, and was found to survive up to 50 days at lower temperatures (+4°C). The aerosolized virus was also detected after 90 min¹⁴.

Due to concern over the allowance of untreated infectious waste to be flushed into sanitary sewers, the US Army Institute of Public Health released additional Standard Operating Procedures for treatment of waste in toilets before flushing. Recommendations include adding one cup of at least 5% or greater sodium hypochlorite, or low alcohol quaternary ammonium, to toilet bowls, and allowing a 15-minute contact time before flushing¹⁵.

The main objective of this study was to evaluate the recommendations for disinfection of waste before flushing on viral contamination of restroom surfaces. In addition to sodium

hypochlorite and quaternary ammonium, hydrogen peroxide and peracetic acid were assessed as disinfectants. The treatments were evaluated for the reduction of virus deposited onto surfaces around the toilet after flushing. The second objective of this study was to compare the efficacies of four disinfectants on reducing the viral concentration in the toilet bowl before flushing.

Methods and Materials

Inoculation and Sample Collection

In order to create a baseline for how the flushing of heavily contaminated organic waste would deposit virus onto commonly touched surfaces around the toilet, 1000 mL volumes of trypticase soy broth (TSB) (BD, Franklin Lakes, NJ, USA) were used to create a replicable and uniform surrogate for human waste. Next, the TSB was inoculated with high titers ($\sim 1 \times 10^{12}$) of MS2 (ATCC 15597-B1), and added to a commercial valve-type toilet bowl containing 2.8 L of water (American Standard, Piscataway, NJ, USA). The bacteriophage was propagated and assayed as previously described by Sassi *et al*¹⁶. After addition of the virus and broth, the toilet was flushed, and surfaces around the toilet were sampled using sponge sticks moistened with 10 mL of letheen broth (3M Brand, St. Paul, MN, USA) (Table 1). An area of 100 cm² was sampled for each site, except the toilet flush handle, which was 90 cm². A succession of water samples was also collected after one, two, and three flushes to determine residual virus in the bowl after flushing. For these samples, 9 mL of water was collected from the toilet bowl and transferred to a sterile 15 mL conical tube (BD, Franklin Lakes, NJ, USA) containing one mL of 10% sodium thiosulfate (Sigma Aldrich, St. Louis, MO, USA) to neutralize any free chlorine in the toilet water. To ensure there was no remaining MS2 on surfaces between trials, the surfaces were cleaned with 70% ethanol, allowed to dry, and, subsequently sampled and assayed.

Disinfectant Additions

Four hospital-grade disinfectants (Table 2) were tested in separate trials to assess the efficacy of reducing the viral load aerosolized onto surfaces after flushing. One cup of each disinfectant (≈ 236 ml) was added to the toilet bowl after the TSB and virus. Two contact times, 15 and 30 min, were evaluated for each treatment to assess the reduction in deposition of virus onto surfaces. Lethen broth (BD, Franklin Lakes, NJ, USA) and sodium thiosulfate were used to neutralize the treatments. The same surfaces were sampled for all trials (Table 1). The reduction of MS2 in the toilet bowl was quantified at three time points for each disinfectant. After the addition of organic matter, virus, and disinfectant, 5 mL samples were collected from the toilet bowl after 1, 15, and 30 min. The samples were then transferred into sterile 15 mL conical tubes containing either letheen broth or 10% sodium thiosulfate.

Sample Processing

Sponge stick samples were individually placed in a sterile plastic bag and eluted using manual pressure application, as previously described in the literature^{16–19}. The volume eluted (approx. 4–6 mL) was recorded and used to calculate a total concentration per sampled surface area. All samples (surfaces and water) were assayed using the double agar overlay method²⁰ in triplicate. Volumes of 1 or 0.1 mL were combined in melted top agar tubes (50°C) with 0.5 mL of host (*E. coli* ATCC 15597) before pouring onto TSA (BD, Franklin Lakes, NJ, USA). When necessary, 10-fold serial dilutions of the samples were made using 0.01M PBS (pH 7.4) (Sigma Aldrich, St. Louis, MO, USA). Plates were then incubated for 24 h at 37°C and viral plaques enumerated. The concentration per mL of sample was determined for water samples collected from the toilet bowl. The concentration per surface sample was calculated by determining the average concentration per mL of eluent and multiplying by the total volume of eluent collected

for the sample. This represents the concentration per 100 cm² for each sample location except the flush handle, which had a concentration per 90 cm². The limit of detection for surface samples was 1 plaque forming units (PFU)/100 cm². This was based off of the volume eluted from the sponge stick and the volume assayed for each sample.

