MOVEMENT OF A VIRAL SURROGATE FROM RESTROOMS TO PUBLIC AREAS IN A HOSPITAL

By

Trevor R. Pivo

_____

A Thesis Submitted to the Faculty of the

DEPARTMENT OF SOIL, WATER AND ENVIRONMENTAL SCIENCE

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2016

STATEMENT BY AUTHOR

The thesis titled *Movement of a Viral Surrogate from Restrooms to Public Areas in a Hospital* prepared by *Trevor R. Pivo* has been submitted in partial fulfillment of requirements for a master's degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: _____

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

_____ 04/29/2016

Charles P. Gerba Date

Professor of Soil, Water and Environmental Sciences

Acknowledgments

I'd like to first thank Dr. Charles P. Gerba for allowing me the opportunity to work in his laboratory over the last three years. Working under you has been a true honor, the knowledge and experience you have given me will never be forgotten. Next, I'd like to thank Dr. Gerardo Lopez. Jerry you have donated a huge portion of your time to me over the last year and your constant support has enabled me to propel myself through this program. Dr. Kelly Reynolds you have a way of challenging your students to find the best within them. Working alongside you has enabled me to reach my true potential. Dr. Hannah Sassi without your constant help throughout my graduate career I wouldn't have been able to accomplish what I was able to. Finally, Daniela Cabrera, you were the best friend and teammate I could have asked for throughout my graduate career. I can truly say I have never had more fun in the lab than when I was working on one of the many projects with you. Working alongside each of you was a true privilege of mine and I want to thank you all from the bottom of my heart.

Dedication

I would like to dedicate this thesis to my family

Dad and Michelle: You both have have invested so much into me and my future. Your constant guidance has led me to this point in my life.

Mom and Richard: You both have given me constant motivation to continue to fight for what I want. My work ethic would be a fraction of what it is without you.

Gary and Carrie: You both have taken me in as your own over the last five years. You have shaped me more than anyone during my collegiate years and because of you I am excited about what the future holds for me.

Ryan: I can't remember a single life event without you being by my side. Your constant love and support since the day I was born has allowed me to keep my head up, even when I had every reason not to.

My only hope is that I have made you all proud

Table of Contents

List of Tables

List of Figures

Abstract

Contaminated fomites are a cause of concern for the spread of health care-associated infections (HAI's). Previous research has placed emphasis on fomites in patient rooms and patient bathrooms with limited focus on the spread of microorganisms on fomites in non-patient care areas. The present study monitored surrogate virus tracer (MS2 coliphage) spread from public restrooms (used by staff and visitors) to waiting areas in a surgical ward in a Level I Trauma Center. The coliphage (virus) MS2 was added onto the entrance door handle of male and female public restrooms. Four hours later, various surfaces in the restroom and waiting area were sampled. Sampling periods were conducted in duplicate consisting of before cleaning, cleaning with the current cleaning product and procedure and cleaning with an intervention (inclusion of a bleach based disinfectant wipe) in addition to the current cleaning product and procedures. Before cleaning took place, the virus tracer was detected on all 21 of the sites sampled in the restrooms and 5/9 sites within the hallway ranging from 15-50 feet from the restroom. These results indicated that a virus could spread from public restrooms to other sites in the restroom and to locations in the surgical ward. The addition of a bleach based disinfectant wipe reduced the virus by another 90% compared to current disinfecting and cleaning procedures. Coliphage MS2 has been used as a model virus for norovirus and rhinovirus since they exhibit similar survival on fomites and resistance to disinfectants. The data generated can be used in quantitative microbial risk assessment models to assess the risk of pathogens spreading from restrooms to patient waiting areas and patient care areas in

healthcare settings. Based on this study, facilities should consider broadening their cleaning and disinfection protocols to include both patient care and non-patient care areas.

Chapter one

Introduction

In the last two decades increased attention has been focused on the spread of health care associated infections (HAI) via contaminated fomites. HAI's caused by methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), and *Clostridium difficile* are associated with high morbidity and mortality (Klevens et al. 2002). An estimated 1.7 million HAI cases occur each year in the United States, resulting in 99,000 deaths per year. This ranks HAI's as the fifth leading cause of of death in the United States (Klevens et al. 2002, Klevens et al. 2007). The cost associated with HAI's range from 28 billion to 45 billion, however new mandates from the center for disease control (CDC) require hospitals to report HAI's in an effort to reduce the risk of infection.

