THE ROLE OF HOSPITAL TOILETS IN MICROBIAL DISSEMINATION AND THE EFFECTIVENESS OF ULTRAVIOLET C IRRADIATION

by

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Abstract

Introduction:

Healthcare-associated infections are a significant public health burden, which affect thousands of Canadians and cost millions of dollars, annually. Flushing toilets can generate pathogen-containing droplets and aerosols, and are a unique challenge in controlling pathogen transmission in healthcare facilities and other settings. This research assessed microbial dissemination of **a)** bacteria and **b)** virus from a flushing toilet in a patient area at Vancouver General Hospital. This project also **c)** evaluated the effectiveness of a permanently installed, automated ultraviolet C (UVC) device for reducing bacterial concentrations in air and on two surfaces in a shared patient bathroom at Lions Gate Hospital.

Methods:

a) Gram negative *Escherichia coli* and Gram positive *Enterococcus faecalis*, were used to simulate human stool during flushing events and viable air samples were collected with the Duo Surface Air System 360 sampler at multiple locations and time points post-flushing. **b)** A norovirus surrogate, MS2 bacteriophage, was also used to assess potential aerosolization of norovirus during flushing. **c)** Airborne and surface bacterial concentrations were compared in a bathroom with UVC and a comparable control bathroom.

Results:

a) Gram negative *E. coli* concentrations exceeded *E. faecalis* immediately post-flush at the location closest to the source, but decreased rapidly at successive time points and further sampling locations. In contrast, the Gram positive *E. faecalis* persisted significantly longer, and sampling location had no effect on its concentrations.

b) Airborne phage was detected at concentrations far above the infectious dose for norovirus of 18 virions, and infectious phage particles were still present up to 60-minutes post-flushing.

c) Airborne and surface bacterial concentrations were significantly reduced in the bathroom with UVC, compared to a comparable control bathroom.

Conclusion:

This work was the first study to evaluate both a Gram negative and Gram positive organism in the toilet plume, and show significantly longer persistence of the Gram positive bacteria. This research also showed that flushing toilets may generate airborne norovirus at concentrations capable of causing infection. Lastly, this work showed that optimized UVC is an effective adjunct to manual cleaning and infection control efforts in bathrooms.

Lay Summary

Flushing toilets can expel microorganisms into the air and onto nearby surfaces, potentially contributing to the spread of pathogens. The goal of this project was to assess airborne spread of two bacterial species and a norovirus-surrogate from a flushing toilet in a patient area at a hospital in Vancouver, Canada. This work showed that certain types of bacteria are able to survive longer in air after flushing than others, and that these organisms may pose a greater risk for the spread of germs from toilets. This research also showed that toilets may play a role in the spread of a common stomach bug, Norovirus. Lastly, this project demonstrated that ultraviolet C (UVC) light can aid in killing germs both in the air and on surfaces in a shared patient bathroom, and that UVC light may be useful in other bathrooms as well.

Preface

The original research idea for this project was first identified by Drs. Karen Bartlett and Elizabeth Bryce. Development of the research idea and design of the research program was a collaborative effort including Drs. Karen Bartlett, Elizabeth Bryce, George Astrakianakis, and myself. I performed all experimental work for the project, with guidance from my supervisor, Dr. Karen Bartlett. Chapters 2 and 3 are based on experimental work conducted at Vancouver General Hospital, with preparatory work performed in the Bioaerosol Laboratory at the School of Population and Public Health at the University of British Columbia. Chapter 4 is based on experimental work conducted at Lions Gate Hospital. Dr. Alexandra Stefanovic also contributed to the development of the research idea and design of the research program for the work performed in chapter 4. With guidance and input from my committee members, Drs. Karen Bartlett, Elizabeth Bryce, and Sarah Henderson, I performed the analysis of the research data. The biosafety number associated with this work is B15-0157.

A version of chapter 4 has been published. Cooper, J., Bryce, E., Astrakianakis, G., Stefanovic, A., & Bartlett, K. (2016). Efficacy of an automated ultraviolet C device in a shared hospital bathroom. American Journal of Infection Control, 44(12), 1692–1694. I conducted all of the testing, and wrote most of the manuscript.

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List of Abbreviations

dH_20 – Distilled water

HAI – Healthcare-associated infection

LEV – Local exhaust ventilation

MRSA – Methicillin-resistant *Staphylococcus aureus*

NS – Normal saline

RH – Relative humidity

TSA – Tryptic soy agar

TSB – Tryptic soy broth

UCL – Upper confidence limit

UVC – Ultraviolet C

VGH – Vancouver General Hospital

VRE – Vancomycin-resistant enterococci

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Many people supported me throughout my thesis work. I would like to give thanks to my supervisor, Dr. Karen Bartlett, who helped me stay focused along the way. This project would not have been possible without your guidance and unrelenting bioaerosol knowledge.

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I would also like to express my gratitude to the Bridge Program, Canadian Institutes of Health Research, and WorkSafeBC for funding the project, and providing me with additional training opportunities along this journey.

To Hyerim Kwon, thank you for your support throughout the writing process.

Lastly, special thanks are owed to my mom and my family, who have supported me throughout my years of education.

I will take with me the lessons learned on this journey into the next chapter of my life.

Dedication

This work is dedicated to the baby girl, Kenya.

Chapter 1: Introduction

1.1 Healthcare-Associated Infections, Routes of Exposure and Transmission

More than 200,00 patients acquire infections while receiving healthcare in Canada each year (PHAC, 2013; Zoutman et al., 2003). These healthcare-associated infections (HAIs) are acquired from both acute and long-term care facilities, as well as home care. Approximately 10% of adults in Canadian hospitals at any given time have an HAI, and 8,000 patients die of these infections annually (Gravel et al., 2007; PHAC, 2013; Zoutman et al., 2003). Unfortunately these figures seem to be increasing in Canada: between 2002 and 2009, in a large network of Canadian hospitals, HAIs increased by almost 12% (Taylor et al., 2016; Zoutman & Ford, 2008). Globally, the World Health Organization estimates that there are hundreds of millions of HAIs annually, costing tens of billions of dollars (WHO, 2011). These infections are caused by a range of different viruses, Gram positive bacteria containing a thick peptidoglycan cell wall, and Gram negative bacteria containing lipopolysaccharide; however yeast and other fungi also cause HAIs (CDC, 2014; Kramer, Schwebke, & Kampf, 2006). Many common healthcare-associated pathogens cause opportunistic infections (Table 1-1), which are infections that occur more frequently in patients with underlying disease, or in hospitalized individuals receiving certain types of medical treatment, particularly antibiotics (CDC, 2014). Although infection from these pathogens is more common in immunocompromised individuals, most of these organisms are also able to colonize healthy people. In such cases the organism is present on the individual, usually on their skin, respiratory or gastrointestinal tract, as part of their normal microbial flora (PHAC, 2013). However, the individual is asymptomatic and therefore unaware that they are serving as a reservoir for the pathogen and potentially contributing to pathogen transmission. There are generally three recognized reservoirs, or sources, of infectious agents: animals, the environment, and humans (PHAC, 2012b). Animal reservoirs are not usually implicated in transmission dynamics in healthcare settings and so will not be discussed here.

Table 1-1 Common healthcare-associated pathogens

Organism	Classification	Common Diseases
Staphylococcus aureus	Gram positive bacteria	Pneumonia, blood and skin infections
Clostridium difficile	Gram positive bacteria	Gastrointestinal disease

Organism	Classification	Common Diseases
Norovirus	Virus	Gastrointestinal disease
Enterococci spp.	Gram positive bacteria	Bloodstream, surgical site, and urinary tract infections
Pseudomonas aeruginosa	Gram negative bacteria	Pneumonia, blood and surgical site infections
Escherichia coli	Gram negative bacteria	Pneumonia, urinary tract infections, and gastrointestinal disease

1.1.1 Routes of Exposure and Transmission

In terms of exposure and transmission dynamics, **exposure** refers to the contact of a susceptible host with a pathogen reservoir, which could be a colonized individual, contaminated environment, or airborne particles (PHAC, 2012b). The organisms do not necessarily have to establish colonization or infection for exposure to occur. **Transmission** is similar, but refers to the transfer of organisms from a reservoir to a new host or environment. Multiple host, pathogen, and environmental factors determine whether transmission leads to the development of disease when transmission involves the transfer of a pathogen to a new human host (PHAC, 2012b). A few of these include host immune status, site of exposure, including dermal, respiratory or gastrointestinal tract, infectious dose of organism, bioburden of pathogen exposure, as well as temperature and relative humidity (RH) of the environment. Exposure and transmission are generally divided into four primary mechanisms: i) vector borne; ii) contact; iii) droplet; and iv) airborne. The latter three are the most relevant transmission mechanisms in typical healthcare settings and will be discussed below. However, organisms can be transmitted by more than one type of transmission mechanism, depending on environmental and host factors (Seto, 2015; Siegel, Rhinehart, Jackson, & Chiarello, 2007).

Contact exposure and transmission occurs either i) directly through physical contact, such as an infected person shaking hands with a new host, or ii) indirectly through contamination of an intermediate object (PHAC, 2012b). An example of the latter could be contamination of a door handle by an infected or colonized source and subsequent transfer from the handle to a new host. However, the most common mode of transmission is indirect transmission through transient

contamination of the hands of healthcare workers, and so many infection control efforts have focused hand hygiene (Duckro, Blom, Lyle, Weinstein, & Hayden, 2005; Monistrol et al., 2012, 2013; Stichler, 2014). After treating an infected or colonized source, the hands can pass pathogens on to a new patient if they are not decontaminated. A number of pathogens responsible for HAIs are transmissible by such contact, including *Clostridium difficile,* methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococcus (VRE), and a number of viruses that cause gastroenteritis, inflammation of the stomach and intesines (PHAC, 2012b).

The next two exposure and transmission mechanisms are droplets and aerosols. These divisions, which are not entirely distinct classifications, but rather exist on a continuum, have been defined by the working group responsible for prevention and control of pandemic influenza in healthcare settings (Figure 1-1) (Annex F Working Group, 2011).

Droplet exposure and transmission occurs through the transfer of microorganisms in small droplets that travel less than 2 meters through the air, and are deposited on the new host (PHAC, 2012b; Xie, Li, Chwang, Ho, & Seto, 2007). Sneezing, coughing, and even talking have been shown to generate droplets (Hamburger & Robertson, 1948), as well as certain medical procedures (PHAC, 2012b). Although small, droplets are large enough that they will settle due to gravity within seconds, preventing them from travelling further in the air (Johnson, Mead, Lynch, & Hirst, 2013). After settling on to surfaces, droplets may contribute to contact transmission by contaminating the local environment (PHAC, 2012b).

Airborne exposure and transmission is similar to droplet exposure and transmission, except that airborne particles are much smaller. Their small size allows them to remain airborne for prolonged periods of time, and potentially to travel long distances, depending on local air currents. Airborne transmission involves a complex interaction of pathogen, host, and environmental factors. Because of this complex interaction, some pathogens show varying abilities to be transmitted via this route, and so airborne transmission can be further subcategorized into obligate, preferential, and opportunistic (Roy & Milton, 2004; Seto, 2015). In the case of obligate airborne transmission, pathogen transmission occurs exclusively through aerosols and not through any other transmission mechanism. This type of transmission is seen

exclusively with *Mycobacterium tuberculosis* (Roy & Milton, 2004). Preferential airborne transmission, which occurs primarily through aerosols, but droplet and contact transmission can also occur, is seen with varicella-zoster (chicken pox) and measles virus (Seto, 2015). Lastly, opportunistic airborne transmission occurs only under certain favorable conditions for some organisms, with other modes of transmission occurring predominately. These are the organisms for which there are likely conflicting findings regarding their ability to transmit via aerosols, depending on the study conditions. Influenza, MRSA, norovirus, and severe acute respiratory (SARS) virus are a few of the organisms that appear to fall into this category (Bonifait et al., 2015; Bos et al., 2016; Hara et al., 2016; Yu et al., 2004). Both the U.S. Centers for Disease Control (CDC) and the World Health Organization (WHO) recognize varicella-zoster, *M. tuberculosis*, and measles virus as airborne transmissible with obligate or preferential classification (Beck-Sagué et al., 1992; Ehresmann et al., 1995; Leclair, Zaia, Levin, Congdon, & Goldmann, 1980; Siegel et al., 2007; WHO, 2014)

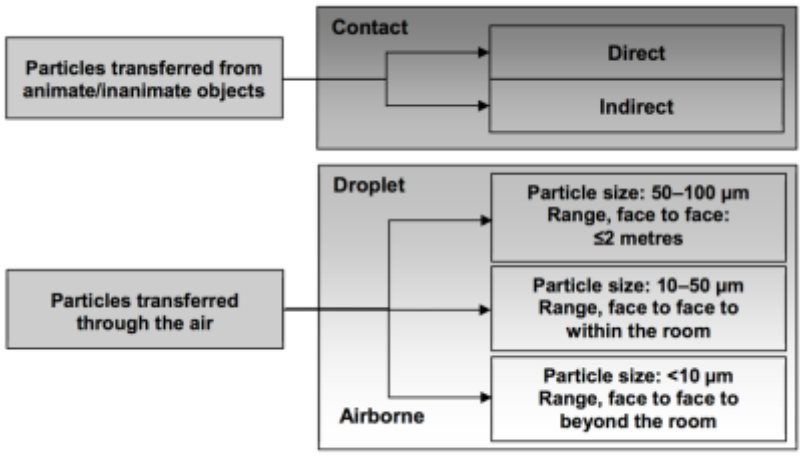

Figure 1-1 Schematic overview of major transmission mechanisms

Source: © All rights reserved. *Routine Practices and Additional Precautions for Preventing the Transmission of Infection in Healthcare Settings.* Public Health Agency of Canada, 2012. Adapted and reproduced with permission from the Minister of Health, 2017.

1.1.2 Pathogen Reservoirs

People and the environment can both serve as reservoirs for pathogens (Table 1-2) (PHAC, 2012b). In addition to transient contamination of the hands of healthcare workers, colonized individuals pose a particular challenge to combatting transmission because they do not display

symptoms and so may represent an unrecognized risk of direct transmission, as well as indirect transmission, through environmental contamination (Knelson et al., 2014; PHAC, 2012b). Even among infected individuals, the person may be shedding pathogen both prior to onset and after resolution of symptoms (PHAC, 2014). Human reservoirs can therefore include anyone who may be present in a healthcare facility: patients, healthcare workers, visitors, and other workers (Boyce, Opal, Potter-Bynoe, & Medeiros, 1993; Muñoz et al., 2002; PHAC, 2012b; Saiman, Siegel, & Cystic Fibrosis Foundation, 2003).

Table 1-2 Pathogen reservoirs

Reservoir Type	Example	Potential Transmission Risk	Reference
Human	Colonized person	Contact, droplet, airborne	(Curry et al., 2013)
	Infected person	Contact, droplet, airborne	(Muzslay, Moore, Turton, & Wilson, 2013)
	Transient hand contamination	Contact	(Monistrol et al., 2013)
Environment	Frequently touched surfaces	Contact	(Eckstein et al., 2007)
	Sink drains	Droplet, airborne	(Bédard et al., 2015)
	Mobile equipment	Contact	(Manian, Meyer, & Jenne, 1996)

The importance of **environmental reservoirs** in pathogen transmission within healthcare facilities has received increased attention in recent years (PHAC, 2012b; Stichler, 2014). Particularly, increased knowledge regarding the survival and growth of organisms on environmental surfaces and the connection between this contamination and infection/colonization has emphasized the importance of good environmental hygiene procedures (Drees et al., 2008; Eckstein et al., 2007; Hayden, Blom, Lyle, Moore, & Weinstein, 2008; Inglis et al., 2010; Kramer et al., 2006). However, human and environmental reservoirs are not static, but rather interact: people contribute significantly to environmental contamination, and in turn the environment has been shown to contaminate the hands of healthcare workers, as well as directly increase the risk of pathogen colonization/infection (Drees et al., 2008; Eckstein et al., 2007; Hayden et al., 2008).

1.2 Bioaerosols, Human Waste, and Toilets

Airborne bacteria and viruses are frequently found as aggregates, along with organic matter, and varying moisture contents, giving the airborne particles a potential range of sizes (Fang, Ouyang, Zheng, & Wang, 2008). Larger droplets may settle onto surfaces due to gravity, and aerosols can remain airborne for prolonged periods of time (Hinds, 1999; PHAC, 2012b; Wells, 1934; Xie et al., 2007). Gravity is a constant acceleration of 9.8 m/s^2 acting upon all matter on earth. It is this constant acceleration that causes a droplet in air to fall to the ground, similar to a baseball falling from the sky. However, one factor that complicates this dichotomization of droplets and aerosols is the fact that droplets can evaporate before settling. This evaporation forms aerosols referred to as droplet nuclei, composed of the tiny particles that remain after partial or total evaporation of moisture within the droplet (Johnson et al., 2013; Wells, 1934). In his seminal work studying the evaporation of droplets, Wells (1934) found that particles with an aerodynamic diameter less than 100 – 125 um would totally evaporate before falling a distance of 2 meters under conditions of 0% RH and 18^{o}C. However, 0% RH is not a condition that would be encountered in indoor environments, and is purely an experimental environment. One of the defining characteristics of a droplet or particle that will determine how quickly it settles is its aerodynamic diameter, which is defined as the diameter of a sphere of water with the same settling time (Stöber, 1971). This finding gave rise to the theory of droplet and droplet nuclei transmission, where droplets larger than 100 um would only travel short distances before settling due to gravity (Wells, 1934; Xie et al., 2007).

When a particle is settling, there are two forces acting on its vertical axis: the acceleration of gravity downwards, and drag forces opposing gravity (Stöber, 1971). When these forces are equal the particle continues to fall, but it does not accelerate because it has reached its terminal settling velocity. For droplet nuclei and small particles, the drag force is large relative to their masses, and so in the absence of any air currents the terminal settling velocities can be quite low (Table 1.1) (Hinds, 1999). However, completely still air is not typically encountered and the fate of the airborne particles is dependent on the local environment. Recent work has shown that airborne particles can be suspended in local turbulent clouds, overcoming the downward acceleration of gravity and remaining suspended in air (Scharfman, Techet, Bush, & Bourouiba, 2016). Prolonged airborne suspension may also facilitate these aerosols to advect in air currents

and potentially travel long distances (Johnson et al., 2013). The 100 um-size definition of droplets from Wells has changed with new studies shedding light on other factors influencing the evaporation rate and fate of particles. Recently Xie et al. (2007) contributed to the work of Wells by developing physical models to look at RH and temperature effects on droplet evaporation (Xie et al., 2007). Relative humidity, a measure of the water content of air, is a ratio of the actual water vapor pressure of air to the saturation, or maximum, vapor pressure at that given temperature and pressure (Shaman & Kohn, 2009). Not surprisingly, Xie et al. found droplets would evaporate more slowly at higher RH, and their calculated critical size for droplet fallout was between 60 -120 um.

