Evaluating the Efficacy of Ebola Human Waste Management Practices for Disposal in Toilets

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Abstract

Background: Past studies have shown that aerosols created during the flushing of infectious materials result in surface contamination in the restroom. The goals of this study were: i) to determine the degree of viral contamination of surfaces in restrooms after disposal of organic wastes through flushing; and ii) to determine the efficacy of four disinfectants on an Ebola surrogate in the toilet before flushing.

Methods: MS2 coliphage (Ebola surrogate) and trypticase soy broth were added to a toilet bowl, and following flushing, bowl water and surrounding surface areas were sampled for the virus. The impact of chlorine bleach, hydrogen peroxide, quaternary ammonium and peracetic acid, added as disinfectants to the bowl were then determined.

Results: After every trial, the toilet bowl rim, toilet seat top, and toilet seat underside were contaminated. All disinfectants significantly reduced MS2 on surfaces when the contact time of the disinfectant in the bowl was 15 min. Peracetic acid and the quaternary ammonium had the greatest log-reductions within the first min of contact in the bowl water.

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

Keywords: Healthcare Associated Infections; Ebola virus; Viral Aerosols; Fomite Transmission, Toilet

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 (Fauci 2014; CDC 2016). It has been widely recognized for some time that transmission of infectious diseases in healthcare environments can occur among patients and healthcare workers (HCWs) (Alexander, 1973). The transmission dynamics and highly infectious nature of EBV are extremely important factors to be considered when protecting HCWs. It is well-established that the primary mode of transmission for EBV is through direct contact with infected bodily fluids. 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 environment. The levels of virus in bodily fluids can range from $10^{5.5}$ to 10^{8} EBV genome copies per mL (Bibby *et al*, 2015). This is well over the suspected median infectious dose of <10 viral particles (Bibby *et al*, 2015).

Human viruses shed in bodily fluids, such as norovirus, adenovirus, and torque teno virus, are known to be aerosolized and deposited on hospital surfaces (Bonifait *et al*, 2015; Verani *et al*, 2014). This release of virus could result in a heightened risk of environmental contact and transmission for HCWs. In the past, outbreaks of EBV have resulted in high infection rates of HCWs. A Sudanese outbreak in 1979 reported that HCWs were up to five times more likely to contract the virus than those who did not practice patient care (Sepkowitz, 1996). 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 workers (Sepkowitz, 1996). 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 outlined (CDC, 2015).

As a preventative measure for environmental spread of EBV to HCWs, the CDC first suggests the use of proper personal protective equipment (PPE) (CDC, 2015). However, in the past it has been demonstrated that PPE can act not only as a barrier, but also as a vehicle for pathogens. In a 2008 study, the ability of MS2 to spread from gloves to bare hands was characterized. MS2 was found on 90% of ungloved right hands, 70% of ungloved left hands, and on other hospital attire (e.g., scrub pants and shirts) (Casanova *et al*, 2008). This study demonstrated the relationship of PPE removal with potential infection events. Therefore, environmental controls, such as elimination through disinfection, should be relied upon before PPE (OSHA, 2016).

Use of an Environmental Protection Agency registered disinfectant with claims against non-enveloped viruses (norovirus, poliovirus, adenovirus) is also a specified recommendation to reduce environmental transmission of EBV (CDC, 2015). Fomite transmission of diseases has become one of the most recognized routes of transmission in healthcare settings (Weber & Rutala, 2013). Because of this, environmental disinfection could be one of the most important steps to containing an EBV outbreak in a hospital setting. Currently, however, flushing human waste contaminated with EBV into a sanitary sewer, without disinfection, is allowed (Bibby *et al*, 2014). If EBV is being aerosolized during flushing, like many other viruses, the deposition of infectious droplets onto surfaces could serve as an environmental transmission route for HCWs. EBV-Zaire has been demonstrated to survive dried onto glass and plastic surfaces for up to 50 days at low temperatures (+4°C) (Piercy *et al*, 2010).