Within indoor settings, enteric and respiratory viruses are responsible for the majority of the illnesses (Sperber et al. 2000). Enteric viruses, such as norovirus and rotavirus are commonly transmitted via the fecal-oral route because the gastrointestinal track is their primary site of replication. Respiratory viruses, like influenza and rhinovirus, can be transmitted by fomites and aerosols (Barker et al. 2001). Enteric viruses have been recognized for having the ability to contaminate fomites in both community and health care facilities (Sifuentes et al. 2014). Under optimal conditions of pH, relative humidity, and temperature viruses can remain infectious on a surface for several days (Boone at al. 2007). Once on a fomite a virus can be transferred from that fomite to an individual's hands and onto a separate fomite. This transmission pattern allows a virus to spread to areas other than its origin. Low

doses of enteric and respiratory virus ($1 – 10^2$ virus particles) can cause infection (Seymour et al. 2001).

Fomites

Fomites are porous and nonporous surfaces or objects that have the ability to harbor a pathogenic microorganism and serve as a mode in transmission. Fomite contamination occurs via direct contact with the body secretions, contact with contaminated hands, settling of aerosolized virus, etc (Boone et al 2007, England et al. 1982, Reynolds et al. 2005, Sattar et al 2001).

The survival of a microorganism on a fomite is influenced by both intrinsic and extrinsic factors. Intrinsic properties include surface composition of fomite (porous vs non-porous material) and the properties of the virus (Boone et al. 2007, Sattar et al 2001, England et al. 1982). Microorganisms are transferred at a higher rate when the fomite is a non-porous surface vs a porous surface such as a sponge or wash cloth under dry conditions. Extrinsic properties that influence the survival of a microorganism on a fomite are temperature, pH, humidity, etc. Viruses are obligate parasites; therefore, the level of viral infectivity on a fomite can only decrease over time (Boone et at. 2007, Ansari 1991, Sobsey et al. 2003). Viruses on surfaces can survive from a few hours to many days on fomites (Boone et al. 2007).

Today, humans living in developed nations are estimated to spend 90% of their lives indoors (Kelley et al. 2013). Disease transmission relies on the accumulation and continued viability of pathogens surface fomites (Gibbons et al. 2015). Understanding the transmission of

microorganisms within indoor settings can be used to develop methods to reduce the risk of exposure and infection.

Chapter 2

Literature Review

Virus Transfer

Transfer of microorganisms between skin and a surface is described quantitatively as the fraction of organism on a contaminated (donor) surface that is transferred on contact to a recipient surface (Julian et al. 2010, Reed 1975, Gwaltney 1982, Ansari et al. 1988, Mbithi et al. 1992 and Rusin et al. 2002).

Julian et al. (2010) tested the ability of three coliphage (MS2,fr, and φχ174) to be transferred from a fingerpad to a non-porous glass surface. The study involved 8 men and 12 women for a total of 688 transfer events. The volunteer's fingerpads were inoculated with 5 µL of either between 100 and 600 or 1000 and 6000 PFU in order to have representation of various titers. Once inoculated the viral suspension was allowed to become visibly dry before the transfer event. The inoculated fingerpad and the surface were placed in contacted for 10 seconds at an average pressure of 25 kPa. The pressure of 25 kPa is comparable to the pressure exerted by a child while gripping an object, the pressure exerted locally on the fingerpads for adults using hand tools, and the pressure used in studies examining transfer of soil from surfaces to skin (Link et al. 1995; Hall 1997; Ferguson et al. 2009, Julian et al. 2012). A cotton tipped swab moistened with phosphate buffer saline (PBS) was used to remove the virus from the non-porous glass surface. The mean fraction of virus transferred was larger for fr than both MS2 ($P< 0.001$) and φχ174 ($P< 0.001$) . The mean fraction of virus transfer for MS2 and φχ174 were found not to be significantly different ($P= 0.16$). The mean fraction of virus transferred f,

was determined to be 0.23 ± 0.22 (mean and standard deviation), concluding that viruses are readily transferred between skin and a model fomite surface.