Statistical Analysis

The concentrations per cm² on surfaces after use of disinfectant were compared to the concentrations deposited onto surfaces without disinfectant using a paired, two-sided t-test. Values were normalized using a log transformation. The log reductions observed after disinfection of the toilet bowl at 1, 15, and 30 min contact times were compared using a multivariate test of means for each disinfectant type. All statistical analyses were performed in STATA 14 (Stata Corp., College Station, TX, USA).

Results

Droplet Deposition on Surfaces

The most heavily contaminated surfaces, on average, were the underside of the toilet seat, the top side of the toilet seat, and the toilet bowl rim (Figure 1). Virus was detected 100% of the time after flushing at these three locations and had the highest concentrations of all surfaces. The least contaminated surfaces after flushing were the flush handle, the wall behind the toilet (i.e., the back wall), and the toilet paper dispenser (Table 3 and 4). The flush handle and toilet paper dispenser were the least frequently contaminated with virus being detected only 17% and 22% of the time, respectively (Figure 1). Virus was only detected in one toilet bowl water sample after a single flushing (1/54).

Comparison of Treatments

The results of the paired t-tests showed that there was a significant reduction in concentration from the baseline (without treatment) with all of the disinfectants, at the 15-minute contact time ($p < 0.05$). With a 30-minute contact time, all disinfectants except hydrogen peroxide showed a significant reduction when compared with no disinfectant (Table 4). When 30-minute contact times were compared with 15-minute contact times, the only treatment that showed a significant further reduction was chlorine bleach ($p = 0.0174$).

Comparison of Viral Reduction on Surfaces

Peracetic acid showed the greatest reduction of all treatments for all contact times. The quaternary ammonium treatment produced a 1.99 log₁₀ PFU/mL reduction within one minute of contact; however, the reduction only increased to 2.21 log₁₀ after a 30 minutes contact time. Hydrogen peroxide exhibited the least reduction for all three contact times (Table 4). When these values were analyzed using a multivariate test of means, the only statistically significant differences in average reduction was seen between hydrogen peroxide and quaternary ammonium ($p = 0.0016$), and between hydrogen peroxide and peracetic acid ($p = 0.0147$). Peracetic acid and quaternary ammonium disinfection resulted in significantly reduced concentrations compared to hydrogen peroxide.

Discussion

Surface Contamination after Flushing

Maximum deposition of virus after flushing occurred on locations closest to the source of the virus (i.e., the toilet bowl). These sites were the toilet bowl rim, the seat top, and the seat bottom. These surfaces have been noted in previous studies to be highly contaminated during flushing events, as well as the floor beneath and next to the toilet^{21,22}. Best *et al.* (2012) found that flushing the toilet with the lid closed significantly reduced the amount of *Clostridium*

difficile spores deposited on surfaces²¹. However, most commercial toilets do not have lids. If a toilet does not have a lid, frequently contaminated surfaces after flushing should be disinfected with a thorough hygiene protocol to reduce environmental contact with EBV and other infectious agents excreted in bodily fluids in a healthcare setting. In contrast, the two least contaminated surfaces were the flush handle and the toilet paper dispenser. These surfaces were the furthest away from the source, suggesting that the droplets were not being ejected with enough force to spread viable virus to more distant locations. It is presumed this is a result of the type of toilet, and will likely vary depending on toilet, bowl volume and flush force. These surfaces should still be targeted using surface disinfectants, however, due to their incidental and infrequent contamination.

Treating infectious waste with any of the tested disinfectants other than hydrogen peroxide showed significant reduction in the concentration of MS2 on surfaces, when compared to the baseline with no disinfection. The reduction of viral contamination during flushing could be an important control point in reducing environmental contact for HCWs, especially in an outbreak setting. Pathogens in aerosols and suspended droplet nuclei, such as norovirus and *C. difficile*, have been identified in air after flushing^{7,21}. *Escherichia coli* in droplets has also been captured on gauze over the toilet bowl during flushing. This study also showed that a lower volume of water in the toilet bowl produced an average higher concentration of *E. coli* suspended in droplets than a toilet with a greater volume²³. The toilet tested in the present study had a relatively low volume in the bowl during testing (2.8 L). When an inoculum of 10⁶ PFU was used in the toilet, no virus could be detected on the surrounding surfaces (data not shown), which demonstrates that the amount of virus being expelled during toilet flushing was less than the assay detection limit (1 PFU/100 cm²). In this study, an average inoculum of 10¹² was used,

which resulted in continual contamination of the toilet seat and toilet bowl rim. Overall, elimination and reduction of virus on surfaces minimizes the risk of exposure to staff through contact with fomites.