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 prior to flushing. Recommendations include adding one cup of 5% or greater sodium hypochlorite or low alcohol quaternary ammonium to toilet bowls and allowing a 15-minute contact time before flushing. Procedures for wiping down surfaces around the toilet are also outlined in this document (US Army Institute of Public Health, 2014).

The main objective of this study was to evaluate the recommendations for in-toilet disinfection of waste before flushing to prevent viral contamination of restroom surfaces. In addition to sodium hypochlorite and quaternary ammonium, two other hospital grade disinfectants were also assessed. The treatments were further 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

Virus Preparation and Assay

Coliphage MS-2 was used as the test surrogate virus. The bacteriophage was propagated and assayed as previously described in Sassi et al (2015). All samples (surface and water) were assayed using the double agar overlay method (Kropinski et al, 2009) in triplicate. Volumes of 1 or 0.1 mL were combined in melted top agar tubes (50 ^{0}C) with 0.5 mL of host (*Escherichia coli* ATCC 15597) prior to pouring onto trypticase soy agar (BD, Franklin Lakes, NJ). When necessary, 10-fold serial dilutions of the samples were done using 0.01M phosphate buffered

saline (pH 7.4) (Sigma Aldrich, St. Louis, MO, USA). Plates were then incubated for 24 h at 37 ^{0}C and viral plaques enumerated.

Fomite Contamination after Flushing

The first sets of experiments were designed to assess the viral contamination of the bowl and adjacent surfaces areas by virus after flushing, without the influence of disinfectants. Trypticase soy broth (TSB) (BD, Franklin Lakes, NJ, USA) (one liter) was added to the bowl followed by addition of the MS2. An average of $1.83 \times 10^{12} \pm 3.8 \times 10^{12}$ plaque forming units (PFU) of MS2 (ATCC 15597-B1) was 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 in Sassi *et al* (2015). Surfaces tested after flushing are shown in Table 1. After addition of the broth and virus, the toilet was flushed, and surfaces around the toilet were sampled 15 or 30 min after flushing using sponge sticks moistened with 10 mL of letheen broth (3M Brand, St. Paul, MN, USA). An average of 100 cm^2 was sampled for each site; however, if the site was less than 100 cm^2, the entire surface was sampled (i.e., toilet flush handle, 90 cm^2). The limit of detection for surface samples was 1 PFU/100 cm^2. This was based on the volume eluted from the sponge stick and the volume assayed for each sample.

A succession of water samples from the bowl 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.

Impact of Disinfectants on Bowl and Fomite Contamination

Four different hospital-grade disinfectants were tested in separate trials to assess efficacy of reducing the viral load deposited on surfaces after flushing (Table 2). To quantify the reduction of MS2 in the toilet bowl before flushing, three contact times were evaluated. After the addition of broth and virus, one cup (~236 mL) of the disinfectant was added and 5 mL water 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 one mL of either letheen broth (BD, Franklin Lakes, NJ,) or 10% sodium thiosulfate, depending on the disinfectant used.

To assess fomite contamination samples were collected using sponge sticks as described in the previous section.

Statistical Analysis

The concentrations per cm^2 on surfaces after use of disinfectant were compared to the concentrations deposited onto surfaces without treatment using a paired t-test, after being normalized using a log transformation. All t-tests were performed as paired, two-sided tests with a null hypothesis that the difference in means was equal (H_0: $\mu_1=\mu_2$). The log reductions observed after disinfection treatments in 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

Virus Droplet Deposition on Surfaces after Flushing

After flushing, with and without addition of a disinfectant treatment, the most heavily contaminated surfaces were the underside of the toilet seat, the top side of the toilet seat, and the toilet bowl rim. 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. Virus was only detected in one toilet bowl water sample after flushing once (1/54). No further bowl water samples were positive after additional flushes.