Ansari et al (1991) measured the percent of total transfer of human parainfluenza virus 3 (HPIV-3) and Rhinovirus 14 (RV-14) using three separate transfer events. The three transfer events were: 1) the fingerpad of a volunteer to a stainless steel disk 2) stainless steel disk to fingerpad and 3) fingerpad to fingerpad. A total of three males and one female served as hosts for the virus. Each human host received instructions prior to the experiment to wash their hands using warm tap water, rinse them with 70% ethanol, and allow them to dry. Each fomite or fingerpad was inoculated using 10 µL of suspended virus and allowed 20 minutes for the viral suspension to become dry. Once dried the donor and recipient surface were pressed together with a force of approximately one kg/cm^2 for a period of 5 seconds. The study concluded that regardless of the donor or recipient surface the range of transfer was from 0.7 to 0.9% PFU/cm^2.

Rusin et al. (2002) studied the ability of surfaces to transfer bacteria and bacterial viruses onto hands and then subsequently from hands onto lips. The study initially compared the ability of Gram-positive bacterium (*Micrococcus luteus*), Gram-negative bacterium (*Serratia rubidea*) and phage PRD1 to be transferred from 8 different fomites. Of the fomites, 6 were porous surfaces consisting of a dishcloth, sponge, carrot, laundry with swatches of 100 % cotton or 50:50 cotton/polyester and ground beef. The remaining two were nonporous surfaces composed of a phone receiver and a faucet handle. Each of the fomites/surfaces were inoculated with a pooled suspension of bacteria/phage at approximately 10^8 CFU or PFU mL^{-1}

and allowed to dry. The hands were disinfected using 70% ethanol followed by washing with liquid hand soap before being dried with a paper towel. The sampling period was after the disinfected hand came into contact with the previously contaminated fomite/surface for a period of 10 seconds. It was observed in all but two events that the Gram-positive bacterium transferred at a higher efficiency than the phage and Gram-negative bacterium, with a range of transfer efficacy from 0.03 to 41.81 %. The two exceptions resulted from the phage's transfer efficiency being higher for the carrot and phone receiver, with transfer rates of 33.5 and 65.8 % respectively. Across all testing parameters the lowest rate of transfer derived from the Gram-negative bacterium, with a range of transfer from <0.01 to 38 %. The highest rate of transfer for all organisms derived from hard non-porous surfaces with a range of transfer from 28 to 66%. Although, the amount of phage recovered from hands after handling porous fomites was still high, ranging from $\log_{10}$ 2.7 to 7. A similar study done by Scott and Bloomfield (1990) also concluded that transfer was greater for Gram-positive bacteria followed by virus and Gram-negative bacterium.

The subsequent transfer from the fingertip to the lip was evaluated as the next component of the Rusin et al (2002) study. Using the same organisms previously mentioned a transfer event between the hands of an individual and the middle of their lower lip was measured. The lip and hand were disinfected using 70% ethanol prior to inoculation. A total of 5 µL of inoculum was applied to the index fingerpad of a volunteer, at an approximate concentration of 10^6 colony forming unit (CFU) or PFU ml^{-1}, and allowed to dry for 30 seconds. The finger was then placed onto the middle of the lower lip for 10 seconds. After the transfer event both the fingerpad and the lip were sampled for transfer quantification. Again the Gram-

positive bacterium, M. *luteus,* resulted in the highest rate of transfer between the index fingerpad and the lower lip with a transfer efficiency of 41%. The study concluded that bacteria and viruses present on a fomite have the ability to be transferred onto the hands of a host through direct contact and subsequently onto their lips.

Virus and Bacteria in the Restroom

Restrooms are shared public spaces with clear potential for disease transmission (Gibbons et al. 2015 and Flores et al.2011). Independent cultivation techniques, such as cloning and gene sequencing, have allowed for a better understanding of the microbial communities within restrooms (Flores et al. 2011, Lee et al. 2007, McManus et al. 2005, Rintala et al. 2008, Kelley et al. 2004). Most of the organisms identified in these studies are related to human commensals suggesting that the organisms are deposited directly (physical content) or indirectly (shedding of skin cells) and not actively growing on the surfaces (Flores et al. 2011).