Table 1-3 Terminal settling velocity of different sized particles

Aerodynamic Diameter (um)	Terminal Settling Velocity (cm/s)	Time to Fall Two Meters
1	0.0035	>15 hours
5	0.078	43 minutes
10	0.31	11 minutes
100	25	8 seconds

(Hinds, 1999)

The work of Wells and Xie has provided valuable information that can be used to inform decision-making regarding transmission precautions in healthcare settings and that has provided a starting point for defining droplets versus aerosols. However, these studies have not been able to capture the entire range of droplet characteristics and environmental conditions that may affect evaporation and settling. Specifically, factors such as the velocity and trajectory of the formed droplets, the constituents and concentrations of the droplets, and local air movement due to exhaust ventilation would all have impacts in addition to RH and temperature. Therefore, the current definition of droplet is largely functional: droplets will settle due to gravity, whereas aerosols have the potential to travel much further distances. It is impossible to assign a specific size because the size will change with environmental conditions and droplet characteristics. To further complicate this issue, there are the biological factors related to the microorganism itself. Regardless of whether a droplet evaporates to form a droplet nuclei, the microorganism must remain viable for the particle to be infectious. Similar to droplet evaporation, multiple factors

affect microorganism viability in air, including temperature, RH, and UV light exposure (Eames, Tang, Li, & Wilson, 2009; Tang, 2009).

Toilets are one potential source of droplets and bioaerosols in hospitals. Human waste including feces, vomit, and even urine have long been known to contain high numbers of microorganisms (Johnson, 2011). The potential for sewage to generate airborne bacteria was demonstrated over 100 years ago by Horrocks (Horrocks, 1907). Not surprisingly, toilets can serve as a reservoir for pathogens, potentially contributing to their spread in the air and onto surfaces within the bathroom (Johnson et al., 2013). This fact is particularly concerning in healthcare settings, where colonization of the GI tract of patients with healthcare-associated pathogens is common (Jarvis, By, & Mayhall, 1996). This colonization poses a risk to the patients themselves, and a risk of transmission to other patients and healthcare workers by serving as a reservoir and contributing to widespread environmental contamination (Boyce, Havill, Otter, & Adams, 2007). To date many efforts have been made to reduce transmission of HAIs by focusing on several key transmission points: point-of-care hand hygiene; reducing aerosol generation while performing medical procedures; and patient room terminal cleaning, the cleaning procedures performed after discharge of a patient (PHAC, 2012b). One area that has not received due attention is the role toilets and bathrooms play in disease transmission, by not only serving as a reservoir for pathogens, but contributing to their spread by the formation of droplets and droplet nuclei during and after flushing.

1.3 Experimental Studies with Artificially Seeded Toilets

Over the past 60 years, a number of studies have evaluated the role toilets play in generating droplets and bioaerosols, and how these disperse with time (Johnson et al., 2013). One of the earliest works done by Darlow and Bale (1959) involved artificial bacterial seeding of a toilet with *Serratia marcescens* (10^{11}-10^{12} CFU inoculum) and bioaerosol sampling following flushing (Darlow & Bale, 1959; May & Druett, 1953). They used a Bourdillon slit sampler and a Porton impinger fitted with a pre-impinger attachment used to separate particles with an aerodynamic diameter greater than 4 um. Slit sampler results indicated bacterial concentrations greater than $7x10^4$ CFU/m^3 in front of the toilet immediately following flushing, and continued bioaerosol detection up to 7-minutes post-flush. The authors concluded from the impinger results that

almost 90% of particles had an aerodynamic diameter less than 4 um. Further, repeated flushing decreased bowl water bacterial concentrations by greater than 99%, but only decreased airborne bacterial levels by 50-60%.

In 1972, Newsom used both gravitational settle plates and the Bourdillon slit impactor to first assess hospital washroom toilets as a source of bioaerosols (Newsom, 1972). Newsom inoculated the toilet with homogenized feces or between 10^{10}-10^{11} CFU of various Gram negative bacteria. In contrast to the earlier work, most samples had fewer than 700 CFU/m^3, 100-fold less than was previously seen, and Newsom concluded that contamination from toilets was low. More recent work by Barker and Bloomfield (2000) investigated dissemination of *Salmonella enteritidis* (10^8 CFUs) from a domestic toilet. They detected minimal contamination of the toilet seat and toilet lid, and airborne bacteria in only one of three replicate samples at a concentration of 34 CFU/m^3 using a portable impinger air sampler (Barker & Bloomfield, 2000). Although bacterial dissemination was minimal, *S. enteritidis* was reportedly detected from the biofilm at the rear of the bowl below the water line up to 50-days post-inoculation, despite flushing twice a day. Five years later Barker (2005) used the same model of sampler to assess the same domestic toilet for dissemination of *Serratia marcescens* (10^{10} CFUs) and MS2 bacteriophage (10^{10} PFUs) (Barker & Jones, 2005). In contrast to his previous work, samples 1-minute post-flush contained high concentrations of bacteria (1370 CFU/m^3) and phage (2420 PFU/m^3) in the small bathroom (2.6m^3), with detectable levels still found in air 1-hour post-flush. Sampling after repeated flushing showed results similar to Darlow and Bale, with continued generation of bioaerosols (Barker & Jones, 2005; Darlow & Bale, 1959). To assess dissemination of *C. difficile* spores, Best et al. (2011) used fecal suspensions of *C. difficile* spores (2x10^9 CFUs) and collected air samples with the lid open and closed (Best, Sandoe, & Wilcox, 2011). Although their results are not reported as a concentration, closing the lid appeared to effectively decrease CFU counts from 35 to 3 at seat height immediately post-flush, suggesting that toilet seats may help decrease the number of emitted bacteria during flushing. Recent work using high-speed videography has enabled visualization of droplets and aerosols emitted during toilet flushing (Traverso et al., 2013). The video shows substantial emission of both large and small droplets during flushing, and exacerbation of emission by use of surfactants meant to aid in cleaning. Visualizations of flush emissions such as these, although not showing microbial dissemination specifically, help

provide a visual understanding of how droplet and aerosol emissions behave directly above the toilet bowl during flushing.

Collectively, results from these studies suggest bioaerosols generated by toilets contain a mixture of i) large droplets that settle relatively rapidly at short distances from the toilet and ii) smaller droplet nuclei that settle more slowly and may follow air currents. The former would settle at locations within the bathroom and, depending on airflow and bathroom design, may contaminate various bathroom surfaces including the sink and door handle. The latter could remain airborne for prolonged periods of time, either remaining in the bathroom or following air currents out of the room. Further, additional flushing may continue to generate bioaerosols, even in the absence of toilet re-soiling. However, although results from these studies show that flushing toilets can mobilize microorganisms in droplet and aerosol form, they do not show whether toilet plumes lead to human infection.

1.4 Bathroom-Associated Pathogen Transmission in Hospital and Other Settings

Numerous epidemiologic studies have implicated bathrooms in the transmission of a variety of pathogens in hospitals, airplanes, and apartment complexes (Best, Fawley, Parnell, & Wilcox, 2010; Hung, 2003; Muzslay et al., 2013; Widdowson et al., 2005). One potential mechanism for bioaerosol dissemination in hospitals is through the feces of infected or colonized individuals. Diarrhea caused by *C. difficile* has been associated with widespread dissemination of epidemic strains through reported spore aerosolization in hospital settings (Best et al., 2010; Roberts et al., 2008). Best et al. showed both airborne contamination as well as environmental surface contamination of a *C. difficile* strain that was present in patient fecal specimens. Roberts et al. found high airborne concentrations of *C. difficile* spores (>400 CFU/m^3) immediately outside the toilet area in an elderly care ward, consistent with other work showing that bathroom surfaces are highly contaminated compared with other areas of the hospital (Fekety et al., 1981; K. Kim et al., 1981; Roberts et al., 2008). Similarly, another study showed airborne contamination with VRE and widespread environmental contamination throughout the room of a colonized patient, whose colonization status was unknown at the time. Specifically, the toilet area was one of the most heavily contaminated areas (Muzslay et al., 2013). Stool analysis later confirmed colonization with the same VRE strain. The authors concluded that given the high airborne concentration and

rapid environmental dissemination of the bacteria, an airborne 'fecal cloud' likely caused the contamination.

In 2003 in the Amoy Gardens apartment complex in Hong Kong there was a SARS outbreak with 321 cases and transmission linked to bathrooms within the complex (Hung, 2003; Yu et al., 2004). A local investigation, later corroborated by an onsite WHO investigation, implicated bathroom floor drain U-traps, along with open windows, that allowed virus-containing droplets to enter into the bathrooms of residents, condense from moisture within the bathroom, and deposit on surfaces (Hung, 2003). Consistently, many patients with SARS excrete coronavirus in their stools, which would have been one of the mechanisms by which virus entered into the apartment complex sewer system, and subsequently into bathrooms (Peiris et al., 2003).

Norovirus outbreaks occur in a range of settings, including healthcare facilities, restaurants, airplanes, and cruise ships (Chimonas et al., 2008; Verbelen et al., 2004; Widdowson et al., 2005). Bathrooms have been implicated in transmission in a number of these settings. On an international flight in 2002, eight flight crew members had acute gastrointestinal illness and reported frequent use of the airplane bathroom (Widdowson et al., 2005). Despite no obvious soiling of the bathroom, passengers who also developed the illness visited the bathroom significantly more than those who did not develop the illness. Stool samples of hospitalized flight members later confirmed the presence of norovirus. Similarly, a confirmed norovirus outbreak on a 2004 Alaskan cruise ship resulted in 359 cases. The investigative team found that the use of a communal bathroom was associated with illness (Chimonas et al., 2008). These findings are consistent with another cruise ship outbreak, where use of a communal bathroom was associated with illness, and access to a private bathroom had a protective effect (Ho et al., 1989).

These results pose two opportunities for the spread of HAIs. First, airborne transmission may contribute to colonization/infection for some persistent nosocomial pathogens (Bonifait et al., 2015; Bos et al., 2016; Hara et al., 2016; Widdowson et al., 2005), so the toilet plume may pose a direct risk of transmission to patients and healthcare workers. Second, even for those organisms that do not cause infection via airborne transmission, droplet dissemination and subsequent

contamination of surfaces or individuals may pose a significant risk of contact transmission (Barker & Jones, 2005; Best et al., 2010; K. Kim et al., 1981; Muzslay et al., 2013). The latter is exacerbated by the fact that many of the pathogens that may be present in the toilet plume can survive on dry surfaces for weeks to months (Cheesbrough, Green, Gallimore, Wright, & Brown, 2000; Kramer et al., 2006).

1.5 Ultraviolet-C Germicidal Irradiation

Ultraviolet-C (UVC) light, with a wavelength between 200-280 nanometers, can cause irreparable damage to bacterial DNA through the formation of covalent bonds between the double-stranded DNA, rendering the organisms non-viable (Anderson et al., 2013; Bentley, Santoro, Gram, Dujowich, & Marsella, 2016). As such, UVC has been used in hospitals as an adjunct to traditional cleaning methods for several decades (Memarzadeh, Olmsted, & Bartley, 2010; G. J. Taylor & Chandler, 1997). Although the efficacy of UVC for killing microorganisms depends partially on the microorganism itself, UVC effectively kills bacteria, viruses and, to a lesser extent, fungi (Fletcher, Noakes, Beggs, & Sleigh, 2004). A number of studies have evaluated the efficacy of UVC using experimental studies with artificial bacterial seeding, as well its effectiveness using *in situ* studies performed in hospital settings (Anderson et al., 2013; Mahida, Vaughan, & Boswell, 2013; Nerandzic, Cadnum, Pultz, & Donskey, 2010; Rutala, Gergen, Tande, & Weber, 2013; Rutala, Gergen, & Weber, 2010). A study using artificially seeded *C. difficile* spores, MRSA and VRE showed greater than 99.8% reduction in spores within 50-minutes of UVC exposure and greater than 99.9% reduction in vegetative cells within 15-minutes of UV exposure (Rutala et al., 2010). Similar results were observed in another study, with greater than 2-3$\log_{10}$ reductions in CFUs for MRSA and *C. difficile* and greater than 3-4$\log_{10}$ reductions for VRE (Nerandzic et al., 2010). Studies performed in clinical settings have shown over 90% reduction in the frequency of MRSA and VRE positive cultures following UVC use, and 80% reduction for *C. difficile* (Nerandzic et al., 2010). Recently, UVC significantly outperformed manual cleaning using accelerated hydrogen peroxide in the removal of MRSA, VRE, and *C. difficile* (Wong et al., 2015). Similar results have been seen with the use of 5-10 minute exposure-time UVC devices, significantly reducing *C. difficile* and MRSA levels (Rutala, Gergen, Tande, & Weber, 2014).

Fewer studies have focused on the efficacy of UVC in killing airborne microorganisms. An experimental study evaluating the efficacy of UVC on aerosolized *S. aureus, P. aeruginosa,* and *Legionella pneumophila* at different RHs showed reductions in viable organisms ranging from 1.7-4.9 $\log_{10}$ units, with greater reduction at lower RHs (Chang et al., 2012). In contrast to the experimental conditions of the Chang study, a more recent *in situ* study published in May of 2015, evaluated an upper-room UVC device for reducing bioaerosol concentrations in elementary school classrooms (Su, Lau & Gibbs, 2015). Sampling was carried out over four months, and the concentrations of airborne bacteria were significantly lower in the classrooms with UVC treatment compared to a control room without UVC during three of the months.

Collectively these results show that UVC irradiation is an effective tool for disinfecting surfaces as well as killing airborne bacteria. For this reason, UVC has been used successfully as an adjunct to traditional cleaning methods for several decades (Memarzadeh et al., 2010). Although a useful cleaning supplement, UVC has several limitations to its more widespread application. In addition to its biocidal effects on microorganisms, UVC exposure is also harmful to people. Acute exposures cause adverse skin and eye effects and chronic exposures increase the risk of skin cancer (Memarzadeh et al., 2010). Because of these harmful effects, UVC devices can only be used when rooms are unoccupied. However, depending on specific UVC device configurations, run times can be long, in excess of 40 and 50 minutes (Nerandzic et al., 2010; Rutala et al., 2010). In busy areas with regular occupancy or rapid turnover of occupants, such as bathrooms, regular use of UVC devices with long run times may not be practical. Although newer devices often have shorter run times, many are portable to facilitate their use in multiple areas, but physically moving and setting up the devices in different sections of healthcare facilities is time consuming. Permanently installed UVC is a recent technology that would overcome these transport issues. Although UVC represents a promising supplementary decontamination tool, we have not overcome these limitations for its most effective administration.

1.6 Limitations of Previous Work

Investigations into bioaerosol generation from toilets have shown huge variability in bioaerosol concentration and dispersal patterns. One reason for this discrepancy is the different sampling

methods used in the studies. The two most commonly used bioaerosol sampling devices were gravitational settle plates and the Bourdillon slit impactor, both of which have significant limitations. Settle plates are non-volumetric, and cannot provide information about the original concentration of organisms in the air (Crook, 1995). Secondly, larger particles are over-sampled and smaller particles under-sampled because particle collection relies on sedimentation, or settling. The sedimentation rate is a function of the terminal settling velocity, which depends on particle size. As such, a disproportionately greater fraction of large particles will deposit on the plates (Crook, 1995). The other sampling device commonly employed, the Bourdillon slit impactor, is volumetric and thus provides information about the bioaerosol concentration present in air. The Bourdillon impactor directly impacts bioaerosols onto a rotating petri dish containing an agar nutrient medium (Johnson, 2011). The petri dish rotates every 30 seconds, yielding a plate with 30-second snap shot images of the bioaerosol concentration. However, when the airborne concentration is high, as can be the case in a bathroom, the early 30-second snapshot cultures are often near confluence, and so the airborne concentration can only be roughly estimated (Darlow & Bale, 1959). Perhaps the 100-fold discrepancy seen between Darlow and Bale's measurements and those of Newsom could be partially explained by these estimation errors.

Another important factor in the mixed results is that each study examined a different type of toilet. Toilets can be classified by a number of different ways, all of which may contribute to aerosol generation during flushing: i) siphonic vs. wash-down flushing mechanism; ii) bowl and flush volume; iii) single vs dual-flush options; iv) round vs elongated bowl shape; and v) country of manufacture. as specifications and requirements differ (MaP, 2014). Not surprisingly, toilet specifications have changed over time and vary from country to country. The flushing mechanism for most toilets in North America is siphonic, meaning that water drains from the tank into the bowl to generate a pushing mechanism to expel wastes, while a jet of water in the S-trap of the toilet also generates a pulling mechanism (American Standard, 2015; MaP, 2014). In contrast, most toilets in Europe have a wash-down flush, and only the pushing mechanism from draining of the tank water into the bowl is used to expel bowl contents. Bound and Atkinson (1966) compared bioaerosol generation from wash-down and siphonic toilets and found that wash-down toilets produce fourteen times as much bioaerosol as siphonic (Bound &

Atkinson, 1966). In a number of the above studies, either some or all information related to toilet characteristics is missing. To date, many of the studies assessing bioaerosol generation from toilets have been conducted in Europe (Johnson, 2011). Even within British Columbia, the Water Conservation Plumbing Regulation has changed within the last 10 years, such that the maximum allowable flush volume for toilets in Metro Vancouver has decreased from 13.25 liters per flush (lpf) to 6 lpf (Province of British Columbia, 2007, 2008).

Another contributing factor to the discrepancy between studies may be related to inoculum contents and concentrations. The studies outlined above use different bacteria in their inocula, including *E. coli, S. enteritidis, S. marcescens, C. difficile,* as well as fecal suspensions. Compounding this issue is the fact that all studies used different inoculum concentrations. These differences in inocula make comparison between the studies difficult with regards to generalizing about the size and fate of the toilet plume generated during flushing. Further, other than the spore-forming *C. difficile*, no studies thus far have evaluated dissemination of Gram positive organisms from a flushing toilet. Gram positive organisms are generally more resilient to desiccation, and are highly abundant in the gastrointestinal tract. (Kramer et al., 2006; Sattar et al., 2016). Although there is significant variation in microbial species gut flora composition between individuals, Gram positive bacteria are one of the most abundant organisms present, accounting for ~60% of the gut microbiota (Moreno-Indias, Cardona, Tinahones, & Queipo-Ortuno, 2014). To fully understand the dissemination and persistence of bacteria from a flushing toilet after human use, and thus understand the potential risk it may pose for the spread of pathogens, studies employing Gram positive organisms as part of the inoculum are crucial.

Given the limitations of various samplers, diverse properties of toilets, differences between countries, and changing standards over time, it is not surprising that the summarized studies, that date back to the 1950s, have shown huge variability in their results. Compounding this issue, the shortcomings of the different inocula have further limited the generalizability of these results to current toilets in use at healthcare facilities in Vancouver, Canada. Although the studies may serve to provide general information regarding toilet plume production, they cannot be used to predict bioaerosol generation.

1.7 Rationale for Current Study

Healthcare-associated infections pose a major public health challenge, afflicting millions of people every year. Toilets likely play a role in these infections, through the generation of infectious droplets and aerosols. Although previous studies have assessed microbial dissemination from toilets, to date, no published studies have assessed the toilet plume in healthcare facilities in Vancouver. Implementation of necessary infection control policies and practices to prevent or minimize pathogen transmission from the toilet plume is vital to protecting the health of both patients and healthcare workers. To this end, information is needed about the extent to which microorganisms are expelled from the toilet, and how long they persist. These organisms include Gram positive bacteria, Gram negative bacteria, and viruses. In addition to understanding the hazards present in the toilet plume, specific controls targeting the plume and contamination stemming from it must be evaluated. An optimized UVC device that i) is automated, ii) has a short-run time, and iii) is permanently installed may be an ideal candidate for use in washrooms to serve as an effective adjunctive infection control measure.

Research Objective 1: Assess Bacterial Dissemination from a Patient Toilet.

Specific Aim: To use an artificial inoculum to determine the concentrations of a Gram negative and a Gram positive organism in air at four locations, and at various time points up to 30-minutes post-flush in a patient washroom at Vancouver General Hospital (VGH).

Research Objective 2: Assess Viral Dissemination from a Patient Toilet.