Comparison of Treatments on Viral Reduction on Surfaces

The toilet seat top and underside, and toilet bowl rim were contaminated during every trial, regardless of the type of disinfectant or contact time. 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 tested, at the 15-minute contact time ($p<0.05$). With a 30-minute contact time, all disinfectants except hydrogen peroxide resulted in a significant reduction of the virus. The only treatment that showed a significant further reduction after 30 minutes was chlorine bleach ($p= 0.0174$).

Comparison of Treatments on Viral Reduction in Toilet Bowl

Peracetic acid formula showed the greatest reduction of all treatments. The quaternary ammonium treatment produced a 1.99 $\log_{10}$ PFU/mL reduction within one minute of contact; however, the reduction saw only a small increase in virus reduction after 30 minutes. 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 were able to significantly reduce the concentrations further than hydrogen peroxide ($p<0.05$).

Discussion

Surface Contamination after Flushing

The deposition of virus on surfaces after flushing occurred to the greatest extent on locations nearest 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, in addition to the floor beneath and next to the toilet (Best *et al,* 2012; Barker and Jones, 2005). Best *et al* (2012) found that flushing the toilet with the lid closed significantly reduced the amount of *Clostridium difficile* spores deposited on surfaces. However, most commercial toilets found in hospitals do not have lids. In contrast, the two least contaminated surfaces, the flush handle and the toilet paper dispenser, were the two surfaces that were the furthest away from the source. This suggests that the droplets were not being ejected with enough force to spread viable virus to more distant locations. These surfaces should still be targeted using surface disinfectants, however, due to their incidental contamination.

Treating infectious waste with any of the tested disinfectants showed significant reduction in the concentration of MS2 on surfaces, when compared to the baseline with no treatment. 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 (Bonifiat *et al*, 2015; Best *et al*, 2012). *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 volume (Gerba *et al*, 1975). The toilet tested in the present study had a relatively low volume in the bowl during testing (2.8 L). When an inoculum of 10^6

PFU was used (data not shown) in the toilet, no virus could be detected on the surrounding surfaces, which demonstrates that the amount of virus being expelled during toilet flushing was less than the assay detection limit (3 PFU/100 cm^2). The levels of virus in bodily fluids can range from $10^{5.5}$ to 10^8 EBV genome copies per mL. Thus, it is likely that disposal of bodily fluids could contain levels of virus which would be at detectable levels ejected from the toilet bowl if they were not disinfected first.

Reduction of MS2 in the Toilet Bowl

The surrogate virus was never inactivated below the limit of detection for all of the disinfectants studied (one PFU/3 mL), which suggests that when present in the high organic matter such as in bodily fluids, viruses are much more difficult to inactivate; the consequence is that infectious virus is still present in the toilet bowl during flushing. Peracetic acid and quaternary ammonium showed the greatest reduction for the one-minute contact time (2.26 and 1.99 $\log_{10}$). It has also been noted that a higher-than-average (30-60 seconds) contact time is likely unrealistic for a healthcare setting, given the demands of staff availability (CDC, 2008).

Recommendations

To reduce environmental contact with EBV and other infectious agents to HCWs, controlling contamination from the toilet is necessary. The concentration of EBV in bodily fluids may be as great as 10^8/mL (Bibby *et al*, 2015). Results from this 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 10^1-10^5, even with treatment before flushing. Thus, even a small amount of bodily fluid or fecal material can be expected to contaminate surfaces in the restroom. Treating waste in the toilet before flushing should be practiced in order to reduce significantly the contamination of surfaces in the restroom. For situations where toilets are not

readily available, EBV waste should be treated before disposal into the environment. In addition, disinfecting highly contaminated restroom surfaces, such as the toilet bowl and seat, should be practiced regularly and after every flushing event to prevent further environmental spread.

