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Persistence of Bowl Water Contamination during Sequential Flushes of Contaminated Toilets

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Abstract

Toilets contaminated with infectious organisms are a recognized contact disease transmission hazard. Previous studies indicate that toilet bowl water can remain contaminated for several flushes after the contamination occurs. This study characterized contamination persistence over an extended series of flushes using both indicator particles and viable bacteria. For this study, toilets were seeded with microbe-size microbial surrogates and with Pseudomonas fluorescens or Clostridium difficile bacteria and flushed up to 24 times. Bowl water samples collected after seeding and after each flush indicated the clearance per flush and residual bowl water contaminant concentration. Toilets exhibited 3 + log10 contaminant reductions with the first flush, only 1–2 logs with the second flush, and less than 1 log thereafter. Contamination still was present 24 flushes post contamination. Clearance was modeled accurately by a two-stage exponential decay process. This study shows that toilet bowl water will remain contaminated many flushes after initial contamination, posing a risk of recurring environmental contamination and associated infection incidence.

Keywords

toilet; aerosol; flush; plume; Clostridium difficile; infectious; transmission

Introduction

Toilets splash and produce droplet aerosols when flushed. Aerosolization of microorganisms from contaminated toilets during toilet flushing has repeatedly been demonstrated for various toilet types and organisms during the past 50 years, as reviewed by Johnson, Mead,

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and coauthors (2013). Large droplet as well as droplet nuclei bioaerosols are produced and can contaminate nearby surfaces and the room air (Barker & Bloomfield, 2000; Barker & Jones, 2005; Bound & Atkinson, 1966; Darlow & Bale, 1959; Gerba, Wallis, & Melnick, 1975; Jessen, 1955; Johnson, Lynch, Marshall, Mead, & Hirst, 2013; Scott & Bloomfield, 1985; Verani, Bigazzi, & Carducci, 2014; Yahya, Cassells, Straub, & Gerba, 1992). This route is a well-recognized contact disease transmission hazard (Sehulster, Chinn, Centers for Disease Control and Prevention, & Healthcare Infection Control Practices Advisory Committee, 2003).

It has been observed that toilet bowl water will remain contaminated for at least several flushes after the initial contamination, and microbial contamination can persist for days or weeks. Barker and Bloomfield (2000) detected residual microorganisms in bowl water 12 days after seeding the toilet with Salmonella, and in biofilm below the bowl waterline for 50 days after the seeding, suggesting a possible role of biofilm as a long-term reservoir and source of pathogenic organisms in the bowl water. Contaminated toilets will produce microbe-carrying aerosols during each flush (Barker & Jones, 2005; Darlow & Bale, 1959; Gerba et al., 1975; Yahya et al., 1992), with associated environmental re-contamination.

A particularly interesting observation by Gerba and coauthors (1975) was that bowl water clearance was incomplete even after seven flushes following contamination of a toilet with either E. coli bacteria or MS2 bacteriophage. They measured approximately 3 logs (1,000-fold) concentration reduction with their first flush after seeding, but only approximately 2 logs with the second flush and less than 1 log with subsequent flushes, consistent with previous and subsequent reports for E. coli (Darlow & Bale, 1959; Yahya et al., 1992), for E. coli and Serratia marcescens (Barker & Jones, 2005), and for E. coli and MS2 bacteriophage (Gerba et al., 1975). Indeed, Gerba and coauthors found that after the first three flushes, an apparent "plateau" bowl water concentration was reached that did not visibly decline over the next four flushes. The investigators attributed this effect to microbial adhesion to toilet bowl surfaces with subsequent re-release after the flush. We could find no reports of research to duplicate and further examine this contamination persistence phenomenon.

Clearly, the persistence of pathogenic microorganisms in a public toilet (such as in a hospital emergency department waiting area), with aerosolization of pathogenic organisms during subsequent toilet uses by others, could pose a contact- or airborne disease transmission risk. Similarly, pathogen persistence in a patient room toilet with subsequent aerosolization of microbes could pose an environmental contamination risk to patient care staff and other patients.

The purpose of our study was to characterize bowl clearance over an extended series of flushes following initial contamination for several modern toilets. Series of up to 24 flushes post contamination were conducted using microbe-size fluorescent polymer microspheres, Pseudomonas fluorescens bacteria, and Clostridium difficile bacteria. The results were compared with those of previous studies with microbial suspensions and also mathematically modeled as a two-stage exponential decay process.