Flores et al (2011) determined the taxonomic composition of bacterial communities on surfaces in public restrooms. The study was able to differentiate the species into three general categories 1) communities found on the toilet surfaces (seat and handle) 2) communities found on the restroom floor and 3) communities found on surfaces routinely touched by hands (faucet handle, soap dispenser etc.). Communities present on the floor were the most diverse with an average of 229 operational taxonomic units (OTU) per sample versus other sites having less than 150 OTU per sample and harbored many low abundance taxa. The abundance of bacterial communities on the floor is assumed to derive from soil particles on shoes containing a highly diverse microbial habitat being tracked into the restroom. Surfaces assumed to be

routinely touched by hands were dominated by skin-associated bacteria. Communities on toilet surfaces were enriched in taxa associated with the human gut (*Firmicutes* and *Bavteroidetes)*. This fecal contamination could have derived through direct contact with uncleaned hands or indirectly as the toilet is flushed and water is splashed or aerosolized. In addition to the presence of gut flora bacteria on the toilet flush handle, communities similar to those found on the floor were also discovered. This discovery suggests that some users may operate the flush handles with their feet.

Aerosols and surface contamination are sources of virus transmission in hospital settings (Verani et al. 2014, Aitken et al. 2001 and Ganime et al. 2012). Verani et al. (2014) studied the link between viral contamination of aerosols and surfaces through the use of toilets. Within a restroom, toilets should also be considered a possible source of indoor air and surface viral contamination because microbial contamination typically occurs after a toilet flush. This is an important source of diffusion for enteric and respiratory viruses because they are often eliminated by the fecal route. A toilet flush generates aerosols varying from 0.3 μm to >20 μm. Particles greater than approximately 5-μm begin to settle after one minute, while those smaller than 5-μm begin to settle after 2 minutes (Johnson et al 2013).

The Verani et al. (2014) study examined five toilets in a hospital and two in an office building. Surface, air and water samples were obtained over the course of the four month study. The isolated nucleic acids from norovirus, enterovirus, rhinovirus, human rotavirus, and Torque teno virus were analyzed using polymerase chain reaction (PCR). The viruses were detected on 135 surfaces (78% of the total tested), in 35 aerosol samples (81%) and in 17 water

samples (89%). This study concluded that the droplets coming from toilet flushing and settling on surfaces is an important source of contamination within the restroom.

Virus Transfer in the Environment

A number of viral tracer studies have been conducted in various environments including day care facilities, hotels, long-term care facilities and households in order to better understand how viruses spread in these environments. The use of a surrogate organism is an effective way to understand the dynamics behind viral dispersion within various environmental settings, where understanding the extent of pathogen spread and the risk of exposure can't be easily determined because of uncertainties in human behavior (Sassi et al 2015, Beamer Pl et al 2015, Sifuentes et al 2014).

Households and Day Cares

Rheinbaben et al. (2000) used bacteriophage $\varphi\chi$174 in order to model the transmission of viruses in a household setting. Initially, the door handle of a room within the home of a volunteer was inoculated with the phage at a concentration of 10^7 PFU. Following a 15-minute drying period the handle was touched by 14 test individuals for a period of 15 seconds. The phage was recovered from the hands of all subjects. There was less than three orders of magnitude reduction in phage recovered from the first to last individual to touch the door handle, with a range of virus recovery from $\log_{10}$ 4.6 – 2.1.

The second component of the Rheinbaben et al. (2000) study used the same phage to inoculate the home of four students. The phage was placed on the door handle of the living

room at an initial concentration of 8 x 10^8 PFU. The phage was allowed 6 hours to transfer throughout the home by its occupants and their guests. The fomites selected for sampling within the home were the door handles, telephones, computer mouse, light switch, refrigerator handle and the water tap. After the 6-hour period the phage was recovered from all fomites sampled within the home except the computer mouse and light switch. The concentration of phage recovered ranged from 1.3 to 3.9 $\log_{10}$.

The occurrence of Influenza A virus on fomites within Arizona day care facilities and homes was studied by Boone et al. (2005). Over the course of 2 and ½ years, a total of 218 samples were obtained from 14-day care centers. A total of 10 fomites were selected from each day care center which included toddler and infant toys, diaper changing areas, toilet seat tops, floor below toilets, kitchen counter tops, bathroom faucets handles, kitchen dishcloths and the drains of the kitchen and bathrooms. The samples were collected using a sterile polyester fiber-tipped applicator swab and the influenza's viral genome was detected using polymerase chain reaction (PCR). A seasonal variation in the presence of the virus on fomites was observed correlating with the influenza season cases within Arizona. During the spring months 53% of the samples were positive for influenza A, while only 23% of the samples were positive during the fall months. Of the total sampling period, influenza A occurred least on toddler toys and most on the kitchen dish cloths with 30% and 57% respectively.