Specific Aim: To determine the concentration of a norovirus surrogate in air at various time points up to 60-minutes post-flush in a patient washroom at VGH.

Research Objective 3: Assess Effectiveness of an Optimized UVC Device in a Patient Bathroom.

Specific Aim: To compare airborne and surface bacterial concentrations in a shared patient bathroom with UVC with those in a comparable control bathroom without UVC at Lions Gate Hospital.

Chapter 2: Bacterial Dissemination from a Flushing Patient Toilet

This study assessed the generation of airborne bacteria from a flushing toilet in a patient area at VGH using bacterial inoculums prepared at concentrations comparable with those seen in human stool. Two bacterial species, one Gram positive and one Gram negative organism were used for the inoculum. Air samples were collected following flushing at four locations, and five time points, up to 30-minutes post-flush. Air temperature, RH, absolute humidity, and ventilation measurements were also determined during the sampling period. A total of 19 experiments were performed, with seven experiments conducted at the second sampling location, and four experiments conducted at each of the other three sampling locations.

2.1 Methodology

2.1.1 Bacterial Inoculum Preparation

2.1.1.1 Preparation of Glass Pie Plate Confluent Colonies

Escherichia coli (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were used for preparing the bacterial inoculums. Both of these organisms are commonly found in the large intestines of humans and are present in feces (Katouli, 2010; Lebreton, Willems, & Gilmore, 2014). Each species has a different shape and cellular structure: *E. coli* is a Gram negative bacillus (rod shaped) and *E. faecalis* is a Gram positive coccus (spherical shaped). To obtain sufficient quantities of bacteria for the inoculation, large glass pie plates were used to grow overnight cultures on tryptic soy agar (TSA) (BD, Ontario, Canada). First, static overnight liquid cultures of each organism were grown in 15 mL of tryptic soy broth (TSB) (BD, Ontario, Canada). The following day, the pie plates were prepared by autoclaving 1,500 ml of TSA and twelve large 23-cm glass pie plates covered with aluminum foil. Approximately 125 mL of TSA was poured into each plate and the agar was allowed to harden. Next, 2 mL of the *E.coli* overnight culture was spread-plated with a glass hockey stick onto each of six pie plates, and the same was done for *E. faecalis* onto the other six pie plates. Plates were then incubated at 37^{o}C overnight and bacterial cells were harvested the following day.

2.1.1.2 Harvesting of Bacterial Cells

Bacterial cells were harvested from the pie plates by adding 10 mL of autoclaved sterilized normal saline, 0.85% NaCl in distilled water (dH_20), to each plate and using glass hockey sticks

to detach cells from the agar. Contents were then pooled from three plates into a sterile 50-mL polypropylene tube (Fisher Scientific, Ontario, Canada), and the three plates sequentially washed with another 10-15 mL of normal saline (NS) to remove any residual cells. This procedure was repeated for all pie plates, to yield a total of four 50-mL tubes containing cells (two for each organism). Bacterial concentration was then measured using an Ultrospec II spectrophotometer (Biochrom Ltd., Cambridge, UK) at a wavelength of 500 nm and then $1x10^{11}$ *E.coli* and *E. faecalis* cells (a total of 10^{12} CFUs) were centrifuged separately at 2080 revolutions per minute (RPM) for 20 minutes (See Appendix A and B for OD Vs. CFU graph). Each pellet was resuspended in 10 mL of NS and added to a 50-mL polypropylene tube containing 40 mL of 0.2% w/v agar (Fisher Scientific, Fair Lawn, NJ) solution to give the bacterial inoculum a consistency similar to that of loose stools. In a review by Sender et al., they compiled fourteen studies dating back to 1966 looking at bacterial counts in stool, and found concentrations ranging from $0.35 – 3.2 \times 10^{11}$ CFU per gram of stool, with a mean of $0.92 X10^{11}$ (Sender, Fuchs, & Milo, 2016). Although there is likely to be significant variation in stool masses between individuals, typical stool masses have often been reported at around 100 grams (Cummings, Bingham, Heaton, & Eastwood, 1992; Rendtorff & Kashgarian, 1967). Thus, our inoculum concentration falls within the same order of magnitude as what may be expected in human stools.

2.1.2 Duo Surface Air System 360 Sampler

The dual headed Surface Air System (SAS) 360 bioaerosol sampler (Bioscience Int., Rockville, MD) was used to measure bacterial concentrations in air. This sampler has two heads, each with 219 holes for impaction of bioaerosols onto the media-containing petri plates loaded into the device. Each head samples at a rate of 180 liters min^{-1}, allowing large volumes of air to be sampled in short time periods. Several studies have used this device, or similar SAS devices in various environmental bioaerosol surveys, indoors and outdoors (Bellin & Schilling, 2001; Coccia, Gucci, Lacchetti, Paradiso, & Scaini, 2010; Cooper, Bryce, Astrakianakis, Stefanovic, & Bartlett, 2016; K. S. Lee et al., 2004; Sanchez-Muoz et al., 2012). One consistent concern with the SAS is the relatively high variability between samples (K. S. Lee et al., 2004; Sanchez-Muoz et al., 2012). Because of this variability, Sanchez-Muoz et al. (2012) recommends multiple replicate samples when using the SAS. The short sampling time of the SAS, allowing multiple samples to be collected within a short time period, made it an ideal sampler for the present study.

To help address the issue of variability, both sample heads were used to compare simultaneously collected air samples, in addition to multiple replicate samples collected at each sampling location.

2.1.3 Study Bathroom One – Training Patient Bathroom at Vancouver General Hospital

This study was conducted in the bathroom of a training patient room (Room 894) in the CP8 unit at VGH (Figure 2-1). The bathroom has a volume of 12.5 m^3, and contains a toilet, handwashing sink, and shower, although the shower was never turned on during any aspect of the study. A 0.013 m^2 ventilation duct is located on the ceiling approximately 2.05 m above the toilet. Two permanently closed windows are directly opposite the toilet and shower. The top of the toilet seat is 0.4 m above the ground. The toilet is a cistern-free Delta Teck II Flushometer (Delta Faucet Canada; Masco Canada Limited, London, Ontario) with a manual flush valve, siphon flush mechanism, and an elongated bowl using of six liters of water per flush (Figure 2-2).

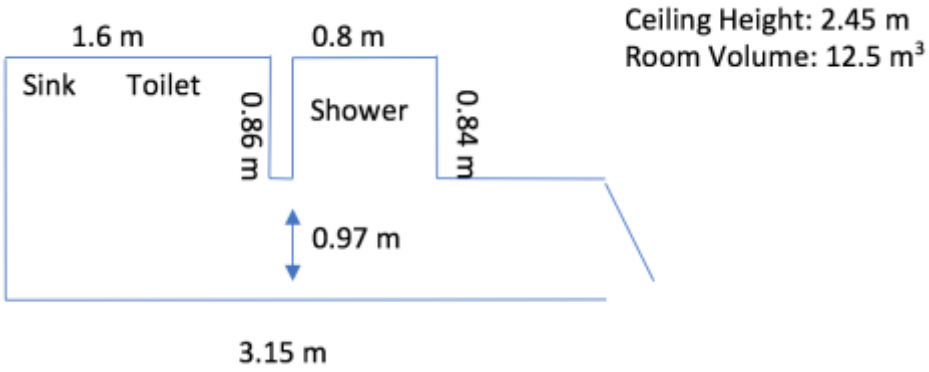

Figure 2-1 Study bathroom one layout

Figure 2-2 Study bathroom one toilet with air sampler

2.1.4 Bacterial Bioaerosol Sampling Procedure

Bacterial sampling was conducted one to two times per week between June and November of 2017. Prior to toilet inoculation with the bacterial culture and air sampling, the toilet was flushed three times to remove any previous contents. After flushing I waited 20 minutes to allow any bioaerosol generated from the flush to settle. Next, two 150-liter pre-inoculation background air samples were collected using both heads of the SAS 360 sampler. Ninety-millimeter petri plates (Phoenix Biomedical Products Inc., Ontario, Canada) containing 30 mL of TSA were used for all bacterial air samples. Immediately prior to each sample collection, the sampler heads were decontaminated with alcohol wipes and the petri plates were loaded into the sampler. After the background air samples were collected, the toilet was inoculated with the two bacterial cultures by pouring approximately half of the 50 mL contents from each tube onto the sides of the bowl, and the remaining half directly into the bowl water. This inoculation method was used to mimic the splashing effects which can occur during acute diarrhea episodes, and has been used elsewhere (Barker & Jones, 2005). After two minutes the toilet was flushed and sequential 150-liter air samples were collected using both sampler heads at the following time points post-flush: < 5 seconds (T_0), 4 minutes (T_4), 8 minutes (T_8), 15 minutes (T_{15}), and 30 minutes (T_{30}).

Between each sample collection the sampler heads were decontaminated with alcohol wipes. This sampling procedure was performed at four sampling locations, at two different sampling heights and two distances from the toilet (Figure 2-3). The sampling heights of 150 cm and 110 cm from the ground were chosen to represent the breathing zone of an adult and a child, respectively. Similarly, the two different sampling distances of 0 cm and 50 cm from the front of the toilet were chosen to capture organisms directly in front of the toilet and organisms that had travelled some distance through the air. From herein the sampling locations are referenced based on their horizontal distance from the toilet and vertical distance from the ground, as follows: H_0V_{110}, $H_{50}V_{110}$, H_0V_{150}, and $H_{50}V_{150}$ (Figure 2-3). All samples were transported back to the University of British Columbia, incubated overnight at 37ºC and colony counts were enumerated the following day. After all samples were collected, the toilet was disinfected by adding 50 – 100 mL of bleach to the toilet bowl water and using a brush to gently scrub the surfaces of the bowl. After 10 minutes the bowl was flushed three times to remove bleach and any remaining contents.

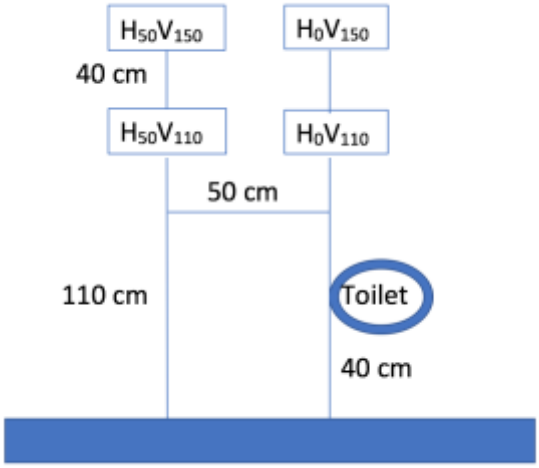

Figure 2-3 Bacterial bioaerosol sampling locations

2.1.5 Bathroom Ventilation, Temperature, and Relative Humidity Measurements

A Velocicalc Plus® anemometer (TSI Inc., Shoreview, MN, USA) was used to measure the flow velocity of the ventilation duct in the bathroom, in order to determine the ventilation flow rate and number of air exchanges per hour. The number of recommended pilot readings required depends on the size of the duct and the agency making the recommendations (Air Monitor

Corporation, 2008). The ventilation duct assessed in our study had a very small surface area, measuring only 7.5 cm across. Because of this small size, the US Environmental Protection Agency guideline of 12 pilot readings was followed, and the flow velocity was measured by taking measurements at three traverses across the short side (7.5 cm) and four traverses across the long side (17 cm), giving a total of 12 pilot readings (Environmental Protection Agency, 1991). When measuring the flow velocity, the probe of the anemometer was held perpendicular to the flow of air from the duct. Readings were taken by allowing the output flow velocity to stabilize, counting to five seconds, and then recording the velocity flow reading from the anemometer. Next, the flow velocity from these readings was averaged, and multiplied by the duct area (128 cm^2) to give the flow rate in volume/time. Lastly, time for one air exchange was calculated by dividing the room volume (12.5 m^3) by the flow rate. The velocicalc plus was also used to measure temperature and RH during each experiment. Relative humidity and temperature measurements were then used to calculate absolute humidity using a standard method previously described (Basu, Feng, & Ostro, 2008; Yantek, 2014).

2.1.6 Colony Enumeration and Bacterial Species Identification

Bacterial colonies were counted after overnight incubation of the sampling plates at 37^{o}C. A three-pronged approach was used for identifying the bacterial species of the colony. First, the colonies were visually inspected, and species identification was often possible because of the distinct colony size, shape, colour, and surface appearance. *E. coli* colonies are roughly five to ten times larger than *E. faecalis* colonies, have a yellowish pigmentation, are roughly circular in shape, and have a more wet, or slimy, appearance. In contrast, *E. faecalis* colonies are much smaller, are white in colour, have a more compact circular shape, and do not have such a wet appearance. At the outset of experimental work, API® strips (Biomerieux Inc., Durham, NC) were used to type the cultures, and these colonies were compared throughout the experiments to the samples collected to serve as the control for quality assurance/quality control during visual inspection of the colonies.

When colonies could not be distinguished by visual inspection, Gram staining (Fisher Scientific, Kalamazoo, MI) was used as the next step in the identification process. Gram staining is a procedure that allows differentiation between Gram positive and negative cells. Prior to Gram

staining, a sterile stab inoculator was used to remove a small amount of material from the colony and spread-plate it onto a TSA plate, which was then incubated at 37°C overnight. This additional grow-up step gave sufficient quantity of colony material for performing the Gram stain technique, described elsewhere (Cornell University - Animal Health Diagnostic Center, 2010).

When the combination of both visual colony inspection and Gram staining could not conclusively identify the colony as *E. coli* or *E. faecalis*, API® strips (Biomerieux Inc.) were used to perform a series of biochemical reactions to confirm colony identification. The API® 20 E strip was used to confirm identification of suspected *E. coli* colonies, and the API® 20 Strep strip for *E. faecalis*.

Once colonies were identified and counted, the positive hole correction factor was applied. The SAS user manual contains a correction table adapted from the original formula from Macher (1989) for a 200-hole impactor head (Macher, 1989). This correction factor accounts for the fact that multiple viable bacterial cells may impact on the agar plate through the same hole in the sampler head and give rise to only one colony, and thus the colony count may underrepresent the actual concentration in the air. This phenomenon is more likely to occur with increasing colonies on the plate, and so the correction table accounts for this fact with a greater correction factor at higher concentrations. Because the correction factor does not consider the specific species present, but rather the total colony counts, when the correction factor was applied, the summed value of the *E. coli* and *E. faecalis* colonies on a given plate were used to find the corrected colony count from the table. The relative proportion of each species was multiplied by the cumulative corrected colony count to give the corrected colony count for each species.

2.1.7 Statistical Analysis

All statistical analyses were performed in JMP (Version 12.0), Numbers (Version 4.2), and Excel (Version 15.32) software. First, a value of 1 CFU/m^3 was used for samples below the limit of detection (LOD). Although a common method for dealing with values below the LOD is to substitute the value of LOD/2 or LOD/$2^{0.5}$ in their place (Finkelstein & Verma, 2001), in this particular case that approach was not used. The repeated airborne bacterial concentrations below

the LOD in the multiple experimental replicates, gave the experimenter confidence that many of the samples below LOD either represented a true absence of the tracer organisms in the air, or at least at concentrations below which LOD/2 or LOD/$2^{0.5}$ would have indicated.

Log-normality of i) the overall bacterial bioaerosol concentration data, ii) the bioaerosol concentration data stratified by species, and iii) stratified by species and location was assessed by considering several factors, including the shape of distribution, Shapiro-Wilks test, kurtosis, skewness, and geometric standard deviation (Table 2-1). Next, arithmetic and geometric measures of central tendency and variability were calculated for both species collectively, and separately. Following this, samples were stratified by species and location to assess the effect of location on airborne concentration for each species using analysis of variance (ANOVA). Next, samples were stratified by species, location, and time point to look at the effects of distance and time on the airborne concentration of each species. Differences between the airborne concentration of the two bacterial species at each time point and location were compared visually by box plots, and numerically using Student's paired, two-tailed t-tests. The choice of paired t-tests comes from the fact that the measured concentrations of the two organisms were coupled by being collected onto the same agar plate, and thus represent a paired sample. Relative humidity, temperature, and ventilation measurements were assessed for normality using the same steps mentioned above, and then measures of central tendency and variability were determined.

Table 2-1 Assessment of data subsets

Data Subset	Assessment
Overall bacterial bioaerosol concentration	Assessment of log-normality, summary statistics
Bacterial bioaerosol concentration stratified by species	Assessment of log-normality, summary statistics
Bacterial bioaerosol concentration stratified by species and location	Assessment of log-normality, summary statistics, ANOVA
Bacterial bioaerosol concentration stratified by species, location, and time.	Box plots, t-tests, geometric means and upper 95% confidence limits
Relative humidity, temperature and ventilation measurements	Assessment of normality, summary statistics

Both the left and right sampler head of the SAS 360 were used for collecting airborne bacterial samples. Two approaches were used to determine whether there was any systematic difference between the heads with regards to measured airborne bacterial concentrations. First, the left head concentrations were subtracted from the right head concentrations, and the distribution of the differences was visualized. Second, for each organism the measured concentrations for the two heads at each sampling location and time point were compared using the Student's paired, two-tailed t-tests.

2.2 Results

All 190 samples were collected between June and December of 2016. Four replicate experiments were performed at each sampling location, except for $H_{50}V_{110}$ where seven replicate experiments were performed. No *E. coli* or *E. faecalis* colonies were identified on the background sampling plates.

2.2.1 Temperature, Relative Humidity, and Ventilation

During the bacterial sampling period temperatures fluctuated between 21°C and 26°C (Table 2-2). Relative humidity showed slightly greater variability, ranging from 37 to 59%, with a mean and median RH of 51%. Absolute humidity ranged from 7.8 to 13 g/m^3. It was approximately six minutes for one air exchange, giving a total of about ten air exchanges per hour.

Table 2-2 Room temperature, relative humidity, absolute humidity and ventilation measurements during bacterial sampling in study bathroom one

	Temperature (°C)	Relative Humidity (%)	Absolute Humidity (g/m^3)	Ventilation (minutes/one air exchange)
N	19	19	19	19
Maximum	26.2	59.1	13.0	6.5
Minimum	21.1	37	7.8	5.8
Mean	22.9	51	10.3	6.1
Median	23	51	10.4	6.0
Standard Deviation	1.25	5.51	1.34	0.19
CAN/CSA Z317.2	22-24	-	-	$\leq$ 6.7

2.2.2 Airborne Bacterial Concentrations and the Effect of Time Post-Flush and Sampling Location

Airborne bacterial concentrations varied markedly with sampling location and time post-flush. Both *E. coli* and *E. faecalis* had maximum airborne concentrations at H_0V_{110} immediately post-flush, with *E. coli* having a concentration of 2853 CFU/m^3, compared with 1733 CFU/m^3 for *E. faecalis* (Table 2-3). Despite a higher maximum concentration, *E. coli* had lower measures of central tendency, both arithmetic and geometric. Reflecting this difference between maximum and average values, *E. coli* showed greater variability than *E. faecalis*, indicated by higher arithmetic and geometric standard deviation values (Table 2-3).

Both *E. coli* and *E. faecalis* airborne concentrations approximated a lognormal distribution, which is common for bioaerosols (Macher, 1999). This claim is supported by several factors: the relatively high geometric standard deviation values (>2.5), low kurtosis and skewness values, and Shapiro-Wilks values of ~0.9 of the log-transformed concentration data stratified by i) species and by ii) species and location (not shown). Additionally, the right-skewed distribution shape of the untransformed and more bell-shaped distribution of the log-transformed above data subsets (not shown) also support this claim.