Methods

Toilets Selected

The three toilet types selected for our study were a dual flush volume high efficiency gravity flow toilet (HET) with selectable flush volumes of either ~3.9 or ~5.3 liters per flush (Lpf), a dual flush volume pressure-assisted toilet (PAT) of either ~4.2 or ~5.1 Lpf, and a commercial type flushometer (FOM) toilet of ~5.5 Lpf. The HET operates by gravity flow of water from a tank mounted at the rear of the toilet base, whereas the FOM operates via a direct connection to the main water supply and has no tank. The familiar FOM toilets are commonly found in commercial, educational, healthcare, and other public access facilities. The PAT is a fairly recent innovation that employs a pressure vessel inside the toilet tank to provide a more vigorous flush than can be achieved by gravity flow alone, though the flush is less vigorous than that of the FOM (Johnson, Mead, Lynch, & Hirst, 2013). All three toilets were of the siphonic type, in which flush water enters the bowl bottom as a submerged jet directed toward the S-shaped outlet trapway, inducing a siphon effect that empties the bowl. A secondary flush water flow passes through perforations spaced around the underside of the bowl rim and rinses the bowl walls during the flush. When water flow stops, the siphon breaks and stops the flush, with some water in the trapway flowing back into the bowl. All three toilet models had been evaluated under the U.S.-Canadian Maximum Performance (MaP) program that tests the clearance performance of toilets using a standard protocol, and achieved the highest MaP clearance performance rating (Gauley & Koeller, 2009).

Fluorescent Microsphere Surrogates

Microbial contamination was simulated using monodisperse suspensions of green fluorescent polymer microspheres of microbial size 0.25, 0.5, or 1.0 μ m after the method of Johnson and Lynch (2008). The toilet bowl water was seeded with a 1 mL aliquot of 1% by volume source suspension and a water sample was collected after mixing. We collected an additional sample within approximately three minutes after each subsequent flush without reseeding. The toilets were installed in a test apparatus that allowed flushed water to be captured for volume measurement. An aliquot of each water sample, diluted as necessary, was filtered through a 25 mm diameter 0.2 μ m pore size mixed cellulose ester (MCE) filter. The filter then was removed and mounted on an oversized 75 × 38 mm microscope slide for top-illumination viewing and particle counting using a Nikon Model Eclipse 80i fluorescence microscope fitted with 10x, 20x, and 40x (Plan-Apochromat) objectives.

Particles were counted manually with magnification (10–40x) and field number (10–75) based on particle size and deposition density. For slides with less than one sphere per field, either half or the entire filter was counted. The particle count divided by the aliquot volume and multiplied by the dilution factor (if any) provided an estimate of the suspension concentration in each water sample. The base-10 logarithm of the ratio of pre-flush to post-flush concentrations was a measure, in logs, of toilet clearance.

In order to avoid potential interferences by naturally occurring fluorescent particles that might be present in the main water supply, flush water was pre-filtered. For the HET and

PAT toilets, we accomplished this by placing a high efficiency cartridge filter in the water supply line. For the FOM toilet, which requires a 1-inch diameter supply line to the flush valve and has a high flow rate, provision of filtered water at a suitable flow rate required using a pressurized tank storage system. Main water at a pressure of 55–70 psi was passed through a high efficiency cartridge filter to a 20-gallon pressure tank for storage until needed for a flush. A 1-inch supply line connected the tank to the toilet. Samples of the filtered water verified that it was particle free. Clearance was assessed for the conditions shown in Table 1.

P. fluorescens and C. difficile

The HET or FOM toilet was seeded with a suspension of either P. fluorescens bacteria or a nontoxigenic strain of C. difficile and flushed 24 times at either the higher or lower flush volume. We obtained University of Oklahoma Biosafety Committee approval prior to commencement of the work. P. fluorescens produces greenish-yellow colonies on Kings B agar that fluoresce brightly under 365 (nm) wavelength ultraviolet light. P. fluorescens was isolated on tryptic soy agar, inoculated in tryptic soy broth, and incubated at 28 °C for 48 hr. The bacteria/broth suspension was diluted with additional broth until a turbidity of 26 nephelometric turbidity units (NTUs) was reached, as measured using a turbidimeter. This resulted in a source seed suspension concentration of ~1.5 × 1010 bacteria/mL.