The second component of the Boone et al. (2005) research studied the occurrence of Influenza A virus in eight homes, five of the homes had children ill with flu like symptoms. During the six month study 92 samples from each home were obtained, with a range of 9 to 14

fomites each. The sites included the kitchen and bathroom faucet handles, doorknobs, phone receivers and handles, computer keyboards, toilet handles, microwave handles, refrigerator handles, light switches, TV remote controls, and door handles. The influenza A virus was only detected in homes containing children experiencing flu like symptoms. Of the homes assessed in March, 59% of the 59 samples were positive for influenza A. The viral RNA of influenza was detected most often on the phone receiver (80%) and least often on the computer (40%). Additionally, the RNA was most often detected on moist surfaces.

Hotels

Viral outbreaks have been linked to hotels in a number of studies, including a norovirus outbreak in Virginia. Love et al. (2002) documented a total of 76 guest and 40 employees that fell ill due to norovirus. Researchers concluded that the virus was spread by the housekeeping staff while guest rooms were being cleaned using standard cleaning procedures. In a study conducted by Sifuentes et al. (2014) two separate scenarios were modeled: 1) The transfer of a bacteriophage virus surrogate from a conference center into hotel guest rooms and communal areas and 2) the transfer of a separate surrogate virus from one guest room to another by the hotel cleaning staff. This study was conducted in a pre- and post-intervention format using a hygiene intervention provided to the house keeping staff. The intervention contained various cleaning tools and a disinfectant. The staff was instructed to use the intervention paired with their traditional cleaning protocol.

The bacteriophage MS2 was seeded onto the outside door handle and kitchen faucet handle while bacteriophage $\varphi\chi174$ was seeded onto the outside door handle and restroom

faucet handle in both at concentrations of 9 x 10^5 PFU/cm^2. The phage $\varphi\chi$174 was transferred by hotel cleaning staff and recovered from 27/169 (16%) of fomites during the pre-intervention sampling periods. The use of the intervention product resulted in virus recovery from 13/175 (7%) fomites. The MS2 was also transferred but no significant difference was demonstrated between the pre-intervention and post-intervention studies. This study concluded that viruses are rapidly transferred by both guests and housekeeping staff. After only one night both viruses were found to spread from the hotel room where the phage was initially placed, into other guest rooms and communal areas, as well as into communal kitchen and living areas.

Long-Term Care Facility

Sassi et al (2015) used MS2 to evaluate the spread of virus during routine long-term care practices. This pre and post-intervention study design focused on the use of hygiene products to combate the spread of the virus surrogate throughout the facility. A total of 37 fomites were selected along with 10 staff members from the facility as sampling sites. Using a single blind study technique, the hands of one staff member were seeded with MS2 at a concentration of 10^{12} PFU, while the other staff members received letheen broth. Staff members were instructed to not deviate from their typical work day. After a 4-hour transfer period, 100-cm^2 were sampled from each fomite and the fingers/palms of both hands of each staff member using a sponge stick(3M Brand, St. Paul, MN).

MS2 was most commonly recovered from a large table located within the nurse's station, which had phage recovered in 5 out of 6 sampling periods. During the pre-intervention phase the MS2 was recovered from an average of 49.1% (52/105) of the fomites. The post-

intervention period resulted in an overall reduction of 99.99% of virus on the fomites, 99.9% reduction on volunteer's hands and >99.9999% reduction on the seeded volunteer's hands. The post-intervention period resulted in a 16.7% decrease in the number of sites from which MS2 was recovered, with an average recovery from the fomites of 32.4% (34/106).

Disinfection

Disinfection is designed to significantly reduce the number of disease causing microbes by 99.99% or more with a contact time specific to the disinfectant or the microorganism intended to reduce. Wipes, sprays and hand sanitizers are three common modes of disinfection.