Table 2-3 Summary statistics of *E. coli* and *E. faecalis* bioaerosol concentrations

	E. coli	***E. faecalis***
N	190	190
Maximum	2853	1733
Minimum	1	1
Median	7	80
Mean	70	137
Standard Deviation	284	208
Geometric Mean	6.1	55.3
Geometric Standard Deviation	7.36	5.34

All values shown are in CFU/m^3 except for geometric standard deviations.

The sampler location had a differential effect on each organism when concentrations were not stratified by time point (Figure 2-4). Airborne *E. coli* concentrations were significantly higher at the closest location (H_0V_{110}) compared with all other sampling locations. In contrast, there was no significant difference between *E. faecalis* concentrations across the four sampling locations.

Thus, the specific sampling location did not affect *E. faecalis* concentrations, but did have an impact on *E. coli*, with lower concentrations observed at further distances from the toilet.

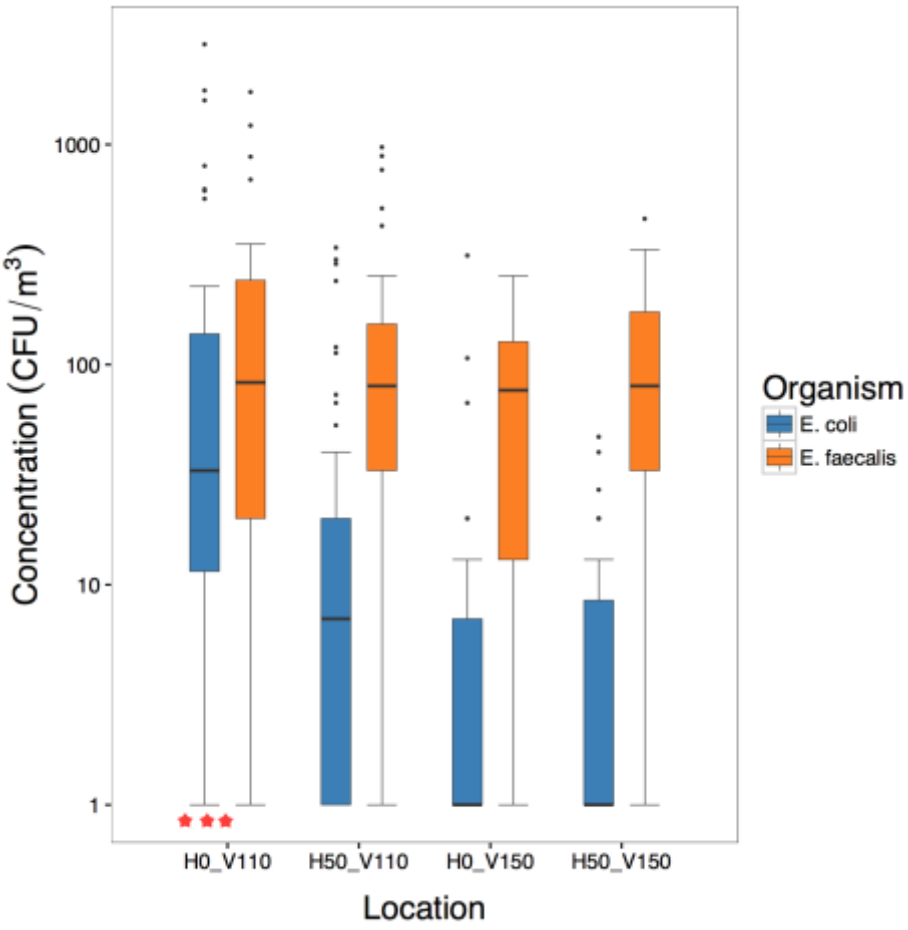

Figure 2-4 Effect of location on airborne bacterial concentrations.
Lower, middle, and upper lines of the box indicate the 25th, 50th, and 75th percentiles, respectively. The upper and lower whiskers represent plus and minus 1.5× the interquartile range, respectively. Three stars indicate a p-value < 0.0001 from ANOVA comparing within species airborne bacterial concentrations across the four locations.

Next, I looked at concentrations at each time point across the four different locations (Figure 2-5). Immediately post-flush (T_0), *E.coli* and *E. faecalis* concentrations were most similar compared with all other time points. The only occasion when *E. coli* concentrations exceeded *E. faecalis* was at this time point (T_0) at the closest sampling location (H_0V_{110}) (Figure 2-5). Looking at the change in concentrations over time, *E. coli* concentrations decreased at each successive time point at all locations. In contrast, *E. faecalis* followed this same pattern only for the closest sampling location (H_0V_{110}). At all other sampling locations, *E. faecalis* concentrations

were similar at 0-, 4-, and 8-minutes after flushing. Not until 15-minutes post-flush was there a clear decrease in *E. faecalis* concentrations. *E. faecalis* concentrations at 4-, 8-, and 15-minutes post-flush showed among the least variability of all the samples, indicated by the short box plots (Figure 2-5).

At time points T_4 through T_{15}, the difference in concentrations between the organisms increased across all sampling locations. Visually, this difference was most pronounced at the higher sampling locations (V_{150}), with *E. coli* concentrations dropping off markedly at the 8- and 15-minute time points, compared with *E. faecalis* (Figure 2-5). At the 8-minute time point, *E. coli* concentrations in the breathing zone (V_{150}) were very low (<10 CFU/m^3), and at 15- and 30-minutes post-flush effectively no *E. coli* was present in the breathing zone (Figure 2-5). In contrast, *E. faecalis* concentrations were significantly higher at 8- and 15- minutes post-flush, with concentrations ranging from 10 – 250 CFU/m^3 (Figure 2-5). By the 30-minute time point, concentrations of both organisms had decreased substantially. Most concentrations at this point were less than 10 CFU/m^3, and the difference in concentration between the two organisms was less than at the 4-, 8-, and 15-minute time points (Figure 2-5).

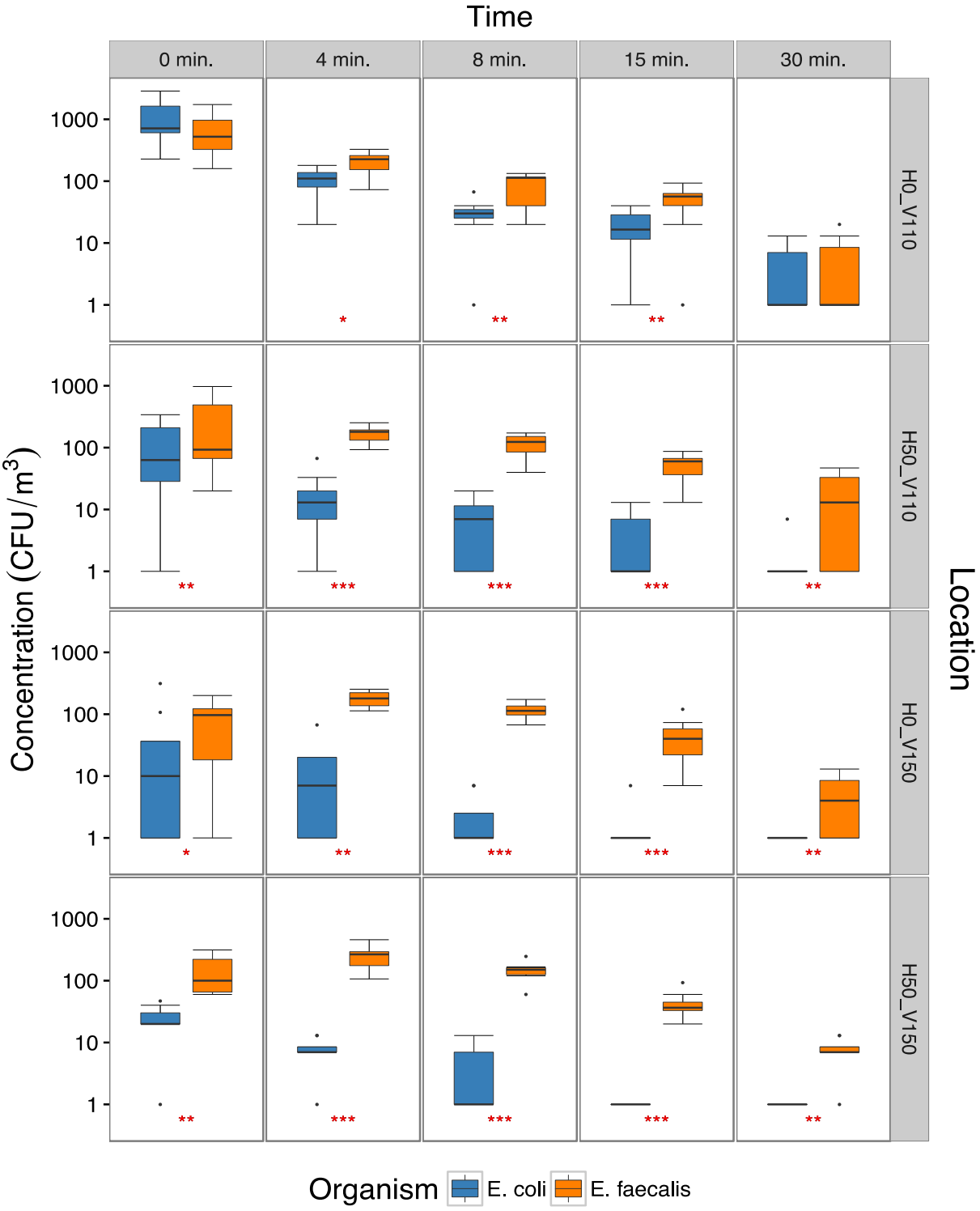

Figure 2-5 Comparison of *E. coli* and *E. faecalis* airborne concentrations at each time point and location. Lower, middle, and upper lines of the box indicate the 25th, 50th, and 75th percentiles, respectively. The upper and lower whiskers represent plus and minus 1.5× the interquartile range, respectively. Three stars indicate a p-value < 0.0001, two stars a p-value < 0.05, and one star a p-value < 0.1.

To assess where the true population mean concentrations may lie, I next looked at geometric means and the 95% upper confidence limits (UCLs) surrounding these for each location and time point (Figure 2-6). Geometric mean concentrations and 95% UCLs were highest for both organisms at the first time point and closest location, with the 95% UCLs for *E. coli* and *E. faecalis* reaching 1692 and 1060 CFU/m^3, respectively. At all other time points and locations, *E. faecalis* concentrations were higher than *E. coli.* Both species appeared to exhibit a characteristic decay in concentration over time. *E coli* geometric mean concentrations steadily decreased with each successive time point at all locations. In the breathing zone (V_{150}), the 95% UCLs for *E. coli* were only about 50 CFU/m^3, and these decreased rapidly for subsequent time points. In contrast, apart from the closest sampling location (H_0V_{110}), *E. faecalis* concentrations were highest at the second-time point (T_4) and gradually decreased after that. The 95% UCLs for *E. faecalis* concentrations remained above 100 CFU/m^3 for all locations up until 8-minutes post-flush, and then dropped to between 50 and 100 CFU/m^3 at 15-minutes. *E. faecalis* was still present in the breathing zone up to 30 minutes after flushing, with the 95% UCL at approximately 15 CFU/m^3.

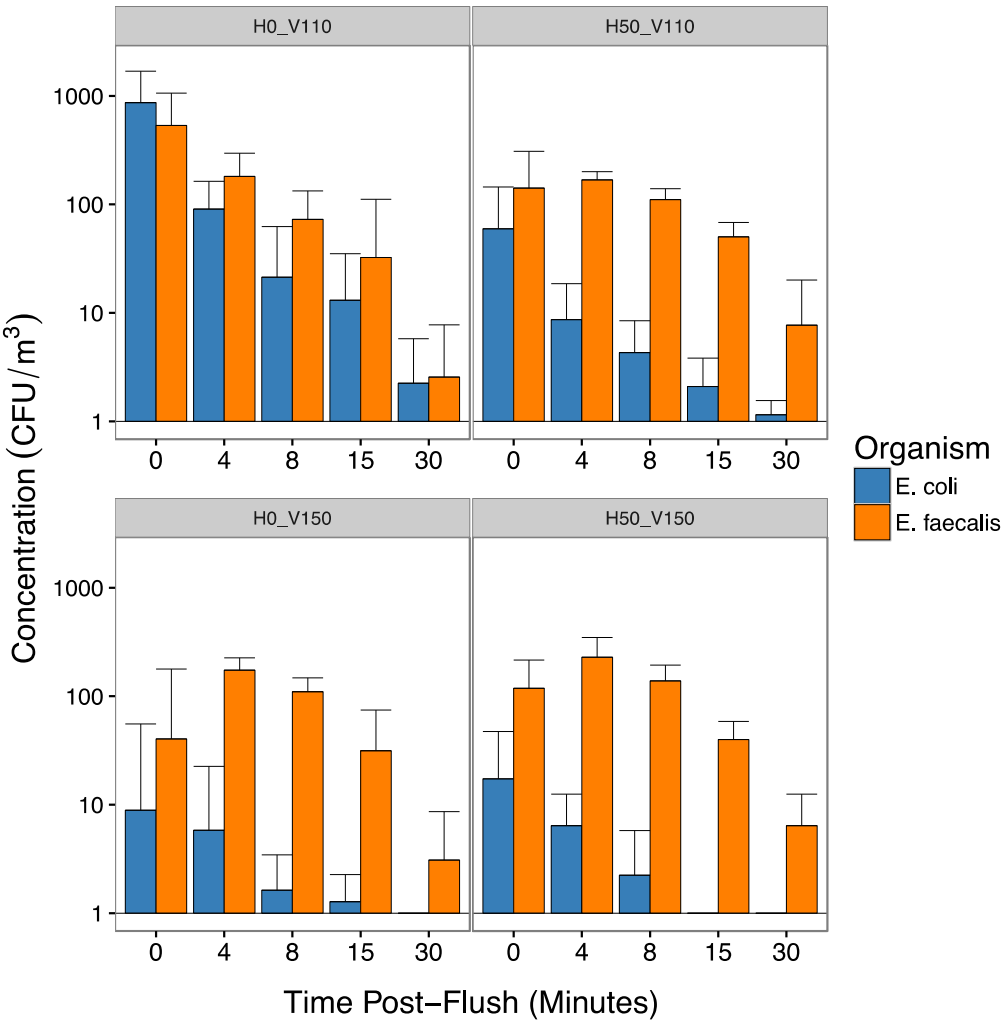

Figure 2-6 Geometric mean bacterial bioaerosol concentrations and 95% upper confidence limits over time stratified by location.

The bars indicate geometric means and the error bars represent the 95% upper confidence limits.

Chapter 3: Viral Dissemination from a Flushing Patient Toilet

This study assessed the generation of airborne virus from a flushing toilet in a patient area at VGH using a norovirus surrogate prepared at concentrations comparable with those seen in human stool. Air samples were collected following flushing at a single sampling location, and seven time points, up to 1-hour after flushing. Air temperature, RH, absolute humidity, and ventilation measurements were also determined during the sampling period. A total of four experiments were performed.

3.1 Methodology

3.1.1 MS2 Bacteriophage

The MS2 bacteriophage has long been used as a model organism for a variety of enteric viruses (Cormier & Janes, 2014). It belongs to the *Leviviridae* family, is approximately 27 nm in diameter, and its icosahedral shape was first described almost thirty years ago (Valegård, Liljas, Fridborg, & Unge, 1990). Norovirus is also icosahedral in shape and is 23-38 nm in diameter (Bertolotti-Ciarlet, White, Chen, Prasad, & Estes, 2002; Prasad et al., 2001). These two properties (size and shape) make MS2 an ideal surrogate for assessing the behavior of norovirus in air. The MS2 bacteriophage (ATCC 15597-B1) and its host bacterial strain *E. coli* (ATCC 15597) used in this study were purchased from Cedarlane (Burlington, Ontario).

3.1.1.1 Growth Media

The double overlay agar assay was used both for propagating virus for preparation of the inoculum, and for preparation of sampling plates to be used during air sampling in the experiments. This technique is commonly used for virus propagation, and involves the use of a firm bottom agar layer and a softer top agar layer seeded with a bacterial culture, in which the virus grows (Cormier & Janes, 2014). Phage broth (used for dilutions) and bottom agar growth media were prepared with TSB (BD) and TSA (BD), respectively, supplemented with: sodium chloride (NaCl) to a final concentration of 8 g/L (Fisher Scientific, Fair Lawn, NJ); glucose to a final concentration of 1 g/L (Becton Dickinson, Sparks, MD); calcium chloride ($CaCl_2$) to a final concentration of 0.294 g/L (Fisher Scientific, Fair Lawn, NJ); and thiamine to a final concentration of 0.01 g/L (Sigma, St Louis, MO). The NaCl was added to growth media prior to autoclaving, while the remaining supplements were dissolved in dH_20, filter sterilized with a

0.45-um filter (Corning Inc., Corning, NY), and added to growth media after autoclaving, once the media had cooled to 50°C in either a hot water bath or on a heating block. Top agar was prepared identically to phage broth, but with the addition of agar (Fisher Scientific, Fair Lawn, NJ) to a final concentration of 0.5 % w/v (added prior to autoclaving).

3.1.1.2 Phage Stock Preparation

The lyophilized phage pellet obtained from Cedarlane was resuspended in 1.2 mL of phage broth, and 100 uL of this was used for generating a large virus stock by first diluting it in 5 mL of phage broth. Next, two large glass pie plates each containing 125 mL of bottom agar were prepared. Following this, 25 mL of top agar was prepared and distributed equally into 15-mL polypropylene tubes (Fisher Scientific, Ontario, Canada) and kept at 50°C using a bench top heating block. Next, 0.75 mL of an overnight culture of the host bacteria grown in phage broth at 37°C was added to each tube containing top agar. Each top agar and host bacteria tube was then added to a glass pie plate containing bottom agar, and the plate was repeatedly tilted until all of the bottom agar surface was covered with top agar. The top agar was then allowed to settle at room temperature. Once the top agar had settled, 2.5 mL of the diluted phage stock was added to each plate, and once again the plate was tilted to allow as much of the agar as possible to be covered with the phage dilution. Plates were then covered with their aluminum foil lids, and incubated overnight at 37°C. The following day, large plaques were observed over almost the entire plate, with bacterial growth only present on the edges where the top agar was not covered with the diluted phage. Phage were harvested by adding 10 mL of phage broth to each plate, and using a sterile glass hockey stick to scrape off the top agar and phage broth. The top agar from both plates was each transferred to separate 50-mL polypropylene tubes, and centrifuged at 4000 RPM for 20 minutes to sediment agar and cell debris. Approximately 10 mL of phage-containing supernatant was then carefully removed from each pellet, passed through a 0.45-um filter and transferred to a 15-mL polypropylene tube. These phage stocks were then quantified (described below) and stored at 4°C.

3.1.1.3 Inoculum Preparation

The phage inoculum used for the flushing experiments was prepared using the same procedures described above, with a few adjustments for upscaling the procedure to produce a larger quantity

of phage. First, instead of two large glass pie plates, twelve were used for preparing the inoculum. From the above prepared phage stock, 1 $X10^{13}$ plaque forming units (PFU) were diluted in 30 mL of phage broth, and 2.5 mL of this was ultimately added to each of the twelve pie plates, after top agar had settled. Following overnight incubation at 37°C, 12 mL of phage broth was added to each plate and scraped with a sterile glass hockey stick. The top agar contents from three plates were pooled into one 50-mL polypropylene tube to give a total of four tubes, which were then centrifuged at 4000 RPM for 30 minutes, and the supernatants passed through a 0.45-um filter (Corning Inc.) into a sterile 50-mL polypropylene tube. The phage concentrations were then quantified and the suspensions stored at 4°C. Viral loads for Norovirus in stool have previously been reported at approximately 8 - 10 Log_{10} PFU per gram of stool (N. Lee et al., 2007). To prepare a comparable phage inoculum, assuming a stool mass of approximately 100 grams, 1.5 $X10^{11}$ PFUs (between 20-30 mL of the phage suspension) were transferred on sampling days to a 50-mL polypropylene tube containing 25 mL of 0.4 % w/v agar solution, to give a final agar concentration around 0.2%. This inoculum was then used that day for the experiment.