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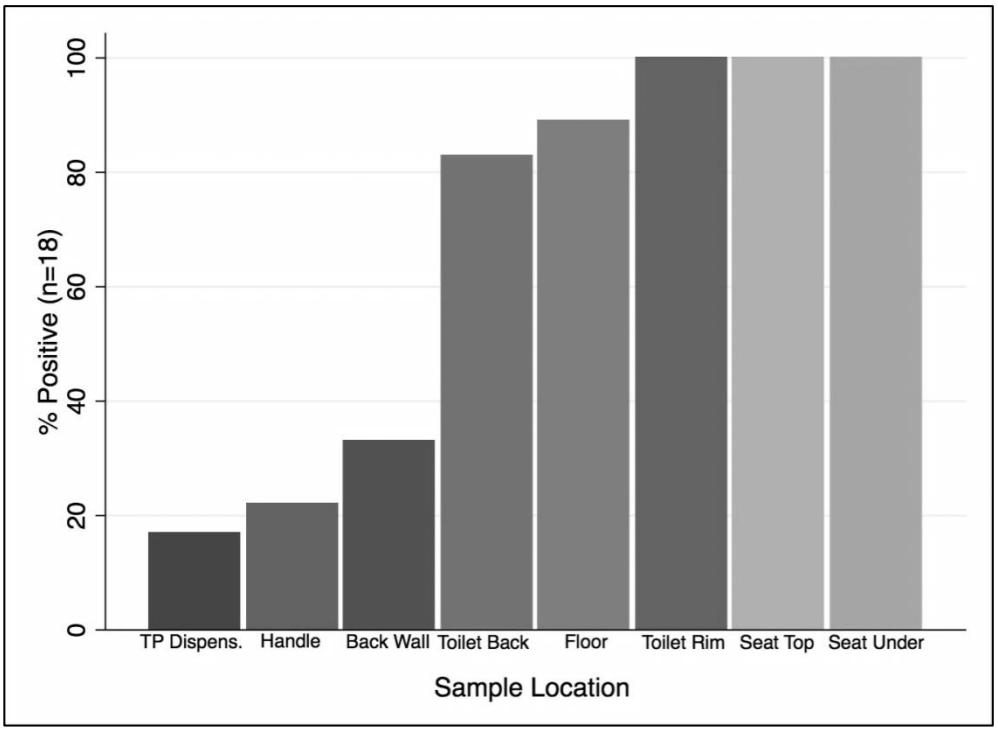
FIGURES 1

Figure 1: Percent positive, by sample location (N=16)



TABLES 1-4

Table 1: Restroom fomite 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)

Table 2: List of disinfectants 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)
Quaternary ammonium	3-5: Alkyl dimethyl benzyl ammonium chloride	Clorox (Oakland, CA)
Peracetic acid	0.23: peracetic acid	Decon (King of Prussia, PA)

Table 3: Concentrations of virus detected on restroom surfaces after flushing for all trials with and without a disinfectant

Arithmetic Mean Concentrations (PFU), by Sample Site per 100 cm^2		
Sample Site	**No Treatment (N=4): Mean ± SD**	**With Treatment (N=16): Mean ± SD**
1-Flush Handle*	1.10E+01 ± 2.20E+01	3.31E-01 ± 8.96E-01
2-Toilet Back	1.56E+04 ± 2.41E+04	3.59E+01 ± 7.56E+01
3-Back Wall	5.58E+03 ± 1.12E+04	4.70E-02 ± 8.78 E-02
4-Floor	4.29E+04 ± 7.05E+04	2.18E+02 ± 4.38E+02
5-Toilet Paper Dispenser	2.83E+02 ± 5.32E+02	7.70E-01 ± 3.05E+00
6-Toilet Bowl Rim	1.06E+06 ± 1.83E+06	1.16E+03 ± 3.06E+05
7-Toilet Seat Top	7.51E+07 ± 1.26E+08	3.72E+03 ± 1.27E+04
8-Toilet Seat Underside	2.88E+06 ± 2.19E+06	2.16E+03 ± 4.44E+03

***denotes 90cm^2**

Table 4: Log_{10} reductions of MS2 per mL by disinfectants in toilet bowl after indicated exposure times

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