Reduction of MS2 in the Toilet Bowl

The surrogate virus was never inactivated below the limit of detection for any of the disinfectants studied (1 PFU/mL), which suggests that when present in high organic matter such as in bodily fluids, viruses are much more difficult to inactivate. Thus, infectious virus were still present in the toilet bowl during flushing. Of all the tested disinfectants, peracetic acid and quaternary ammonium showed the greatest reduction for the one-minute contact time (2.26 and 1.99 log₁₀). In a previous study, EBV was completely reduced by sodium hypochlorite concentrations of 5 and 10 mg/L in sterilized wastewater after 20 seconds. At 1 mg/L, EBV was reduced overall by 3.5 log₁₀ in 20 seconds²⁴. This contact time is significant because a higher-than-average (30-60 seconds) contact time is likely to be unrealistic in a healthcare setting, given the demands of staff availability²⁵.

Recommendations

One of the major concerns with EBV infectious waste is the high concentration of viral particles shed in bodily fluids (10⁸/mL)⁴. In 2017, recommendations on critical control points and containment for EBV waste were outlined in a WHO publication. Latrine use, and cleaning/disinfection were included as potential critical control points for environmental contact²⁶. Results from this current study indicate that when high concentrations of virus are present in the toilet bowl, detectable levels of virus on fomites in the restroom can occur in concentrations of 10¹-10⁵, even with disinfection before flushing. Thus, even a small amount of bodily fluid or fecal material can be expected to contaminate surfaces in the restroom. Treating

237 waste in the toilet before flushing should be practiced in order to significantly reduce the
238 contamination of surfaces in the restroom. For situations where toilets are not readily available,
239 EBV waste should be treated before disposal, and disinfection of highly contaminated restroom
240 surfaces should be practiced regularly, and also in between flushing events. In order to
241 effectively reduce and/or eliminate contact between infectious particles and HCWs, controlling
242 contamination from the toilet is essential.

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326 **FIGURES 1**

327 *Figure 1: Percent positive, by sample location (n=18)*

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330 **TABLES 1-4**

331 *Table 1: Restroom sample locations*

Sample	Location	Description
1	Handle	Toilet flush handle
2	Toilet Back	Back of toilet, mounting
3	Back Wall	Wall where toilet is mounted
4	Floor	Floor underneath toilet
5	TP Holder	Toilet paper dispenser
6	Toilet Bowl-In/Rim	Composite of toilet rim and under rim
7	Toilet Seat Top	Top of the toilet seat
8	Toilet Seat Under	Under toilet seat (actual seat piece)

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334 *Table 2: List of treatments and percent active ingredient*

Disinfectants		
Treatment Type	% Active Ingredient	Manufacturer
Bleach	5-10: sodium hypochlorite	Clorox (Oakland, CA)
Hydrogen peroxide	0.5-2: hydrogen peroxide	Clorox (Oakland, CA)
	3-5: Alkyl dimethyl benzyl	
Quaternary ammonium	ammonium chloride	Clorox (Oakland, CA)
		Decon (King of
Peracetic acid	0.23: peracetic acid	Prussia, PA)

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336 *Table 3: Concentrations of virus detected on restroom surfaces after flushing for all trials (N=18)*

Mean Concentrations, by Sample Site (n=18) per 100 cm²		
Sample Site	Geometric Mean $\pm$ SD (Log10 PFU)	Arithmetic Mean $\pm$ SD (PFU)
1-Flush Handle*	1.65 $\pm$ 0.91	2.65E+01 $\pm$ 7.64E+01
2-Toilet Back	2.89 $\pm$ 1.04	3.7E+03 $\pm$ 7.3E+03
3-Back Wall	1.63 $\pm$ 1.36	1.2 E+03 $\pm$ 5.3 E+03
4-Floor	3.44 $\pm$ 1.08	1.9E+04 $\pm$ 4.2E+04
5-Toilet Paper Dispenser	1.49 $\pm$ 1.41	68.5 $\pm$ 2.9E+02
6-Toilet Bowl Rim	3.88 $\pm$ 1.59	3.1E+05 $\pm$ 9.2E+05
7-Toilet Seat Top	4.21 $\pm$ 1.26	3.4E+05 $\pm$ 1.2E+06
8-Toilet Seat Underside	4.22 $\pm$ 1.26	2.5E+05 $\pm$ 4.6E+05

337 *denotes 90cm²

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342 *Table 4: Mean log₁₀ reductions of MS2 per mL by disinfectants in toilet bowl after indicated*
 343 *exposure times (N=3)*

Treatment	1 minute	15 minute	30 minute
Chlorine Bleach	0.48	1.4	2.83
Hydrogen Peroxide	0.01	0.03	0.06
Quaternary Ammonium	1.99	1.93	2.22
Peracetic Acid	2.26	3.37	3.43

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