3.1.1.4 Quantifying Phage Concentrations

Phage concentration was determined by serially diluting the phage suspension in phage broth, and plating 100 ul of the various dilutions using the double agar overlay technique. First, 90-mm petri plates (Phoenix Biomedical Products Inc.) were prepared with 10 mL of bottom agar. Next, 2.5 mL of top agar was aliquoted into glass tubes and kept at 50°C. One drop of an overnight host bacterial culture was then added to each top agar tube, the tube was vortexed for 5 seconds, and then poured onto a bottom agar-containing petri plate. Once the top agar had settled, 100 ul of the diluted phage was spot-pipetted onto half of the plate, and this was repeated on the other half of the plate with another dilution. Plates were then incubated overnight at 37°C and the plaques enumerated the following day.

3.1.1.5 Sampling Plates Preparation

Plates for sampling airborne phage were prepared similar to plates used for quantifying phage concentrations. The same 90-mm petri plates (Phoenix Biomedical Products Inc.) used for bacterial air sampling were also used for phage sampling. First, 25 mL of bottom agar was added

to each plate. Next, 2.5 mL of top agar was aliquoted into glass tubes, kept at 50°C, and one drop of an overnight host bacterial culture was added to each tube. After vortexing, the 2.5 mL of top agar was added to a bottom agar-containing petri plate and allowed to settle. These plates were then immediately used for sampling that afternoon.

3.1.2 Duo Surface Air System 360 Sampler

As described in Section 2.1.2. the dual headed SAS 360 sampler was used to collect 150-liter air samples at each time point, using the plates prepared in Section 3.1.1.5. There were two changes for the phage sampling protocol compared with the bacterial sampling protocol outlined in Section 2.1.4. First, sampling was only performed at one location: H_0V_{110}. Second, in addition to the sampling time points for the bacterial sampling (T_0, T_4, T_8, T_{15}, and T_{30}), samples were also collected at 45- and 60-minutes post flush.

3.1.3 Study Bathroom

As described in Section 2.1.3, study bathroom one is a patient bathroom at VGH with a volume of 12.5 m^3, a 0.013m^2 exhaust ventilation duct located on the ceiling, and contains a Delta Teck II Flushometer (Delta Faucet Canada; Masco Canada Limited, London, Ontario) with a manual flush valve and a flush volume of six liters per flush.

3.1.4 Bathroom Ventilation, Temperature, and Relative Humidity Measurements

As described in Section 2.1.5., ventilation measurements, temperature, and RH were measured with a Veclocicalc Plus® aneomometer (TSI Inc.). Ventilation measurements were then used with the room volume to determine the time for one air exchange. Relative humidity and temperature measurements were used to determine absolute humidity during the sampling period.

3.1.5 Plaque Enumeration

Plaques were enumerated the following day after sampling. Only macroscopically visible plaques were counted. Because of the specificity of the infection process of the phage with the host bacterial cell, there was no step required for speciating the plaques, as there was for the colonies of the bacterial bioaerosols.

3.1.6 Statistical Analysis

As described in Section 2.1.7, statistical analyses were performed in JMP, Numbers, and Excel software. A value of 1 PFU/m^3 was substituted for values below the LOD. Log-normality of the overall phage bioaerosol concentration data was assessed by considering several factors, including the shape of distribution, Shapiro-wilks test, kurtosis, skewness, and geometric standard deviation. Arithmetic and geometric measures of central tendency and variability were then calculated, along with upper 95% confidence limits.

3.2 Results

All 28 samples were collected between December, 2016 and January, 2017. Four replicate experiments were performed at the sampling location (H_0V_{110}), with samples taken at seven time points. No plaques were observed on any of the background sample plates.

3.2.1 Temperature, Relative Humidity, and Ventilation

Relative humidity was significantly lower than during the bacterial sampling period, likely reflecting a failure of the heating, ventilation, and air conditioning system to adequately humidify the dry winter outdoor air. Relative humidity ranged from 16.8 to 28.7%, with a mean of only 22% (Table 3-1). Temperatures fluctuated very little, ranging from only 21.3 to 22.7°C, and ventilation measurements were similar to those during the bacterial sampling period, at approximately six minutes per air exchange.

Table 3-1 Room temperature, relative humidity, and ventilation measurements during MS2 phage sampling in study bathroom one

	Temperature (°C)	Relative Humidity (%)	Absolute Humidity (g/m^3)	Ventilation (minutes/one air exchange)
N	4	4	4	4
Maximum	22.7	28.7	5.6	6.3
Minimum	21.3	16.8	3.1	6.0
Mean	22.1	21.8	4.2	6.1
Median	22.2	20.8	4.2	6.1
Standard Deviation	0.53	4.86	0.98	0.12

CAN/CSA Z317.2	22-24	-	-	$\leq$ 6.7

3.2.2 Airborne Phage Concentrations and the Effect of Time Post-Flush

Airborne phage concentrations ranged from 173 PFU/m^3 to below the limit of detection of 7 PFU/m^3 (Table 3-2). Similar to the bacterial bioaerosol distributions, airborne phage concentrations approximated a log-normal distribution based on the relatively high geometric standard deviation (>2.5), low kurtosis and skewness values, and Shapiro-Wilks values of ~0.9 of the log-transformed data (not shown), and the general shapes of both the untransformed and log-transformed phage distributions.

Table 3-2 Summary statistics for MS2 bacteriophage concentrations

Maximum	173
Minimum	1
Median	20
Mean	37
Standard Deviation	47
Geometric Mean	12.5
Geometric Standard Deviation	5.8

All values shown are in PFU/m^3 except geometric standard deviation.

Next geometric mean and 95% UCLs were calculated for each time point (Figure 3-1). Airborne phage concentrations appeared to decrease over time in three sequential steps, based on the geometric means. From immediately post-flush to 4-minutes the geometric mean phage concentrations increased from 63 to 96 PFU/m^3, respectively. Airborne phage concentrations then decreased to 14 and 19 PFU/m^3 for the 8- and 15-minute time points, respectively, and finally to below 5 PFU/m^3 from 30-minutes to 1-hour post-flush. However, looking at the upper confidence limits, phage concentrations may remain as high as almost 225 PFU/m^3 up until 8-minutes post-flush, and 113 PFU/m^3 up to 15-minutes after flushing. From 30 to 60-minutes post-flush the upper confidence limits fluctuate, peaking at 70 PFU/m^3 at 45 minutes, and then decreasing to 16 PFU/m^3 one hour after flushing.

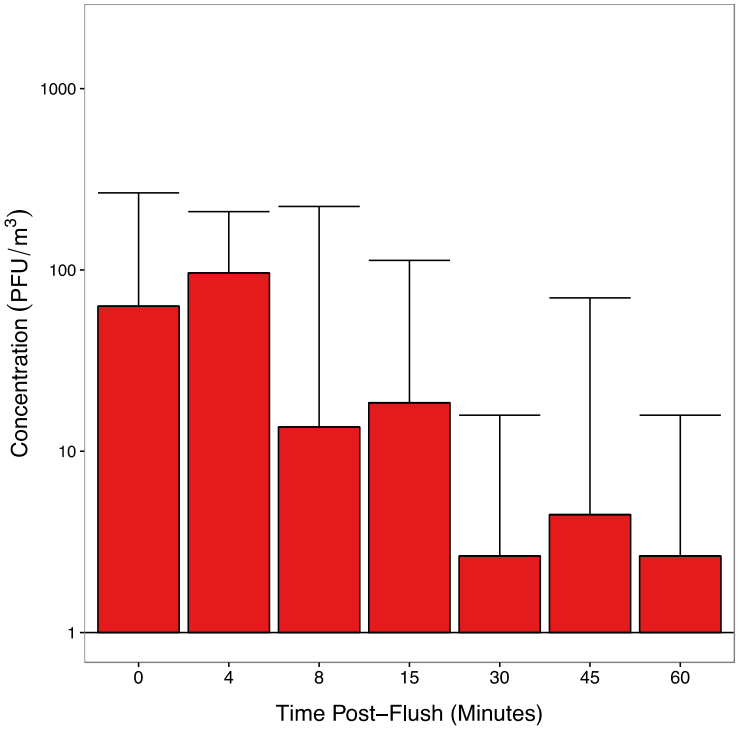

Figure 3-1 Geometric mean airborne MS2 phage concentrations and upper 95% confidence limits over time. The bars indicate geometric means and the error bars represent the 95% upper confidence limits.

Chapter 4: Effectiveness of Permanently-Installed UVC in a Shared Bathroom

This study assessed the effectiveness of permanently installed UVC at controlling airborne and surface bacteria in a shared patient bathroom at Lions Gate Hospital. Air and surface samples were collected in a bathroom with UVC and in a comparable control bathroom without UVC.

4.1 Methodology

4.1.1 Study Bathroom Two (UV and Control) – Shared Patient Bathroom at Lions Gate Hospital

This study was conducted in two common hallway bathrooms in Lions Gate Hospital medical ward. Both bathrooms were shared by up to eight patients and have identical layouts (Figure 4-1). One bathroom has a UVC instrument located directly above the door (UV-bathroom), and the other bathroom does not have UVC (control bathroom). The control bathroom (Room 452.3) is slightly shorter than the UVC bathroom (Room 462.1) (Figure 4-1), with a length of 2.42 m and 11.7 m^3 volume, compared with 12.7 m^3 for the UVC bathroom. Both bathrooms contain small (0.02 m^2) ventilation ducts on the ceiling and 6.0 liters per flush, siphon-jet flush toilets with an elongated bowl (TOTO, Morrow, GA).

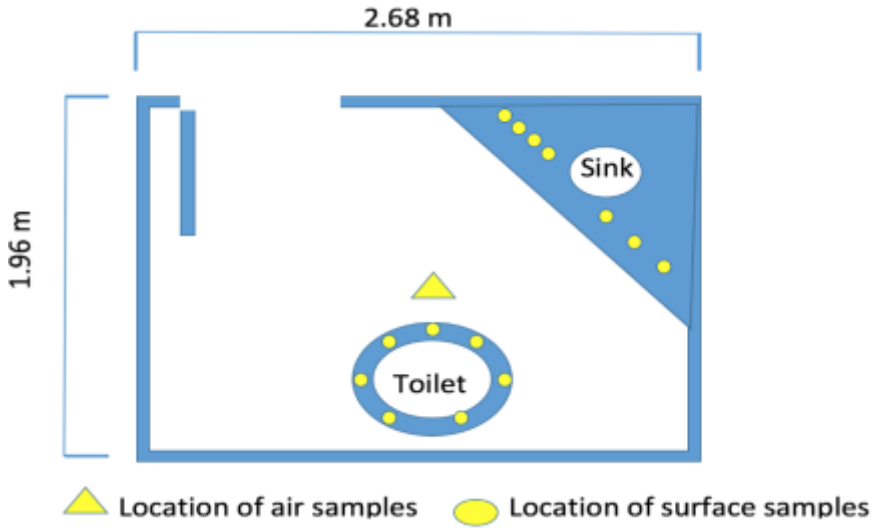

Figure 4-1 Ultraviolet C evaluation study bathroom

4.1.2 Aseptix 1 - Wall-Mounted Ultraviolet C Irradiation

The permanently installed, wall-mounted automated UVC (254 nm) device (ASEPT.1X; Sanuvox, Saint-Laurent, QC, Canada), is located directly above the door in the UV-bathroom. The decontamination cycle initiates when no motion has been detected by the infrared sensors for 30 seconds, after which the UVC cycle will run for 5 minutes. The instrument has an automatic shutoff safety feature that terminates the cycle if the bathroom door is open mid-run. If no motion is detected in the bathroom for a period of four hours, the device will run another 5-minute decontamination cycle to further reduce microbial loads. Manufacturer specifications state an average intensity of UVC measured 7.6 cm (3 inches) from the middle of the bulb to be 4.15 mW/cm^2. A report assessing the same device in another patient bathroom measured irradiances and 5-minute doses at the sink (0.108 mW/cm^2 and 324 J/m^2, respectively) and toilet seat (0.028 mW/cm^2 and 84 J/m^2, respectively) (Hunt & Anderson, 2016). Although UVC can react with atmospheric oxygen to form ozone, previous assessments of Aseptix1 have shown minimal ozone generation and minimal risk to bathroom occupants.

4.1.3 Bacterial Sampling Procedure and Analysis

Air and surface samples were collected from each bathroom five minutes and thirty seconds after patient use – a standardized time that allowed for comparison between the two bathrooms, and for the decontamination cycle to complete in the UVC bathroom. Air samples (150 liters) were collected using both heads of the Duo SAS 360 sampler (Bioscience International) at a height of 1.5 meters directly in front of the toilet. The right head was loaded with 5% sheep blood agar plates (Oxoid, Nepean, ON, Canada) for sampling aerobic airborne bacteria, and the left head was loaded with Brucella blood agar plates (Oxoid) for anaerobic bacteria. Obligate anaerobic bacteria far outnumber aerobic and facultative anaerobic organisms in the human intestine (Gorbach, 1996), and so sampling included both groups of organisms, aerobic and anaerobic. Immediately after sampling the anaerobic plates were placed in a candle jar to maintain a reduced oxygen environment, until incubation at 37°C anaerobically for 48 hours. Aerobic plates were incubated at 37°C for 24 – 48 hours, aerobically. Surface sample locations were matched between the two bathrooms, with samples taken at random locations on the counter and on the top of the toilet seat. Samples were collected with 65-mm Replicate Organism Detection And Counting (RODAC) plates (American Precision Plastics, NorthGlenn, CO, USA) containing

TSA (BD) with a contact time of 10-12 seconds. Plates were then incubated at 37ºC overnight. Total bacterial counts for all samples were enumerated by visual inspection of the plates following incubation. Statistical analyses were performed in JMP. A value of 1 CFU/(10cm)^2 was substituted for surface bacterial concentrations below the LOD. Log-normality of the overall phage bioaerosol concentration data was assessed by considering several factors, including the shape of distribution, Shapiro-wilks test, kurtosis, skewness, and geometric standard deviation. Arithmetic and geometric measures of central tendency and variability were then calculated. Geometric mean bacterial concentrations were then compared between the UV-bathroom and the control bathroom using Welch's t-test.

4.2 Results

All 130 samples were collected in November of 2015 over three sampling days. A total of 34 anaerobic bacterial bioaerosol samples, and 32 surface counter and toilet seat, as well as 32 aerobic bacterial bioaerosol samples were collected.

4.2.1 Temperature, Relative Humidity, and Ventilation

Bathroom air temperature ranged from 20.2 to 24.4ºC, with a mean and median of approximately 23ºC (Table 4-1). Relative humidity fluctuated between 36.8 and 50.9%, with a mean of 41.2%. The ventilation in both the UV-bathroom and the control bathroom had a higher air exchange rate than the bathroom at VGH, with 4.7 and 4.2 minutes per air exchange, resepectively, or just over 14 air exchanges per hour.

Table 4-1 Room temperature, relative humidity, and ventilation measurements during bacterial sampling in study bathroom two

	Temperature (ºC)		**Relative Humidity (%)**		**Absolute Humidity (g/m^3)**		**Ventilation (minutes/one air exchange)**	
Bathroom	UV+	UV-	UV+	UV-	UV+	UV-	UV+	UV-
N	12	12	12	12	12	12	3	3
Maximum	23.9	24.4	50.9	44.4	10.3	8.6	4.7	4.2
Minimum	21.4	20.2	37.1	36.8	7.4	7.3	4.1	3.8
Mean	22.7	22.8	42.3	39.9	8.5	8.1	4.4	4.0
Median	22.6	23.2	41.1	39.7	8.4	8.1	4.3	3.9
Standard Deviation	0.89	1.29	4.68	2.16	0.92	0.39	0.31	0.21

	Temperature (ºC)	Relative Humidity (%)	Absolute Humidity (g/m^3)	Ventilation (minutes/one air exchange)
CAN/CSA Z317.2	22-24	-	-	$\leq$ 6.7

4.2.2 Effect of UVC on Surface and Airborne Bacterial Concentrations

UVC was associated with significantly lower airborne and surface bacterial concentrations (Table 4-2 and Figure 4-2). The UVC-treated bathroom had a 35.2% reduction in geometric mean aerobic bioaerosol concentration compared with the control bathroom (Table 4-2). This difference was even more pronounced for anaerobic bacterial bioaerosols, where the UVC-treated bathroom had a 47.7% reduction compared with the control bathroom. The mean bacterial concentration on the UVC-treated counter was reduced by approximately 95%, compared with the control bathroom. The greatest effect was seen for surface bacteria on the toilet, with a 97% reduction in the UVC-treated bathroom compared with the control (Table 4-2). The effect of UVC was also apparent when looking at other summary statistics: for nearly all conditions UVC reduced maximum concentrations, as well as measures of central tendency and variability (Table 4-2).

Table 4-2 Summary statistics for aerobic and anaerobic bacterial bioaerosols and toilet seat and counter surface bacterial concentrations

	Aerobes (CFU/m^3)		Anaerobes (CFU/m^3)		Counter ($CFU/((10cm)^2)$)		Seat ($CFU/((10cm)^2)$)	
	UV +	UV -	UV +	UV -	UV +	UV -	UV +	UV -
N	16	16	17	17	16	16	16	16
Maximum	313	547	207	513	12	102	304	2305
Minimum	53	127	7	7	1	1	1	1
Median	200	223	40	133	1	41	6	478
Mean	175	253	63	133	2	45	36	576
Standard Deviation	84	102	51	120	3	28	78	665
Geometric Mean	153	237	45	86	2	32	8	224
Geometric Standard Deviation	1.7	1.4	2.4	2.8	2.2	3.1	5.5	7.5
t-test	2.6, p=0.0075		1.96, p=0.0294		5.76, p<0.0001		5.11, p<0.0001	

T-tests were performed on the geometric means

Visualizing the effect of UVC with box plots clearly shows its differential effect on airborne and surface bacterial levels (Figure 4-2). Although UVC reduced airborne aerobic and anaerobic bacterial concentrations significantly (Table 4-2), the shift in air concentrations was much smaller than the shift in surface concentrations (Figure 4-2). The interquartile range for each of the anaerobic and aerobic airborne bacteria clearly overlaps between the UVC and the control bathroom (Figure 4-2). However, for both the counter and toilet seat locations, there was a large separation between the interquartile ranges between the two bathrooms. Although effective against both airborne and surface bacteria, UVC has a much stronger effect on surface bacteria compared with airborne bacteria.