C. difficile was inoculated into a Cooked Meat Medium and incubated at 37 °C under anaerobic conditions for 48 hr. The bacteria/medium suspension was diluted with additional medium until a turbidity of 100 formazin attenuation units (FAU) was reached, as measured using a Hach DR/890 colorimeter. This resulted in a source seed suspension of 3 to 4×108 CFUs/mL.

Prior to seeding, we disinfected the toilet bowl by pouring bleach in the bowl and allowing it to sit overnight. The chlorine concentration was approximately 5,000 mg/L (5,000 ppm) in the bowl. We flushed the toilet a minimum of 10 times to clear the chlorine, and then tested the water for total chlorine using a Chlorine Pocket Colorimeter II. We took a 50-mL toilet tank water sample before the initial seeding for microbial plating to verify the absence of the study microbe in the supplied flush water. We then seeded the bowl water with 50 mL of source suspension and stirred, and took the first (pre-flush) 50-mL water sample. We resampled the bowl water at approximately three minutes after each of the subsequent 24 flushes, and took another tank sample after the 24th flush. A fraction of residual chlorine, typically less than 0.05 ppm, entered the bowl with each flush due to the chlorine content of the main water supply; therefore, samples were de-chlorinated with the addition of one drop of sodium thiosulfate solution. We measured the volume of ejected water after each flush. We performed three to six replicate trials at each flush condition.

We filtered each 15-mL water sample utilizing the membrane filtration technique (Messer & Dufour, 1998). We diluted samples from each flush to avoid cultures that were too numerous to count (TNTC). Diluted samples were filtered through 47 mm diameter 0.45 μ m pore size MCE membrane filters utilizing a three-place vacuum filtration manifold and disposable filter cups. For P. fluorescens, we placed each filter on Kings B agar and incubated them at 28 °C for 24 hr (Alemu & Alemu, 2013). We observed colonies on slower-growing plates

again 48 hr after filtration. We counted colonies under ultraviolet light at 365 nm. For C. difficile, each filter was placed on cycloserine-cefoxitin-fructose agar with sodium taurocholate medium and cultured anaerobically at 37 °C for 48 hr, after which colonies were counted. We also observed plates after 72 hr to confirm counts.

We calculated bowl water concentration in CFUs/mL from the plate count, dilution factor, and volume filtered. We performed scoping trials to determine the dilutions needed to ensure a countable filter for each water sample. Only the pre-flush and first few post-flush samples required dilution.

Results

Approximate mean flush volumes for the lower (LO) and higher (HI) flush volume conditions for the dual-flush toilets were HET LO 3.9 Lpf, HET HI 5.2 Lpf, PAT LO 4.1 Lpf, and PAT HI 4.9 Lpf. The FOM mean flush volume was approximately 5.5 Lpf.

Figure 1 presents bowl water concentration decay results expressed as fraction of initial concentration remaining (mean of all trials). For all toilets, all flush conditions, and all particle suspensions except C. difficile, the toilet bowl water remained contaminated throughout the extended series of flushes. Fractional clearance patterns were similar, though not identical, for all conditions, with ~3 logs particle concentration reduction with the first flush, $\sim 1-2$ logs reduction with the second flush, and < 1 log reductions thereafter. This pattern is consistent with findings reported by others for S. marcescens (Barker & Jones, 2005; Darlow & Bale, 1959), E. coli (Gerba et al., 1975; Yahya et al., 1992), and MS2 bacteriophage (Gerba et al., 1975). Cumulative reductions for the first two flushes were typically 4–5 logs. C. difficile appeared to clear faster and more completely than either microspheres or P. fluorescens in the FOM toilet, and was not detected after the 12th flush. The pattern of persistence exhibited only gradual concentration decline after the first several flushes, consistent with observations by Gerba and coauthors (1975) for seven-flush experiments with E. coli and MS2 bacteriophage. The 3 logs first flush concentration reductions exceeded what would be expected for simple dilution even with perfect mixing, indicating an essentially "plug flow" clearance action as suggested by Darlow and Bale (1959). Cumulative clearances through 4, 12, and 24 flushes varied by toilet type, flush condition, and particle type as shown in Table 2.

