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Major Article

Evaluation of hospital-grade disinfectants on viral deposition on surfaces after toilet flushing

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Background: Past studies have shown that infectious aerosols created during toilet flushing result 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 MS2 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 minutes. 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.

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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 health care setting.[1,2] It has been widely recognized for some time that infectious disease transmission in health care environments can occur among patients and health care 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 milliliter.[4-6] 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 9 L of stool per day, discharging copious amounts of virus into the environment.[4]

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 convergences.[9] 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 5 times more likely to contract the virus than those who did not practice patient care.[10] Fifteen years later, during the 1995 outbreak of EBV in the Democratic Republic of the Congo, at least 32% of the infected individuals

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(N = 296) were health care workers.[10] Since these outbreaks, the Centers for Disease Control and Prevention 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 outlined.[11]

Use of an Environmental Protection Agency–registered disinfectant with claims against nonenveloped viruses (noroviruses, enteroviruses, and adenoviruses) was specified in recommendations by the Centers for Disease Control and Prevention as a method to reduce environmental fomite transmission of EBV.[11] Fomite transmission of diseases has become one of the most recognized routes of transmission in health care settings.[12] Because of this, environmental disinfection could be one of the most important steps to containing an EBV outbreak in a hospital or health care setting. The current standard for disposing of human waste materials is flushing into a sanitary sewer without prior disinfection.[13] 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 minutes.[14]

Because of concern over the allowance of untreated infectious waste to be flushed into sanitary sewers, the U.S. Army Institute of Public Health released additional standard operating procedures for treatment of waste in toilets before flushing. Recommendations include adding 1 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 flushing.[15]

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 4 disinfectants on reducing the viral concentration in the toilet bowl before flushing.

METHODS AND MATERIALS

Inoculation and sample collection

To create a baseline for how the flushing of heavily contaminated organic waste would deposit virus onto commonly touched surfaces around the toilet, 1,000 mL volumes of trypticase soy broth (TSB) (BD, Franklin Lakes, NJ) were used to create a replicable and uniform surrogate for human waste. Next, the TSB was inoculated with high titers (~1×10^{12}) of MS2 (ATCC 15597-B1; ATCC, Rockville, MD), and added to a commercial valve-type toilet bowl containing 2.8 L of water (American Standard, Piscataway, NJ). 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) (Table 1). An area of 100 cm^2 was sampled for each site, except the toilet flush handle, which was 90 cm^2. A succession of water samples was also collected after 1, 2, and 3 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) containing 1 mL of 10% sodium thiosulfate (Sigma Aldrich, St Louis, MO) 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.

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	Toilet paper 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)

Table 2
List of treatments and percent active ingredient

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

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 (approximately 236 mL) was added to the toilet bowl after the TSB and virus. Two contact times, 15 and 30 minutes, were evaluated for each treatment to assess the reduction in deposition of virus onto surfaces. Letheen broth 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 3 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 minutes. 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 (approximately 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 method[20] in triplicate. Volumes of 1 or 0.1 mL were combined in melted top agar tubes (50°C) with 0.5 mL of host (*Escherichia coli* ATCC 15597; ATCC) before pouring onto TSA. When necessary, 10-fold serial dilutions of the samples were made using 0.01 M phosphate-buffered saline (pH 7.4) (Sigma Aldrich). Plates were then incubated for 24 hours at 37°C and viral plaques enumerated. The concentration per milliliter 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 milliliter of eluent and multiplying by the total volume of eluent collected for the sample. This represents the concentration per 100 cm^2 for each sample location except the flush handle, which had a concentration per 90 cm^2. The limit of detection for surface samples was 1 plaque forming unit (PFU)/100 cm^2. This was based off of the volume eluted from the sponge stick and the volume assayed for each sample.

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