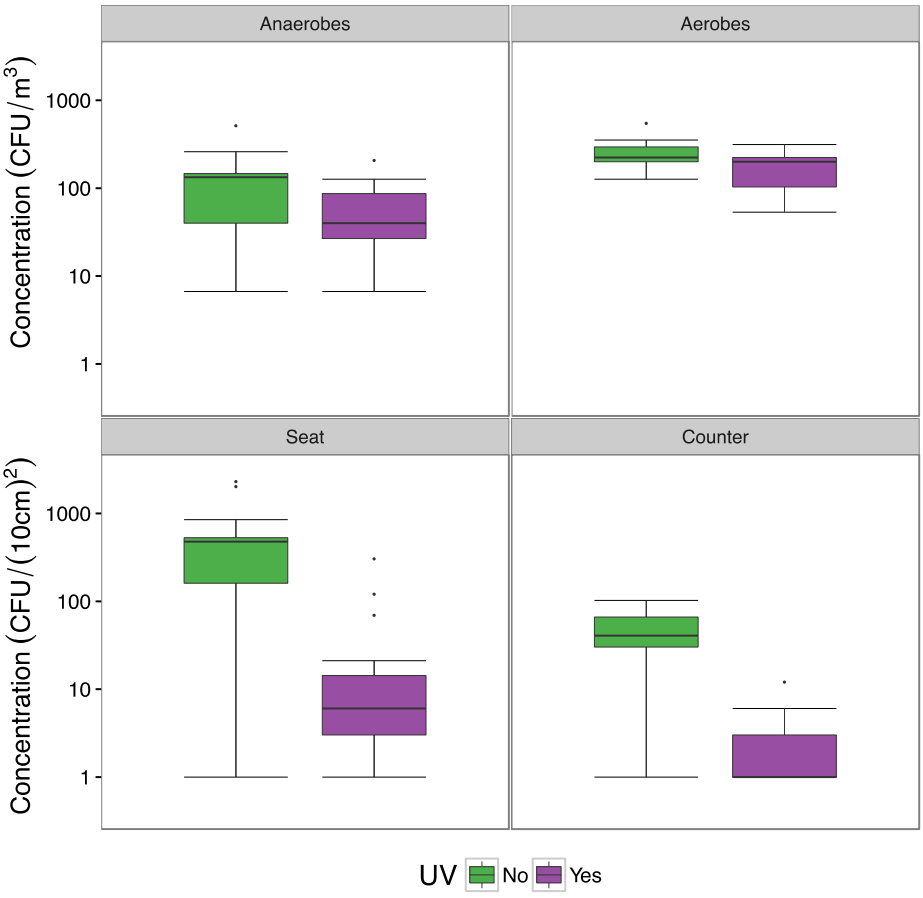

Figure 4-2 Effect of ultraviolet C on airborne and surface bacteria.
Lower, middle, and upper lines of the box indicate the 25th, 50th, and 75th percentiles, respectively. The upper and lower whiskers represent plus and minus 1.5× the interquartile range, respectively.

Chapter 5: Conclusion

5.1 Overview of Major Findings

5.1.1 Toilet Flush-Generated Airborne Bacteria

The first study bathroom was used to assess the generation of airborne bacteria and virus from a flushing toilet in a patient area using bacterial and viral inoculums at concentrations comparable with those seen in human stool. Airborne bacterial concentrations were highest immediately after flushing at the closest location, with *E. coli* concentrations reaching 2.9 x 10^3 CFU/m^3 and *E. faecalis* reaching 1.7 x 10^3 CFU/m^3. Sampling location had no effect on airborne *E. faecalis* concentrations, but did affect *E. coli*, with concentrations significantly higher at the location nearest the source. *E. faecalis* showed greater persistence in air than *E. coli*, with concentrations remaining relatively constant up to 8-minutes after flushing, and then gradually decreasing at 15- and 30-minutes post-flush. In contrast, airborne *E. coli* concentrations decreased more quickly, with essentially no *E. coli* present in the breathing zone at 15-minutes post-flush. This differential survival of Gram positive and negative bacteria is consistent with previous studies looking at indoor and outdoor airborne bacteria (Fang, Ouyang, Zheng, Wang, & Hu, 2007; Heo et al., 2010; Lonc & Plewa, 2010; Rendon, Garcia, & Vital, 2016; Zhu et al., 2003). All studies have shown significantly higher concentrations of Gram positive organisms compared with Gram negative, with Gram positive bacteria accounting for 80-90% of the bacteria present in air in several of the studies (Fang et al., 2007; Rendon et al., 2016). Experimental studies looking at survival of bacteria on surfaces have also shown similar results – longer survival of Gram positive bacteria (Hirai, 1991; Pettit & Lowbury, 1968; Xie, Li, Zhang, & Fang, 2006). The much thicker peptidoglycan cell wall of Gram positive bacteria is believed to contribute to their hardiness by protecting against environmental stresses, including desiccation (Fang et al., 2007; Heo et al., 2010; Lonc & Plewa, 2010; Rendon et al., 2016).

It is difficult to compare my results for flush-generated airborne bacteria with previous findings due to differences in air samplers used, toilet types and flush mechanisms, bathroom dimensions and local ventilation, as well sampling location and sampling time points. The most similar comparison across studies would be looking at maximum concentrations, which typically occur immediately after flushing. In this study, max airborne *E. coli* concentrations were 2.9 x 10^3 CFU/m^3 and *E. faecalis* were 1.7 x 10^3 CFU/m^3, both of which are middle of the range compared

with previous studies (Table 5-1). Although comparing airborne concentrations is challenging due to significant differences in methodology, one commonality between all studies is that toilet flushing generated bacteria-containing droplets (Barker & Bloomfield, 2000; Barker & Jones, 2005; Bound & Atkinson, 1966; Darlow & Bale, 1959; Gerba, Wallis, & Melnick, 1975; Newsom, 1972) and aerosols (Barker & Jones, 2005; Best et al., 2011; Darlow & Bale, 1959; Gerba et al., 1975), which remain persistent for up to 30-minutes after flushing.

Table 5-1 Maximum airborne bacterial concentrations from previous studies

Authors	Year	Test Organism	Airborne Concentration (CFU/m^3)
Darlow & Bale	1959	*Serratia marcescens* (10^{11}-10^{12} CFU)	$>7 \times 10^4$
Bound & Atkinson	1966	*E. coli*	21
Newsom	1972	*Achromobacter* (10^{11}-10^{12})	7×10^3
Barker & Bloomfield	2000	*Salmonella enteritidis* (10^8)	34
Barker & Jones	2005	*Serratia marcescens* (10^{10})	1.4×10^3
Best et al.	2011	Fecal suspensions of *C. difficile* spores	35
Cooper et al.	2017	*E. coli*	2.9×10^3
		E. faecalis	1.7×10^3

5.1.2 Toilet-Flush Generated Airborne Phage

Flushing of the MS2 norovirus-surrogate inoculum generated a maximum airborne phage concentration of 173 PFU/m^3. Airborne phage concentrations appeared to decrease in three sequential steps, with mean concentrations between 50-100 PFU/m^3 at 0- and 4-minutes post-flush, between 10-20 PFU/m^3 at 8- and 15-minutes post-flush, and less than 5 PFU/m^3 at 30-, 45- and 60-minutes. However, based on the 95% UCL, the airborne phage concentrations may exceed 200 PFU/m^3 up to 8-minutes after flushing, and remain as high as 70 PFU/m^3 up to 45-minutes post-flush, far above the infectious dose of 18 virions for norovirus (Bonifait et al., 2015). Barker & Jones (2005) also assessed MS2 dissemination from a flushing toilet and reported markedly higher airborne concentrations than were found in this study: 2.4×10^3 PFU/m^3 at 1-minute post-flush, 1.8×10^2 PFU/m^3 at 30-minutes, and 27 PFU/m^3 at 60-minutes

post-flush (Barker & Jones, 2005). The authors, however, provided little information regarding specifications of the toilet used in the experiments. They also did not specify whether the reported concentrations represent mean or maximum concentrations from the three replicate experiments they performed. Additionally, they collected air samples in closer proximity to the toilet, only 20 cm above the toilet seat. Lastly, during sampling, virus particles were collected onto an entrapment medium, which they assayed using the overlay technique. Likely a combination of different experimental methodology, as well as different toilet characteristics contributed to the discrepancy seen between my results and theirs. Although there is a difference in absolute concentrations between their study and mine, the decay rate at 30-minutes is similar between the two studies. Both studies found airborne concentrations at 30-minutes to be less than 10% of the initial airborne concentration: approximately 4% in my study, and 7% in theirs. Although the toilets in the two studies generated different concentrations of airborne virus, once airborne the virus appeared to decay in a similar fashion between the two studies. These similar decay rates suggest similar environmental conditions between the bathroom in their study and the one in this study, although they provide very few details. Interestingly, their study bathroom was much smaller, with a volume of only 2.6 m^3, compared with 12.5 m^3 for the bathroom assessed in this study.

5.1.3 Effectiveness of Permanently-Installed UVC in a Shared Bathroom

Comparison of airborne and surface bacterial concentrations in a bathroom with a permanently-installed, automated UVC device, with bacterial concentrations in a comparable bathroom without UVC showed significantly lower bacterial concentrations in air and on surfaces in the UVC bathroom. Ultraviolet C irradiation has been used for several decades to complement manual cleaning in hospitals (Memarzadeh et al., 2010; Taylor & Chandler, 1997). Numerous studies have shown its efficacy in experimental lab studies (Anderson et al., 2013; Mahida et al., 2013; Rutala et al., 2010), and its effectiveness in *in situ* studies in hospital settings (Nerandzic et al., 2010; Rutala et al., 2014; Wong et al., 2015). My study was the first to evaluate this novel application of UVC as a permanent decontamination tool in a shared patient bathroom, and to show its effectiveness on both airborne and surface bacteria.

The UVC bathroom had 35% and 48% reductions in aerobic and anaerobic airborne bacterial concentrations, respectively, compared with the control bathroom. The reductions on surface bacteria were markedly more pronounced: 95% and 97% reductions in counter and toilet seat surface bacteria concentrations, respectively, compared with the control bathroom. One potential explanation for this discrepancy could be due to a protective effect of water molecules associated with the cells. Higher RH has been shown to have a protective effect for bacteria against UVC irradiation, potentially by requiring higher UVC energies to damage cellular DNA (Chang et al., 2012). Perhaps many of the airborne bacteria had been aerosolized by the most recent flushing, and their droplets still contained significant water content. In contrast, the surface bacteria may have been present in the bathroom for longer, and so more of their water content associated with the exterior of the cell could have evaporated, making them more susceptible to UVC irradiation. Some studies have used reflective paint to show an increase in the biocidal effect of UVC compared to rooms with normal paint (Jelden et al., 2017). Perhaps reflection at the surfaces increases the intensity of UVC on surface bacteria compared with airborne organisms, causing an increase in the biocidal effect of UVC for surface bacteria not seen for airborne organisms. Alternatively, the higher relative concentrations of bacteria on the surfaces compared with in the air may also account for some of the discrepancy in the germicidal effect of UVC. Su et al. (2015) reported the effect of UVC on airborne bacteria to be less pronounced when airborne concentrations were lower (Su; Josephine Lau; Shawn G. Gibbs, 2015). The higher biocidal effect of UVC on surface bacteria may be partly explained simply by their greater abundance compared with bacteria in the air. However, it is unclear why the efficiency of UVC would be greater when the bacterial concentrations are higher. Another explanation could be related to the orientation of the bacteria, and the effective UVC dose the cells receive in the 5-minute exposure time. Airborne bacteria are moving in three dimensions, and likely changing orientation while airborne. In contrast, surface bacteria would have more limited mobility. It is possible that surface bacteria had a higher UVC dose, which is the irradiance multiplied by the exposure time. The greater mobility and changing orientation of the airborne bacteria may have decreased their effective dose compared to the relatively less mobile surface bacteria.

5.2 Transmission Risk of the Toilet Plume

5.2.1 Gram Positive and Negative Bacteria

This study showed that both Gram positive and negative bacteria are aerosolized during a toilet flushing event. Gram positive organisms persisted in air for up to 15- and 30-minutes post-flush, while the concentrations of Gram negative organisms decreased more rapidly. However, the presence of pathogens in the air does not necessarily mean they will be transmitted to a susceptible a host. It can be difficult to ascertain exactly where the infection of a patient or healthcare worker originates from without genomic testing. Several studies, however, support toilets playing a role in transmission, either through airborne, droplet, or contact transmission. Senn et al. (2016) recently reported a prolonged four-year outbreak of MRSA between 2008-2012 in a tertiary care hospital involving 1,600 patients (Senn et al., 2016). They used whole genome sequencing to identify the specific strain and to show clonal spread of the organism. This specific strain had several distinct characteristics that likely contributed to the prolonged outbreak, and are consistent with toilets playing a role in transmission. First, there was a particularly high enteric colonization rate: 70% of patients carrying the specific MRSA strain who were tested were positive for enteric colonization, compared with 49% of tested carriers for other MRSA strains. Second, asymptomatic carriers played a significant role in sustaining the outbreak, particularly during the early stages, prior to identification of enteric carriage. Although the researchers did not discuss the role of toilets in the outbreak, it is highly likely they contributed to disease transmission. Toilet use and subsequent flushing by both symptomatic and asymptomatic carriers would have generated airborne and environmental contamination and, in the case of asymptomatic carriers, this risk would have been unrecognized. Consistent with this mechanism of pathogen transmission, the researchers found that common 5-bed rooms with shared toilet facilities were the “epicenter of the spread” in numerous wards. Further, compared with other clones, this strain was significantly more transmissible between patients sharing a room. Intensified patient screening for MRSA carriage and increased environmental disinfection were used to control the outbreak. In my study I showed that a Gram positive organism can persist in the air for up to 30-minutes after flushing, and others have shown that organisms present in the toilet plume can settle on surfaces and contribute to environmental contamination (Best et al., 2011; Johnson et al., 2013; Newsom, 1972). In fact, previous studies have shown that MRSA-positive patients with the organism present in their stool generated higher

environmental contamination than MRSA-positive patients whose stool was negative for the organism (Boyce, Havill, & Maria, 2005; Boyce et al., 2007). As such, GI colonization with MRSA has been described as an unrecognized reservoir that may contribute to MRSA transmission in healthcare facilities. Toilet flushing is a likely mechanism for some of the observed environmental contamination in these cases. In the prolonged outbreak described above (where 5-bed rooms with shared facilities served as the focal point for transmission) toilets likely contributed to both direct and indirect transmission. Direct transmission would include the formation of infectious droplets and aerosols that could have been transmitted to other susceptible patients within the shared rooms, as there appears to be evidence of airborne transmission of MRSA under certain conditions (Bos et al., 2016), This airborne MRSA may have contributed to nasal carriage, as well as autoinoculation by healthcare workers and patients. Indirect transmission would include contamination of environmental surfaces and subsequent fomite transmission. Likely a combination of all these factors contributed to the prolonged outbreak.

Clostridium difficile is another healthcare-associated pathogen, which form spores that are resistant to a number of biocidal agents, presenting a significant challenge to controlling environmental contamination. In B.C. there were 2,893 *C. difficile* infections reported in the 2015/16 fiscal year, an increase of 28% from 2014/15 (PICNet, 2016). Transmission of *C. difficile* appears to be associated with toilets, although few studies have directly examined this relationship. Several studies have shown, however, an association between the density of *C. difficile* in stool and the contamination of skin and environmental surfaces (Donskey et al., 2014; Sethi, Al-Nassir, Nerandzic, Bobulsky, & Donskey, 2010). Donskey et al. (2014) found that higher *C. difficile* density in stool was associated with increased *C. difficile* concentrations on the groin, chest, and abdomen, as well environmental contamination on the bedside table and rail (Donskey et al., 2014). Sethi et al. (2010) showed similar results, and even found that the changes in *C. difficile* concentrations in stool over time mirrored the levels of environmental contamination during the same test period prior to, during, and following antibiotic treatment, (Sethi et al., 2010). Of course, there are several mechanisms that may explain this association, including the fact that simply a person with more diarrhea will have greater potential for environmental contamination. But toilet flushing is a probable mechanism for facilitating that

contamination, and is likely one major contributor to the observed contamination, although it was not addressed in either study. Consistent with the role of toilets in *C. difficile*-contamination of the environment, numerous studies have shown surfaces within bathrooms to be among the most contaminated with *C. difficile* in both homes and hospitals (Alam, Anu, Walk, & Garey, 2014; Grünewald et al., 2001; K. Kim et al., 1981; Shaughnessy et al., 2016). One study in particular looked at surfaces in multiple hospital wards, as well as environmental contamination in homes with *C. difficile*-infected persons (K. Kim et al., 1981). They found bathroom surfaces, including sinks, sink cabinets, toilets, toilet seats, and floors to be among the most contaminated. Although these studies found that bathrooms had significant *C. difficile* contamination, they did not discuss the role of toilets and flushing in dissemination of the organism. My study showed that a flushing toilet generates a mixture of droplets and aerosols. These toilet-generated droplets are likely responsible for the environmental contamination seen in the above studies. However, droplets have limited mobility, and so likely *C. difficile*-containing aerosols are also significantly contributing to transmission dynamics.

Although most studies of *C. difficile* have focused on environmental contamination of surfaces, a few have looked at bathrooms and aerosolized *C. difficile* spores (Best et al., 2011; Roberts et al., 2008). Roberts et al. (2008) measured airborne *C. difficile* concentrations outside a bathroom in a multi-bed bay, and found concentrations exceeded 400 CFU/m^3, with almost a third of all samples exceeding 200 CFU/m^3 (Roberts et al., 2008). More recently Best et al. (2011) used fecal suspensions of *C. difficile* spores to inoculate a hospital toilet and measured airborne and surface bacteria after flushing (Best et al., 2011). *C. difficile* was detected in the air up to 1-hour post-flush, and on settle plates at almost all locations in the bathroom. Consistent with these findings, Dulny et al. (2013) looked at risk factors for *C. difficile* infection for patients, and found staying in a multi-bed room or in a private room without an *en suite* toilet significantly increased the risk of infection (Dulny, Zalewska, & Mlynarczyk, 2013). My study supports the theory that a shared toilet facility may increase the risk of pathogen transmission from an infected/colonized patient to a susceptible host. Given the environmental resilience of *C. difficile* spores, their presence in stool, and their potential for aerosolization, toilet flushing represents a significant risk for *C. difficile* transmission, particularly in the case of shared bathroom facilities. This risk has received surprisingly little attention to date.

Another group of organisms that may pose a transmission risk during toilet flushing are the enterococci. *Enterococcus* are a genus of Gram positive cocci ubiquitous in GI tracts, of which *Enterococcus faecalis* and *Enterococcus faecium* are the most common opportunistic pathogens (Lebreton et al., 2014). In the US, enterococci cause 66,000 infections annually, of which 20,000 are resistant to the 'last resort' antibiotic vancomycin (CDC, 2013). One study found widespread contamination of both surfaces and air with *E. faecium* in a 4-bed open bay (Muzslay et al., 2013). The most heavily contaminated surfaces were in the patient bathroom, at almost 3 CFU/cm^2, which was nearly five-times higher than for any other surface tested. Airborne *E. faecium* concentrations in the open bay area exceeded 100 CFU/m^3 during 'peak' contamination. The authors suggested that the 'fecal cloud' may have contributed to the widespread contamination observed, but did not comment on any specific contamination mechanisms. In the same study, the room of an isolated patient with *E. faecalis* colonization also had significant surface and airborne contamination with *E. faecalis*, and the *en suite* toilet area contained the most heavily contaminated sites. In their study, both the open bay and isolated patient room contamination were consistent with spread originating in the bathroom. Toilet flushing may have i) generated bacteria-containing droplets that contributed to the high level of surface contamination observed in the bathrooms, and ii) generated aerosols that spread throughout the open bay and isolated patient room.