Discussion

These concentration decay patterns observed by Yahya and coauthors (1992) for 3 flushes with E. coli, by Barker and Jones (2005) for 4 flushes with S. marcescens, and by Gerba and coauthors (1975) for 7 flushes with E. coli and MS2 bacteriophage were replicated in several toilet types for 4–24 flushes with fluorescent microspheres of various sizes and for up to 24 flushes with two types of viable bacteria. For both microspheres and bacteria, there was a rapid initial decline in the first two flushes, totaling generally ~5 logs, but only gradual concentration decline thereafter. The continuing gradual decline, not discernible by Gerba and coauthors (1975) in their 7-flush experiments, was seen to continue throughout the 24-flush series.

The apparent faster attenuation of C. difficile counts relative to P. fluorescens can be explained by a number of factors: 1) C. difficile might have a reduced affinity for attaching to bowl surfaces (less surface charge), 2) C. difficile might be more prone to exist as clumps of cells that are easier to flush due to their larger size, or 3) recovery of C. difficile might be lower due to culture methods. P. fluorescens colonies are easy to detect visually due to their fluorescent nature, while C. difficile colonies are more visually obscure. Furthermore, the recovery of C. difficile has been shown to vary among different culture media. The taurocholate cycloserine cefoxitin agar medium used in this study has been shown to have lower recoverability for C. difficile than some other media (Carson, Boseiwaqa, Thean, Foster, & Riley, 2013; Eckert, Burghoffer, Lalande, & Barbut, 2013). Nevertheless, we observed C. difficile to persist in the toilet for at least 12 flushes. This persistent bowl water contamination over an extended number of flushes has clear implications for infectious disease transmission risk should the contaminant be a pathogen that can survive under bowl water conditions, such as C. difficile.

The physics of the toilet flush is fairly simple, and it would be expected that fractional clearance would be consistent across flushes. As shown in this and other works, however, it clearly is not consistent. Surface adhesion with subsequent resuspension to the bowl water after the flush seems the most plausible explanation for persistent bowl water contamination, and the similar behavior of inert polymer microspheres and bacteria suggests a physical rather than biological attachment mechanism.

Our water samples were collected within a few minutes of the flush, so resuspension would have to be occurring rapidly. It was unclear, however, whether such resuspension might be limited to a sudden event corresponding to the time period of fluid shear (i.e., the flush duration) or perhaps might continue for some longer time. To explore this question, we conducted a simple follow- up experiment in which we seeded a toilet, flushed four times, and collected bowl water samples from the center of the bowl approximately 2 in. below the water surface at intervals for 30 min following the fourth flush. The results indicated an ongoing resuspension of particles for at least 30 min after the flush, with a doubling of bowl water concentration in that time, from ~300–600 particles/mL, in a linear manner (data not shown). The resuspension might therefore be occurring in a two-phase manner—a rapid release during and/or immediately after the flush, plus a more gradual but still substantial release in the undisturbed bowl water for some period thereafter, likely from submerged bowl surfaces.

The clearance data were modeled from first principles as a two-stage exponential decay due to flush clearance and erosion of an adhered surface layer. Solution of the resulting differential equation yielded:

$$C(x) = \left(C_o - \frac{R_o}{a-b}\right)e^{-ax} + \frac{R_o}{a-b}e^{-bx}$$

where x is the flush number, Co is the initial bowl water concentration (particles or CFUs/mL measured), Ro is an estimated initial reseeding potential (particles or CFUs/mL), and a and b are empirical constants that would be a function of the experimental conditions

(particle type, toilet type, and flush condition). Least squares fit on the logarithms of the concentrations using Excel Solver for each data set resulted in fits similar to those shown in Figure 2 (R2 > .97) for all but one trial). The model does not contain a third term to account for the continued release after the flush, which should improve the fit.

Implications for Infectious Disease Transmission in Healthcare Settings

The persistence of bowl water contamination over many flushes after an initial toilet contamination has important implications for infectious disease transmission in healthcare and other facilities. As reviewed by Johnson, Mead, and coauthors (2013), numerous studies have shown that flushing a contaminated toilet will produce droplet and droplet nuclei bioaerosols that can contaminate surfaces and expose persons by contact or air currents. Multiple flushes of a given toilet will occur between cleaning and sanitation even in a healthcare setting, and once contaminated, the toilet will produce bioaerosols with each flush. This information could be particularly important in a healthcare environment if the toilet is used by a patient with, for example, C. difficile or norovirus infection. Sethi and coauthors (2010) reported pretreatment fecal loadings of 105-106 C. difficile per g of stool in symptomatic patients, while Atmar and coauthors (2008) reported 108–109 norovirus per g of stool, and Caul (1994) reported 106 norovirus per mL of vomit. The potential for droplet and droplet nuclei aerosolization of these and other gastrointestinal pathogens during sequential flushes of a contaminated toilet, with the associated surface contamination and airborne transport, has not yet been characterized and would be a worthwhile avenue of future research.