The risk of infection from both respiratory and enteric viruses has been demonstrated to decrease upon good hand hygiene involving hand washing and/or the use of alcohol-based hand sanitizers (ABHS) (Tamimi et al. 2014, Prazuck et al. 2010; Stebbins et al. 2011, Warren-Gash et al. 2012). A study conducted by Tamimi et al. (2014) was designed to assess the impact an ABHS has on reducing the transmission of a virus throughout a household. The bacteriophage MS2 was used in this study as the surrogate virus. Seven households with children living in the home were selected for the study with 19 fomites, in addition to the hands of the family members, selected as sampling sites. In this pre and post-intervention study design the hands of an adult family member was inoculated with the bacteriophage at a concentration of 1×10^8 PFU. The family was then allowed to conduct their daily routines for an 8-hour period. After the 8-hour period, the hands of all family members and the 19 fomites were sampled. During the pre-intervention sampling periods the families were asked to not use

antimicrobials on surfaces or their hands but normal hand washing practices with soap and water were allowed. Without the use of antimicrobials, the virus spread to the hands of all family members of each household and most fomites within the households.

In the post-intervention period the addition of alcohol based sanitizers, active ingredient 70% ethanol, were made available to all members of the family. The members of the family were then instructed to use ABHS 3 times a day for phase 1 and once a day for phase 2. The reduction of virus while using ABHS found on hands and fomites was determined to be statistically significant ($P<0.0005$). The percent of sites where phage was recovered in the pre-inoculation stages were 98% and 97% for phase 1 and 2 respectively. After the use of an ABHS the sites where phage was recovered decreased to 65% in phase 1 and to 52% in phase 2. The use of ABHS once and three times a day reduced the virus concentration on fomites and hands within the households by ≈99%. In addition, the phage was only detected from half of the fomites.

The efficacy of a disinfectant wipe to reduce the microbial load on various surfaces was quantified by Lopez et al. (2014). The wipe contained a quaternary ammonium compound (QAC). E*scherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* spores and poliovirus 1 were applied to ceramic tile, laminate and granite. From $10^7 – 10^9$ CFU or PFU were added to each type of surface. The fomites were then treated with the wipe. Reduction of the B. *thuringiensis* spores was always less than the other three microorganisms with a mean $\log_{10}$ reduction of 1.9 to 2.5 compared to 3.5 to 5.0 CFU/2 cm^2 or PFU/2 cm^2 respectively. This study concluded that disinfectant wipes reduce the microbial load is similar to the studies of Siani et

al. 2011, Williams et al. 2007, Williams et al. 2009, Panousi et al. 2009, Berendt et al. 2011 and Rutala et al. 2006.

The reduction of rotavirus using a Lysol disinfectant spray (LDS) was examined by Ward et al. (1991). Culture-adapted human rotavirus (CJN strain) at a concentration of 1.5×10^8 PFU/ml dried in 5% nonfat dry milk (NDM) (0.1 ml) which was spread over an area of 6 cm^2 on a petri dish. When LDS treatment was applied at 32 inches and 25 inches from the surface of the fomites the virus was reduced by 3 and 5 logs respectively. It was concluded that the use of the LDS is an effective method to reduce the amount of virus harbored on a surface.

Chapter 3

Material and Methods

Study Location

The study was performed in one of the surgical wards of a Level I Trauma Center. The public has access to this area and contained both women's and men's restrooms, used by hospital staff and patrons alike. A total of 10 sites were selected in the women's restroom and 11 in the men's restroom as shown in Table 1. Both women's and men's restrooms were equipped with a single sink with manual (hand operated) fixtures, manual soap dispensers and an automatic paper towel dispenser. The women's restroom had two stalls each with manual flush handles while the men's restroom had one stall with a manual flush handle and one urinal with automatic flushing.

Nine additional fomites were selected from the exterior of the restrooms (Table 1). Fomite selection was based on observation of individual tendencies after exiting the restrooms. Observations were made over the course of two hours and consisted of an individual watching patrons from the hallway as they exited the restroom. The end destinations were documented and used to determine areas of the facility to be used for sampling.