Although toilets appear to play a role in the transmission of Gram positive bacteria, there is little evidence of toilets contributing significantly to transmission of Gram negative organisms. Although the lack of evidence may be partly due to investigative infection control efforts focusing on Gram positive organisms, my findings are consistent with the above: airborne Gram negative bacteria concentrations rapidly decreased after flushing. As previously mentioned, one likely explanation for the differential survival of the organisms could be related to desiccation of the less resilient Gram negative bacteria (Bardsley, 1948; Fang et al., 2007; Heo et al., 2010; Lidwell & Lowbury, 1950). Moist environments may be more favorable to supporting growth of these organisms. In fact, the drains in handwashing sinks have been implicated as a source of transmission in numerous prolonged outbreaks of Gram negative organisms (De Geyter et al., 2017; Inglis et al., 2010; Willmann et al., 2015). Although toilets may contribute to the spread of

Gram negative organisms into such moist environments, it is unlikely that toilets contribute significantly to widespread airborne and surface contamination of Gram negative bacteria.

5.2.2 Norovirus

One of the most relevant pathogens associated with the toilet plume is norovirus, a group of viruses which, globally, are the most common cause of gastroenteritis – inflammation of stomach and intestinal lining, causing acute diarrhea and vomiting (CDC, 2016). They are responsible annually for 685 million cases and 50,000 child deaths (CDC, 2016). Bathrooms are frequently implicated in norovirus outbreaks, with toilets likely playing a key role in pathogen dissemination. On a 2002 international flight, numerous crew members who frequented the in-flight bathroom developed acute gastrointestinal illness, later confirmed to be norovirus (Widdowson et al., 2005). Passengers who developed illness reported more frequent use of the bathroom compared with those who did not get sick. Similarly, the use of communal bathrooms has been associated with an increased risk of infection on cruise ships with norovirus outbreaks, while use of private bathrooms has had a protective effect against development of illness (Chimonas et al., 2008; Ho et al., 1989). Although these case-control studies do not definitively show that bathrooms and, specifically, toilets caused pathogen transmission, they are consistent with flushing toilets playing a role.

In 2002, a large, prolonged norovirus outbreak in a long-term care facility afflicted approximately half of all residents and employees (Wu et al., 2005). The authors state that extensive environmental contamination contributed to the outbreak (Wu et al., 2005). Sixty percent of norovirus-positive sites were either in a bathroom or in close proximity to one. Once again, a probable mechanism for transmission is through the generation of norovirus-containing droplets and aerosols from flushing toilets. Norovirus poses a particular challenge in preventing transmission because as few as 18 virions are capable of causing infection (Bonifait et al., 2015; Shamkhali Chenar & Deng, 2017). Although the evidence is still unclear, it is also possible that norovirus may be transmissible via aerosols. Bonifait et al. (2015) used a norovirus animal model, murine norovirus, to show that aerosolized virus particles remained infective using murine cell lines (Bonifait et al., 2015). This finding has been supported by several epidemiological investigations as well (Kuo et al., 2009; H. Xu et al., 2013). Additionally,

norovirus can survive on surfaces for prolonged periods of time, and is resistant to a number of chemical disinfectants (Rajagopalan & Yoshikawa, 2016). These factors – low infectious dose, possible airborne transmissibility, and environmental resilience – make norovirus a particularly challenging pathogen for controlling in the toilet plume. My results showed that toilet flushing produced an aerosolized norovirus surrogate, and that airborne concentrations far exceeded the infectious dose for norovirus. Further, airborne concentrations may remain above the infectious dose for up to 45-minutes post-flush, posing both a direct airborne transmission risk, as well as a possible secondary contact transmission risk.

Norovirus also poses a significant risk for healthcare workers, particularly in long-term care facilities where half of all norovirus outbreaks occur (CDC, 2015). Home care and long-term care workers face unique occupational hazards, including exposure to the toilet plume. Workers often assist patients with getting on/off the toilet (Hittle, Agbonifo, Suarez, Davis, & Ballard, 2016), which may potentially expose them to aerosolized pathogens. Not surprisingly, workers make up a significant proportion of the affected individuals in many norovirus outbreaks in long-term care facilities (Heijne et al., 2012; Wu et al., 2005; Zheng et al., 2015). In fact, attack rates for healthcare workers often exceed those for patients, particularly when the patients are heavily dependent on the care-providers (Heijne et al., 2012; Zheng et al., 2015). For healthcare workers in British Columbia, there were 1,687 lost-time claims due to norovirus infection from 2011-2015, almost three times as many lost-time claims than for any other disease in healthcare. During this period norovirus claims accounted for 45% of all disease-related claims within healthcare. Perhaps most alarming is the trend of norovirus claims in healthcare over the past five and fifteen year periods. From 1990-2005, there was a total of 214 norovirus claims, compared with 3,427 claims from 2006-2015. More recently, 2014 and 2015 saw the highest number of claims recorded at 487 and 339, respectively. Although some of the increase in claims may be due to better surveillance, awareness, and improved reporting, this increase may also represent a true increase in incidence among healthcare workers.

5.3 Controls and Recommendations

5.3.1 Heating, Ventilation and Air Conditioning

Specifications for temperature, ventilation, and RH in Canadian health care facilities are

described in the documented national standards, CAN/CSA-Z317.2-15. Although most measurements were within the specified ranges, the RH measurements in the first study bathroom during the phage sampling were significantly lower than those during other sampling periods, with a mean of approximately 22%. In the above CSA standard, the specified RH range for most hospital rooms is 30-60%, but an RH range for patient bathrooms is not specified. Although the RH is technically not out of range, since no range is specified for bathrooms, it likely reflects a failure of the heating, ventilation, and air conditioning (HVAC) system to adequately humidify outdoor air on those sampling dates, which was during cold, dry weather in January 2017. Water's saturation vapor pressure of air increases exponentially with temperature (Shaman & Kohn, 2009), so cold air has a drastically lower ability to hold moisture compared with warmer air. These challenges in tempering outdoor air are likely faced by many healthcare facilities with older buildings, where retrofitting all the HVAC components may be costly. The low measured RH is also noteworthy because it significantly impacts the fate of the generated bioaerosols.

The HVAC systems in healthcare facilities, including RH control, are important for their direct effects on human health and for their indirect effects via aerosol and droplet physics. The fate of a droplet, which can settle on a surface or evaporate to form a droplet nuclei, is partly determined by its surrounding air temperature and RH (Xie et al., 2007). At lower RHs, droplets are more likely to evaporate to form droplet nuclei and so to remain airborne for prolonged periods of time, while the opposite is true at higher RHs. In our study, the generation of droplets from the toilet flush can be observed at the location nearest the source and first time point for *E. faecalis* (Figure 2-6). Xie's (2007) revision of Wells' evaporation curve, which includes differing RHs, predicts a critical particle size of 100 um at an RH of 50%, which was the average measured RH during bacterial sampling. Below this size, particles are predicted to totally evaporate before falling a distance of 2 meters, and thus remain suspended. Above this size particles are predicted to fall out within 20 seconds (Xie et al., 2007). Therefore, at the 4-minute time point at an average RH of 51%, particles in this study would have evaporated to droplet nuclei suspended in air. As such, we can assume that the concentrations immediately after flushing represented both droplets and aerosols, while the concentrations 4-minutes post-flush represented primarily aerosols. At the location closest to the source, H_0V_{110}, these *E. faecalis* concentrations were 534

and 181 CFU/m^3, respectively, suggesting that twice as many droplets were generated during the flush as were aerosols (Figure 2-6). However, these droplets settled quickly and appeared to be present only at the H_0V_{110} sampling location. In contrast, at all sampling locations, a persistent aerosol concentration of 100-200 CFU/m^3 can be observed up to 8-minutes post-flush for *E. faecalis.* Regardless of whether a droplet settles or evaporates, if the microorganism within the droplet does not remain viable, the particle is no longer infectious. The differences in concentration over time observed between *E. coli* and *E. faecalis* are likely not due to droplet/aerosol settling, but rather due to organism viability, which is also directly affected by temperature and RH.

Generally, RH values from 40-60% are most lethal to airborne bacteria and viruses, though the presence or absence of an outer lipid membrane appears to affect the impact of RH for viruses (Arundel, Sterling, Biggin, & Sterling, 1986). A number of enveloped viruses, including influenza (Harper, 1961; Schaffer, Soergel, & Straube, 1976), respiratory syndrome virus (Hermann et al., 2007), and Japanese B encephalitis virus (Larson, Dominik, & Slone, 1980) are most stable at low RH (<30%). On the other hand, a number of non-enveloped viruses including poliovirus (Harper, 1961; Khalid Ijaz, Sattar, Johnson-Lussenburg, Susan, & Doane, 1985), rhinovirus (Ijaz, Karim, Sattar, & Johnson-Lussenburg, 1987; Karim et al., 1985), and picornavirus (Akers & Hatch, 1968) are most stable at higher RHs (>70%) (Tang, 2009; Verreault, Marcoux-Voiselle, Turgeon, Moineau, & Duchaine, 2015). However, these findings are not universally applicable as numerous enveloped and non-enveloped viruses, including Human Coronavirus 229E (Ijaz et al., 1987), pseudorabies (Schoenbaum, Zimmerman, Beran, & Murphy, 1990), and rotavirus (Ijaz et al., 1985; Sattar, Ijaz, Johnson-Lussenburg, & Springthorpe, 1984) show stability at intermediate RHs. Even different studies looking at the effect of RH on the same virus have shown mixed results, including experimental studies looking at norovirus survival (Kim, Si, Lee, & Ko, 2012; Lamhoujeb, Fliss, Ngazoa, & Jean, 2009). Although the presence of a lipid envelope may impact stability based on RH, it is difficult to determine an absolute effect of RH on virus stability. Instead, other structural components, including viral proteins found within the outer capsid or lipid envelope may have differing susceptibilities to RH, contributing to virion stability (Verreault et al., 2015). Most of the studies cited above were conducted at temperatures between 20-24ºC.

The interpretation of RH can be difficult, given its dependence on temperature. Saturation vapor pressure of air, which is the maximum water vapor pressure of air, increases exponentially with temperature (Shaman & Kohn, 2009). Because RH is the ratio of the partial pressure of water vapor to the saturated pressure of water vapor, identical RHs at two different temperatures can have vastly different absolute humidities. One method to overcome this challenge is to hold temperature constant in laboratory experimental settings. However, *in situ* studies cannot always control the ambient temperature variable. Shaman & Kohn (2009) looked at relative and absolute humidity effects on airborne survival and transmission of influenza in guinea pigs (Shaman & Kohn, 2009). Looking at vapor pressure as a measure of absolute humidity, compared with RH, absolute humidity had a much stronger relationship with both virus survival and transmission, accounting for 90% of virus survival variance. A recent study looking at the effect of absolute humidity on norovirus survival found that it was a better predictor of virus survival than RH, and that absolute humidity below 0.007 kg H_20/kg air was associated with increased virus survival and infection (Colas de la Noue et al., 2014; Shamkhali Chenar & Deng, 2017). Within each section of my study, there was minimal variability in both RH and absolute humidity, so no significant effect of these parameters was seen on airborne virus and bacteria concentrations. However, it is important for future studies to consider both RH and absolute humidity when looking at droplets/aerosols and microorganism survival, particularly in instances when there are large fluctuations in temperature.

Maintaining moderate RH levels can help control transmission of a range of pathogens, prevent fungal growth, and minimize generation of persistent droplet nuclei. Relative humidity above 60% can contribute to the fungal growth (Arundel et al., 1986), while lower RH can facilitate evaporation of droplets to droplet nuclei, and create favorable conditions for transmission of a number of enveloped viruses (Harper, 1961; Hermann et al., 2007; Larson et al., 1980). To minimize the risk of pathogen transmission from the toilet plume, maintaining RH between 30 and 60% would have the most protective effect against the broadest range of pathogens (Arundel et al., 1986; Verreault et al., 2015).

Ventilation is another important engineering control that can help remove pathogens aerosolized during flushing. A minimum of 9 air exchanges per hour in patient bathrooms is specified in the above CSA standard, and all ventilation measurements exceeded this minimum requirement. Although exhaust ventilation would help reduce airborne microbial concentrations, the location of the ventilation ducts in the study bathrooms could potentially expose persons in the bathroom to pathogens present in the plume. Aerosolized pathogens would likely pass through the breathing zone of an individual before being captured by the exhaust ventilation duct located on the ceiling, leading to potential exposure. Ideally, ventilation ducts would be located in areas that prevented pathogens from entering the breathing zone, for example on the wall directly behind the toilet. However, the best option would be ventilation that captures airborne microorganisms before they exit the toilet. One company has developed several toilets capable of exhausting the airspace within the bowl above the water, known as down draft toilets (PRM Services, 2017). These toilets use natural air spaces within the toilet, along with additional duct connections and an external fan to provide local exhaust ventilation (LEV) to the toilet bowl. With recommended flow rates of 5-10 liters/second, these toilets would be able to replace the air within the bowl numerous times in a short period. However, the expense of retrofitting hospital toilets with LEV toilets may be a substantial barrier to their widespread use, with each toilet costing in the range of $1500-2000 including installation (Chessor, 2017). Currently, there are no studies evaluating how effectively these toilets remove organisms present in the toilet plume. Prior to their use in hospitals, an evaluation of these toilets would assist in any cost/benefit analyses to determine their feasibility for use in hospitals or other settings.

5.3.2 Toilet Design

Toilets in British Columbia are regulated with regards to maximum flush volume of water allowed, and toilets in hospitals must be in accordance with the specifications outlined in CSA – Z317.1-16. Specifically, this standard requires hospital toilets to have a maximum flush volume of 6 liters, have a siphon-jet flush mechanism with an elongated bowl, and produce ‘minimal aerosol’. Numerous industry-funded studies have assessed water use, clearing efficiency of toilet contents, cost effectiveness and a number of other toilet characteristics (Capital Regional District, n.d.; Province of British Columbia). However, there is currently no mandated testing for emissions of toilet contents during flushing, and the toilet plume does not appear to be one of the

characteristics assessed in most industry-funded studies. Therefore, when purchasing a toilet either for residential or commercial use, including hospitals, it is not possible to know the extent to which that toilet will generate potentially infectious aerosols. Although numerous studies have assessed bioaerosol generation during flushing, significant differences in methodology and incomplete information about toilet characteristics make it impossible to extrapolate the information from those studies to predict bioaerosol generation from a new toilet.

One of the simplest ways to improve toilet design from the perspective of bioaerosol generation would be to require toilet manufacturers to perform a basic assessment during flushing. A simple, standardized sampling protocol, either performed by the manufacturer or a consultant, could help identify specific toilet designs, flush mechanisms, bowl shapes, or other toilet characteristics that may be associated with releasing a large quantity of bowl contents into the air. This information would be particularly useful in healthcare settings, with vulnerable populations and people potentially infected/colonized with pathogens that may be transmitted via the fecal/oral route. Although the above CSA standard requires toilets to have minimal aerosolization, there are no specific requirements given as to what is meant by 'minimal'. Mandated toilet testing could ultimately lead to the development of an industry standard with regards to a maximum allowable bioaerosol concentration originating from the flush. Such a standard would be a good first step in ensuring that toilets, particularly in healthcare facilities, are not unnecessarily exposing patients and workers to potential pathogens.

5.3.3 Toilet Lids

Perhaps the simplest engineering control would be to install toilet lids on hospital toilets to prevent release of aerosols and droplets from the toilet during flushing. Several studies have assessed the impact of closing lids on the generation of airborne bacteria from flushing, but unfortunately the results have been mixed. Darlow and Bale (1959) showed that closing the lid prior to flushing on a wash-down toilet decreased bacterial bioaerosol concentrations by approximately 2.5-fold at seat height immediately following flushing (Darlow & Bale, 1959). This reduction was primarily of larger particles. Bound and Atkinson also assessed the effect of closing two different types of lids (Bound & Atkinson, 1966). However, unlike the previous work of Darlow and Bale, they found that for both lid types, closing the lid had no significant

effect on airborne bacterial levels at seat height immediately following flushing. Similarly, Barker and Jones (2005) found that lid closure did not affect airborne bacterial concentrations 30 cm in front of the toilet, at 20 cm above seat height (Barker & Jones, 2005).

More recently, in agreement with the earlier work of Darlow and Bale, Best et al. (2011) found that flushing with the lid down resulted in a nearly 12-fold reduction in airborne *C. difficile* spores at seat height immediately following flushing (Best et al., 2011). However, measurements taken 10 cm above seat height showed similar concentrations with the lid open compared with closed.

Several factors likely contributed to these mixed results observed for closing the toilet lid. First, test conditions for each study varied markedly: different test organisms, different toilets each with varying flush characteristics, and different air samplers used. Whether or not lid-closure has any effect on the release of organisms from within the bowl is likely dependent on the toilet itself and the plume it generates. Most toilet lids do not form a tight seal around the top of the toilet, but leave a space of approximately 10-20 mm from which aerosolized microorganisms may escape. Depending on i) the size of droplets and aerosols, and ii) air currents within and around the bowl generated during the flush, airborne microorganisms may impact onto the lid, onto another surface within the toilet, or escape through the airspace. Although no studies to date have looked at the impact of specialty lids that do not contain an airspace, it is likely that such lids would at least reduce the escape of droplets and possibly aerosols. Perhaps some of the discrepancy in the results mentioned above regarding the effect of lid closure could partially be due to different airspace sizes between the lids.

Despite the mixed results seen thus far, it is reasonable to believe that lids would at least help prevent the release of larger droplets, whereas the fate of smaller droplets and aerosols may be harder to predict. Given the relatively low cost of installing toilet lids on hospital toilets, compared with some of the other interventions discussed here, toilet seats may be a feasible control for the toilet plume. Of course, a practical issue with widespread installation of toilet lids would be the burden of additional cleaning and maintenance issues for healthcare workers, as well as an additional contact transmission risk for the workers. To prevent potential exposures of

healthcare workers to pathogens that may be associated with the toilet lids, proper training and education regarding the risks and best practices would be required.

5.3.4 Ultraviolet C Irradiation

My study showed that an automated, permanently installed UVC device with a short run time effectively reduced both surface and airborne bacterial levels in a shared patient bathroom. Several features of this technology make it promising for decontamination in a variety of bathrooms, beyond hospitals. First, the total automation and permanent installation of the device allow it to clean without the need for a person to manually operate the device. Together with the short run time, these features enable the device to cycle regularly, potentially minimizing bacterial loads in bathrooms between most occupant visits. The automated shut off safety feature, which is triggered when a person enters the bathroom mid-cycle, minimizes disruptions in bathroom use by preventing bathroom closures during cleaning, while still maintaining occupancy safety. Questions have been raised, however, regarding the possibility of an occupant remaining motionless in the bathroom for the 5-minute wait period before the cycle initiates. In this case, the machine would begin its cycle while the bathroom is still occupied. However, the manufacturer has developed a similar device which senses body heat to prevent this safety concern.

Shared, single occupancy bathrooms are an ideal location for these devices, where occupants may be exposed to pathogens from a previous user. In fact, some commercial airliners, such as Boeing, have begun installation of UVC devices in their in-flight bathrooms (Jensen & Longacre, 2016). In hospital settings, where shared patient bathrooms likely contribute to pathogen transmission, these devices would greatly supplement more time-consuming manual cleaning methods, and allow for more frequent decontamination. However, there are several limitations to UVC that may limit its more widespread application. First, UVC is harmful to people, so bathrooms must be vacant while UVC devices are in operation. In multi-occupant and very busy single occupant bathrooms, this limitation may limit the frequency at which the device can run. Shadows or dark areas in bathrooms may also be an issue. The biocidal effect of UVC is limited if the organism is not directly exposed to UV light (Jelden et al., 2017). Decontamination of unexposed surfaces would be minimal, and would require additional attention during manual

cleaning. If these types of surfaces were abundant in a bathroom, UVC may be partially ineffective. Ultraviolet C-resistant bacteria may be another concern, if this technology was widely implemented. Photolyase-containing bacteria possess repair enzymes that render them resistant to UVC treatment (Marizcurrena et al., 2017), and long term use of UVC could select for such resistant bacteria. If these genes were horizontally transferrable, that is, passable from one cell to another via a plasmid, then the resistance could clonally spread through a population of bacteria, similar to antibiotic resistance. Despite these potential limitations of UVC, the device assessed in this study is a promising decontamination supplement to manual cleaning methods in single occupancy bathrooms, and is an effective tool for helping to control both airborne and surface bacteria originating from the toilet plume. Perhaps the next generation of this technology would be applying UVC directly in the toilet itself, and killing microorganisms before they are transmitted to people or the environment.