Conclusion

The sequential flush data developed in this work for three different toilet types using viable bacteria and nonviable surrogate particles showed a consistent bowl water clearance pattern that was quite similar to that reported by other investigators using bacteria and bacteriophages. The clearance pattern in this and other studies suggests a robust and persistent auto-reseeding mechanism that likely involves surface adhesion of particles with subsequent resuspension to the bowl water during and after the flush. The exact auto-reseeding mechanism could not be determined from these data, but it seems likely that a two-phase physical process of surface adhesion and subsequent detachment by hydrodynamic fluid shear during the flush, followed by a slower particle release over many minutes, might be at play. The similarity in clearance patterns for inert polymer microspheres and viable bacteria suggests a physical rather than biological attachment mechanism.

The implication of these results is clear: contaminated toilets are a potential source of recurring surface contamination and droplet nuclei bioaerosol production that could be contributing to healthcare-associated infections. A single toilet flush produces thousands of aerosol droplets, hundreds to thousands of which entrain microbes as large as bacteria and subsequently evaporate to droplet nuclei size and remain airborne for extended periods. It seems highly improbable that such droplet and droplet nuclei bioaerosols produced by toilets contaminated with gastrointestinal pathogens would not be contributors to healthcare-

associated infection incidence, especially for persistent spore-formers such as C. difficile. Additional research is needed to characterize the mechanism of persistent bowl water contamination and identify means to control it, thereby minimizing toilet flush bioaerosol generation and the risk of toilet-related infectious disease transmission by contact or airborne routes.

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Figure 1. Bowl Water Clearance of Fluorescent Polymer Microspheres and Bacteria During Series of Flushes

Results are presented as mean fraction of initial concentration remaining versus flush number for number of replicates (*n*) indicated.

HET = high efficiency gravity flow toilet; FM = fluorescent polymer microspheres; HI = higher flush volume; LO = lower flush volume; PF = *Pseudomonas fluorescens*; PAT = pressure-assisted toilet; FOM = flushometer toilet; CD = *Clostridium difficile*.



Figure 2.

Modeled and Observed Bowl Water Clearance for *Pseudomonas fluorescens* in Flushometer Toilet Obtained via a Least Squares Fitting Procedure on the Logarithms of the Concentrations

Table 1

Experimental Conditions

Toilet Type	Flush Volume Condition	# of Flushes	Particle	Replicate Trials
PAT	Low	12	0.25 µm FM	1
	High			1
HET	Low	24	0.25 µm FM	3
			0.5 µm FM	3
			1.0 µm FM	3
	High	4	0.25 µm FM	3
HET	Low	24	Pseudomonas fluorescens	3
	High			3
FOM	_	24	0.25 µm FM	1
FOM	_	24	Pseudomonas fluorescens	6
FOM	_	24	Clostridium difficile	3

PAT = pressure-assisted toilet; HET = high efficiency gravity flow toilet; FOM = flushometer toilet; FM = fluorescent microspheres.

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Toilet Type and Suspension	4 Flu	shes	12 Fl	ushes	24 Fh	Ishes
	го	IH	ОТ	IH	ОТ	HI
PAT 0.25 µm FM	5.0	5.0	5.1	5.0	-	I
FOM 0.25 µm FM	-	5.4	—	5.7	-	6.0
HET 0.25 µm FM	5.9	5.0	7.0		7.4	I
HET 0.5 µm FM	5.3	I	7.2	-	7.3	I
HET 1.0 µm FM	6.2	I	7.5	-	7.8	I
HET PF	4.5	5.4	5.2	5.8	5.5	6.0
FOM PF	5.	1	5.	6	2.	9
FOM CD	8.	0	.8	6	IN	
* Calculated as loo10 (1)fraction	remain	ino afte	ər <i>n</i> fluc	(set		

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PAT = pressure-assisted toilet; FM = fluorescent polymer microspheres; FOM = flushometer toilet; HET = high efficiency gravity flow toilet; PF = *Pseudomonas fluorescens*; CD = *Clostridium difficile*; LO = lower flush volume; HI = high efficiency gravity flow toilet; PF = *Pseudomonas fluorescens*; CD = *Clostridium difficile*; LO