Table 1. Fomites Selected for Sampling

Women's Bathroom			Men's Bathroom			Outside Bathrooms		
ID	**Fomite**	**Fomite Area Sampled (cm^2)**	**ID**	**Fomite**	**Fomite Area (cm^2)**	**ID**	**Fomite**	**Fomite Area (cm^2)**
1	Restroom Entrance Handle	45	**11**	Restroom Entrance	45	**22**	Elevator Push Buttons	100

								(Composite 8)	
2	Middle and Top of Outside Stall Door	100	**12**	Middle and Top of Outside Stall Door Handle	100	**23**	Door to Surgery Physician offices	100
3	Middle and top of Inside Stall Door	100	**13**	Middle and Top of Inside Stall Door	100	**24**	Front Half Waiting Room 1 Chair arms (composite 3)	100
4	Inside Stall Lock	100	**14**	Inside Stall Lock	100	**25**	Waiting Room 1 Check in Pen	30
5	Manual Toilet Handle	50	**15**	Manual Toilet Handle	50	**26**	Waiting room 1 Counter top	100
			16	Automatic Urinal Handle	100	**27**	Front half Waiting room 2 Chair arms (composite 3)	100
6	Front, Left and Right of Counter top	100	**17**	Front, Left and Right Counter top	100	**28**	Waiting room 2 Counter top	100
7	Manual Soap Dispenser Push Button	100	**18**	Manual Soap Dispenser Push Button	100	**29**	Staff Coded Hallway Door Handle	100
8	Manual Sink Faucet/ Handle	100	**19**	Manual Sink Faucet/ Handle	100	**30**	Nurses station 1 Counter top	100
9	Automatic Paper Towel Dispenser Signal	100	**20**	Automatic Paper Towel Dispenser Signal	100			
10	Restroom Exit Handle	100	**21**	Restroom Exit Handle	100			

Study Design

The sampling was composed of three phases, a control period followed by Pre- and post-intervention sampling. The control sampling period was conducted without the restrooms

being cleaned during the four-hour exposure period. During the pre-intervention phase of the study the hospital cleaning staff was instructed to clean the restrooms using their current cleaning product and protocol at the end of the four-hour exposure period. Restrooms were cleaned 2-3 times a day using Virex II spray (Medline Industries, Mundelein, IL) in shifts consisting of day, night and weekends. The post-intervention phase of the study included the addition of Bleach Germicidal Wipes used as an intervention cleaning product provided by The Clorox Co. (Oakland, CA). The cleaning staff was instructed to use their current cleaning protocol in addition to the intervention product within the restrooms.

It was requested of the cleaning staff to use the provided disinfecting wipes on all surfaces cleaned with the Virex II. Each sampling event was performed in duplicate allowing 48 hours between sampling events. In the pre-intervention phase, a 100 cm^2 area was sampled for each fomite except the restroom entrances, toilet handles and pen at the check in counter where only 45, 50 and 30 cm^2 respectively could be sampled. A sponge stick (3M brand, St Paul MN) pre-inoculated with 10 mL letheen was used to recover the virus from the fomite. The fomites were then cleaned using hydrogen peroxide based disinfecting wipes and allowed approximately 10 minutes to dry to inactivate any remaining virus. Following the drying period, background (before the addition of the MS2 virus to the door handle) samples were collected from each fomite, ensuring no residual MS2 was present from the previous sampling periods. Following another 10-minute drying period, 100 µL of MS2 was seeded on the restroom entrance handle (concentrations ranging from $1.6 \times 10^{10} – 1.21 \times 10^{11}$) of both male and a female restroom. After a 4-hour period each of the fomites were sampled again.

Intervention

During the pre-intervention phase of the study the hospital cleaning staff used their current cleaning product and procedures. The current procedure was to clean the restrooms twice during the day and twice during the night. The restrooms were cleaned by one individual during the day and a separate individual during the night. The only cleaning product used in the restroom was Virex II, a quaternary-based hospital grade disinfectant (Medline Industries, Mundelein, IL). The spray was applied directly to the surface for a contact time of 10 minutes and wiped off using a cloth. The active ingredients in this product are Didecyl dimethyl ammonium chloride n-Alkyl (50% C14, 40% C12, 10% C16) and dimethyl benzyl ammonium chloride.