5.3.5 Administrative Controls

A seemingly practical administrative control for reducing the risk of pathogen transmission from toilets would be to implement policies targeted at patients with known infections that are transmitted via the fecal-oral route. For example, if a patient is known to have a *C. difficile* infection, it may be prudent to require that patient to use a designated bathroom with or without UVC decontamination, and to curtail use by patients without similar infections. However, one obvious limitation to this type of control may be a lack of sufficient bathrooms required for this sort of designated use. Another, perhaps more challenging, limitation is the role of asymptomatic carriers. A recent study found that new *C. difficile* cases were linked to asymptomatic carriers as often as they were linked to *C. difficile*-infected individuals (Curry et al., 2013). Similarly, Knelson et al. (2014) found that VRE or MRSA asymptomatic carriers more frequently had contaminated surfaces in their rooms compared with infected patients, and the contaminated surfaces had a greater bacterial burden (Knelson et al., 2014). The situation is further complicated by reported colonization rates ranging from 11- 20% for *C. difficile* (Alasmari, Seiler, Hink, Burnham, & Dubberke, 2014; Truong et al., 2017), and much higher for enterococcus (Agudelo Higuita & Huycke, 2014). It is not always clear who may be contributing to the spread of pathogens via the toilet plume. Similarly for norovirus, people may start shedding the virus in their stool before the onset of symptoms, and up to two weeks after

recovery (PHAC, 2012a). Unless all patients were to be assessed for pathogen carriage, only targeting known cases for designated bathroom use would miss a large proportion of individuals. Alternatively, for patients who are severely immunocompromised, restricting bathroom use to designated bathrooms that are not used by other patients, and perhaps supplementing cleaning with UVC, may help protect these vulnerable patients. However, there may be issues of feasibility with regard to a limited number of available bathrooms.

Modifying cleaning procedures to include the addition of certain water additives to increase surface tension would be another potential administrative control. Many cleaning products, including surfactants, decrease the surface tension of water which contributes to increased fragmentation of particles from the surface (Pennisi, 2014). The Bourouiba Research Group at the Massachusetts Institute of Technology used high-speed video recordings to show an increase in large and small droplet production during toilet flushing after addition of surfactants (Bourouiba Group, 2014). Compounded by the fact that surfactants can vastly increase bowl water concentrations of bacteria by dislodging adsorbed bacteria (Gerba et al., 1975), surfactants could actually worsen conditions. Alternatively, additives that increase the surface tension of water would decrease fluid fragmentation, and thus reduce droplet and aerosol formation during flushing (Pennisi, 2014). Electrolyte solutions are known to have such an effect (Hoorfar, Kurz, Policova, Hair, & Neumann, 2006). For example, the addition of a slow-release sodium chloride solution to the toilet bowl should decrease droplet and aerosol formation during flushing, and may be a financially feasible control to implement.

5.3.6 Personal Protective Equipment

Although personal protective equipment (PPE) is the last choice in the hierarchy of controls, its use may be warranted under the following circumstances to protect healthcare workers from pathogens present in the toilet plume. First, if healthcare workers are in close intimate contact with patients, such as homecare or long-term care workers, PPE may help protect workers from exposure to aerosolized pathogens. Specifically, if workers are physically assisting patients with getting on and off the toilet, then PPE may be warranted. Second, if patients are known to have, or recently have had, infections transmitted via the fecal-oral route, PPE may provide protection during patient bathroom use. Third, if there is evidence of droplet or aerosol transmission of the

infectious agent, as there is with many of the pathogens present in the toilet plume, then PPE use would be prudent. Of course, these recommendations would not protect healthcare workers from exposure to pathogens colonizing but not causing infections in patients. In order to protect against colonizing pathogens that may also be present, PPE would be required at all times when assisting patients with using toilet facilities. Although this approach would be prudent, compliance issues may arise and PPE use may be neglected altogether. Use of PPE only around patients with known infections would provide protection to the worker when most needed, and would help to avoid non-compliance.

Ideally the PPE would protect both against inhalation of droplets and aerosols, as well as contact transmission risks. At the same time, it is important to not overburden the user with unnecessary PPE, such that its use is cumbersome and time consuming, resulting in non-compliance. To this end, hand protection, such as latex or nitrile gloves, along with respiratory protection in the form of an N95 disposable respirator, would be sufficient to protect the worker, while not overburdening him/her. Gloves would serve to protect the worker from contact transmission risks while touching the patient, as well as other surfaces in the bathroom where pathogens may have deposited. Although the worker would likely be wearing gloves while assisting a patient, their use is strongly recommended while assisting patients with toilet use. However, there is always the potential for pathogen transmission from the gloved hand of the worker to surfaces within or outside of the bathroom. The worker would have to be fully informed of the potential for contact transmission from his/her hands to surfaces, and take precautions to prevent it. N95 disposable respirators have a 95% filter efficiency against aerosol droplets of the most penetrating particle size, those with a diameter of 0.3 um, (CCOHS, 2017). Airborne particles with an aerodynamic diameter of 4 um and smaller, referred to as the respirable fraction, are small enough that they are able to penetrate down into the alveoli of the lungs during breathing (Brown, Gordon, Price, & Asgharian, 2013). N95 respirators would be able to protect workers against inhalation of such particles. Currently, the Government of Canada and the United States Center for Disease Control recommends their use for healthcare workers in contact with patients who have infections transmitted via aerosols, such as tuberculosis (CCOHS, 2017). Although there is not definitive evidence that many of the pathogens present in air after toilet flushing are transmitted via aerosols, multiple studies have suggested that this may be the case (Bonifait et

al., 2015; Kuo et al., 2009). N95 respirator use by healthcare workers assisting patients in bathroom settings would help protect against such exposures.

5.4 Future Studies

My study evaluated the generation of airborne bacteria and viruses from a flushing patient toilet, the persistence of these airborne microorganisms over time at four locations, and the efficacy of UVC in controlling surface and airborne bacteria in a shared patient bathroom. Although this work provided valuable information regarding the degree to which microorganisms are aerosolized, and the differential survival of a Gram negative and positive organisms, there is still a paucity of information regarding toilet flushing and aerosolization of microorganisms. Along with the work presented here, previous studies have begun to shed light on microbial dissemination from flushing toilets, but research with a more standardized methodology is needed to help elucidate some of the variation in results seen thus far, or at least help identify some of the factors contributing to this variation.

Until now studies assessing the toilet plume have seen large differences in initial airborne microbial concentrations, their persistence over time, and subsequent surface contamination (Barker & Jones, 2005; Best et al., 2011; Gerba et al., 1975; Johnson et al., 2013; Newsom, 1972). The persistence over time and surface concentrations are partly determined by the toilet itself, but are also largely influenced by the local environment within the bathroom, which can be difficult to describe in sufficient detail. Additionally, these conditions will inevitably vary from one bathroom to another, meaning that such information, although useful for that particular environment, will have limited generalizability to other settings. The initial airborne concentration, however, is primarily a function of the toilet itself. There is a distinct need for studies to employ a more standardized methodology to assess how different toilet characteristics, such as bowl shape, flush volume, flush mechanism, and bowl volume affect bioaerosol generation. Key methodological aspects that must be standardized include test organisms, sampling location, type of sampler, volume of air sampled, and sampling media. Information from these studies could then be appropriately applied to a range of different bathrooms, depending on the toilet present, to help predict the expected bioaerosol generation.

Several studies to date have assessed the effect of closing toilet lids on aerosolization of microorganisms during flushing, however the results have been mixed (5.3.3 Toilet Lids). Employing standardized methodology outlined above, future studies should address whether the toilet lid prevents airborne microorganisms from escaping the toilet bowl. A small-scale pilot study could assess whether lids are effective with the toilets at VGH, and these results could be used to inform future decisions as to whether wider spread installation of lids would be helpful in controlling the toilet plume. Similarly, studies assessing i) LEV-toilets, and ii) the effect of additives that increase water surface tension would provide information as to whether these interventions can help control the toilet plume. Particularly in the case of LEV-toilets, which have a substantial initial cost, information regarding their effectiveness would assist decision makers in cost-benefit analysis.

Although most commercial bathrooms where HVAC systems are tightly controlled may not experience large variations in RH, poorly ventilated bathrooms in private residences likely would experience very high RHs while the shower is running. Future studies assessing the effect of high (and low) RHs on bioaerosol generation would help determine whether such environments pose an increased risk of pathogen exposure by potentially facilitating survival of airborne microorganisms, or whether droplets settle rapidly in these environments due to the high moisture content.

My study showed differential airborne survival of a Gram positive and negative bacteria aerosolized during flushing. The greater survival of the Gram positive organism is consistent with previous work showing Gram positive bacteria to be more resilient to various environmental stressors than Gram negative organisms (Fang et al., 2007; Heo et al., 2010). Future studies assessing aerosolization of other Gram positive and negative organisms in the toilet plume would help confirm my finding that Gram positive bacteria are more persistent in the toilet plume, and that the differential survival observed here is not due to another characteristic of the organisms distinct from their cell wall.

In my study, airborne bacteria and viruses were collected and grown on agar plates to determine airborne concentrations. Although this method provides information regarding airborne

concentrations, it does not provide any information in terms of the infectivity of the airborne organisms. To date, no studies have conclusively shown pathogen transmission of flushing toilets, either in humans or in animal models. Animal studies using model pathogens to assess the risk of infection from the toilet plume would provide invaluable information regarding actual infectivity of aerosolized organisms. Murine norovirus shares structural and genetic similarities with human norovirus (Bonifait et al., 2015). Studies employing mice to assess murine norovirus transmission aerosolized during flushing would provide information regarding actual transmission risk and would be the first to show definitively airborne transmission of pathogens in the toilet plume. These studies would also generate much-needed attention from researchers and practitioners regarding the role of toilets in pathogen transmission, and help facilitate further research in this area.

5.5 Limitations

Although this work provided valuable information regarding aerosolization and survival of microorganisms from a flushing patient toilet at VGH, there are several limitations to this study. First, as mentioned above, this work did not evaluate actual infectivity of the aerosolized organisms. Another limitation could be the collection of sequential air samples. In this study, air samples were collected sequentially at five different time points. It is possible that each sample collected affected the following sample by decreasing the number of organisms present in air. For example, samples collected immediately after flushing may have decreased the abundance of organisms present at the 4-minute time point. This phenomenon would increasingly underestimate the concentrations at increasing time points. To overcome this limitation, a study that collected samples only at one time point per site visit would be required. However, such a study would increase the number of site visits 5-fold, as each of the five sampling time points would require individual sampling days.

Another limitation of this study was the potential impact of the researcher on the air flow patterns in the bathroom. During sample collection, I had to operate the air sampler, change sample collection plates, sterilize the sampling heads, and perform several other tasks required for the experiment. My movements within the bathroom may have affected air currents around the toilet plume and helped disseminate the airborne organisms within the room and potentially

settled on myself. However, until automated and remotely controllable air samplers are available, manually performance of these tasks is required. In an attempt to minimize my impact on dissemination of the toilet plume, I never entered the area in front of the toilet where samples were being collected.

One limitation of the study evaluating UVC in a shared patient bathroom was that no consideration was given to how the occupant used the bathroom. Flushing stool would expectedly generate greater bacteria-containing droplet and aerosol concentrations than flushing urine. It is possible that in the control bathroom, occupants more frequently passed stools than in the UVC bathroom, partially accounting for the higher airborne and surface contamination seen in the control bathroom. Although there is no reason to believe that patients were systematically using one bathroom for specific evacuation, more than 30 samples were collected over multiple days to account for the possibility of differences in the UVC bathroom being observed by chance alone. Although not definitively shown, the difference in concentrations likely reflects the biocidal activity of UVC, and not differences between how the bathrooms were used.

Another limitation of this study is that did not directly assess particle size. Samplers such as the six stage Andersen separate particles based on size, and thus provide size-specific information about the bioaerosol concentration (Xu et al., 2013). In this study, rough particle size estimates were inferred based on the work of Xie et al. (2007), the measured RHs, and the concentrations at various time points (Xie et al., 2007). However, using a size-separating sampling device would provide a direct measurement of the range of particle sizes at each time point, and would confirm Xie's revisions of Wells' evaporation-settling curve.

I was unable to show settling of bacteria or virus-containing droplets in my study. Stainless steel plates were placed on the window ledge approximately 1.5 meters in front of the toilet and sampled for droplet deposition using RODAC plates. When this was unsuccessful, settle plates were then used on the window ledge to sample once again for droplet deposition. Plates were exposed for up to 90 minutes, but bacterial concentrations were comparable to the control plates, and the tracer organisms were not detected. Lastly, settle plates were placed on the ground surrounding the toilet in an attempt to capture droplets depositing in close proximity to the toilet.

However, this attempt was also unsuccessful, and there are several possible explanations as to why. First, during the early attempts at detecting settling of organisms on the window ledge, air samples were also collected in the same experiments. It is possible that the sampler created air flow patterns that resulted in unexpected dispersion of the organisms onto surfaces that were not sampled. However, bacteria settling experiments were also conducted while the air sampler was not used, and during these days settling was also not observed. Another possible explanation is that the droplets did not travel all the way to the window ledge. However, in this case, it would be expected that settling would be observed in the experiments when plates were placed on the floor. It is possible the movement of the experimenter contributed to dispersion of the droplets such that they did not deposit on the settle plates. Other studies have reported on settling of organisms in the toilet plume (Barker & Jones, 2005; Best et al., 2011), employing similar sampling methodology. This study was conducted in a large, single occupancy bathroom with a volume of approximately 12.5m^3. It is likely that air flow patterns generated by the in-bathroom ventilation partially contributed to dissemination of the droplets such that they did not settle in the designated sampling locations. Additionally, the toilet assessed in this study may also have ejected droplets with a trajectory such that they did not settle in the anticipated locations. Likely a combination of these factors contributed to droplets not being detected on the settle plates.

5.6 Conclusion

Flushing toilets represent a unique challenge in controlling pathogen transmission. Their frequent use generates bacteria and virus-containing droplets and aerosols, though their precise role in the spread of disease is unclear. Toilets cause environmental contamination of surfaces, as well as aerosolize microorganisms that can remain suspended in air for prolonged periods of time. Although some studies have evaluated this phenomenon, their results have been mixed and, in general, this topic has not received due attention. While this study focused on the importance of toilets in healthcare settings, toilets are found in most buildings, so their role in environmental contamination is relevant to a wide range of contexts. Currently, there is no mandated testing by manufacturers to ensure that the spread of toilet contents is minimal. Given the ubiquity of toilets, costly interventions to help reduce their environmental contamination are not feasible in most circumstances. Unfortunately, it is unclear as to whether toilet lids, the most basic control, are helpful so further studies are urgently needed to evaluate their effectiveness. Automated

UVC is an effective control for both surface and airborne bacteria in single occupancy bathrooms, but is not likely to be feasible for use in multi-occupancy bathrooms. Development of an industry standard for toilet manufacturers with regards to a maximum allowable aerosol emission would be instrumental in helping infection control practitioners selecting appropriate toilets for use in settings with vulnerable populations.

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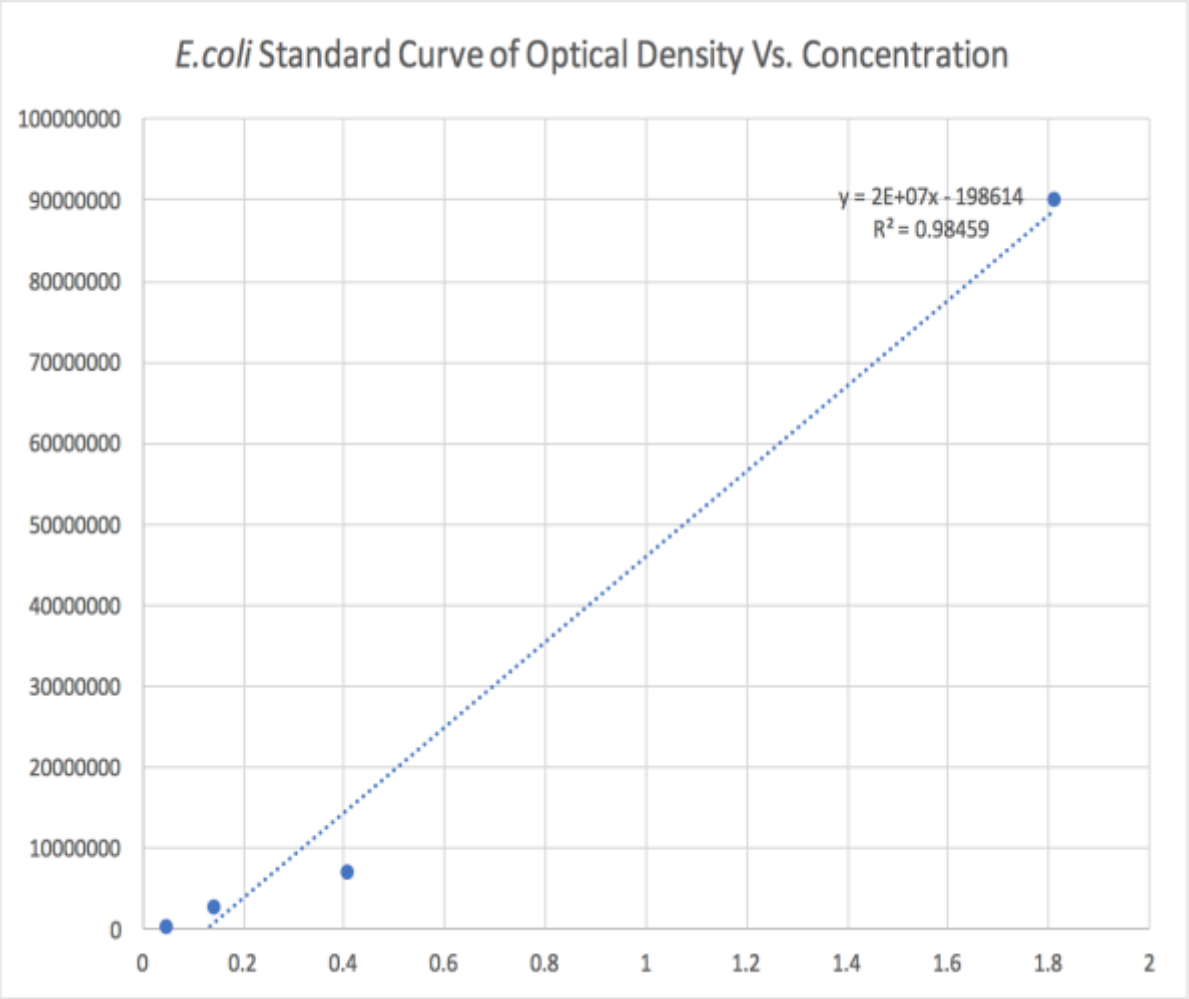
Appendix A Standard Curve for *Escherichia coli*



Appendix B Standard Curve for *Enterococcus faecalis*