Following the pre-intervention sampling period, Clorox Bleach Germicidal Wipes (Clorox Professionals, Oakland, CA) were added in addition to the current cleaning product being used by the hospital. The bleach wipes contain 0.55% sodium hypochlorite as the active ingredient and require a 3-minute contact time. Prior to the implementation of the Clorox bleach wipe intervention, a training was conducted by a Clorox representative at the hospital. During this 30-minute training hospital cleaning staff was instructed on the proper use and implementation of the intervention wipes. The staff was then instructed to use the new Clorox wipes in addition to the current Virex II spray for a period no fewer than 14 days. Following the 14-day intervention period the post-intervention samples were collected again in duplicate allowing 48 hours between sampling events.

Preparation of MS2

Bacteriophage MS2 (ATCC 15597-B1) was propagated using *Escherichia coli* (ATCC 15597) as the bacterial host. MS2 was propagated on TSA at 37^{o}C following standard methods of cultural and top agar assays (Strauss et al. 1963).

Following the 24-hour incubation period 6 mL of sterile 0.01 M phosphate buffered saline (PBS) was added to each of the plates and agitated every 30 min for two hours. The eluent was collected from each plate and placed evenly into 2-50 mL polypropylene conical tubes. The solution was then placed into a centrifuge for 10 minutes to allow the bacterial cellular debris to pelletize. The supernatant was collected and filtered using a Steriflip® loaded with a Millipore Express® PLUS Membrane, 0.22 µL pore sized filter (Millipore Corporation, Billerica, MA). The stock virus concentration was determined using a 10-fold serial dilution in PBS. Diluted samples were plated and quantified using the agar overlay method (Kropinski et al. 2009).

Sample Collection and Enumeration

The samples were collected by two individuals, in order to keep the variability of the sampling technique to a minimum, both used a sponge stick (3M brand, St Paul MN) pre-inoculated with 10 mL letheen. The sponge sticks were removed from a sterile plastic bag and the fomites were swabbed in a unidirectional motion. The sponge sticks were placed back into a sterile plastic bag and onto ice for transport to the laboratory. The sponge sticks were eluted by separating the sponge from the stick and applying a firm pressure directly onto the sponge in a sterile plastic bag. The fluid volume was then recorded and assayed. Rose et al (2011)

determined the recovery efficiency of the sponge stick to range from 20 - 31%. Samples were assayed and enumerated by the double agar overlay method (Kropinski et al. 2009).

Statistical Analysis

The data was input into a database in order to be read using the R Language, recovery per cm^2 was then used for statistical analysis (R Core Team 2013). The database consisted of the following fields: a unique ID key, location of sampling (Women's, Men's or Outside), Arm (Arm 1, Arm 2 or Arm 3) and concentration of MS2 per cm^2 of swabbed surface area from each fomite. Two types of statistical analysis were performed: Descriptive Statistics using Microsoft Excel Spreadsheet program as part of Office Professional 2016 (Microsoft Inc. Redmond, Washington); and analysis of variance (Ott and Longnecker, 2001) using the R-Language (R Core Team, 2013). The statistical analysis for the MS2 data sets were divided into three arms.

Control

The restrooms were inoculated with MS2 phage at 8:00 a.m. and allowed 4 hours for the virus to transfer within the restroom and into the outside hallway. During this time the restrooms were not cleaned by the custodial staff.

Pre-Intervention

The restrooms were inoculated at 8:00 a.m. and allowed 4 hours for the virus to transfer within the restrooms and into the outside hallway. The restrooms were cleaned at 12:00 p.m. using Virex II and the hospitals current cleaning procedure. Samples were obtained immediately after the cleaning staff exited the restroom following the cleaning.

Post-Intervention

The restrooms were inoculated at 8:00 a.m. and allowed 4 hours for the virus to transfer within the restrooms and into the outside hallway. The restrooms were cleaned at 12:00 p.m. using Virex II and Clorox bleach wipes. Samples were obtained immediately after the cleaning staff exited the restroom following the cleaning.

Graphical representation of the data was developed for the different arms of the study. MS2 counts are represented in three ways: 1) Arithmetic means, used when there was a common difference such as measuring concentrations of a specific site 2) Geometric means, used in the presence of a common ration such as fomites in the women's restroom and 3) standard deviations to indicate the deviation of a group as a whole.

Analysis of Variance

For each of the MS2 datasets, a one-way ANOVA with two or more factors will be performed. Analysis of variance was conducted for each phase of the study and between the different phases to determine statistically significant differences based on a rejection region of 5%. An F statistic was calculated based on MS2 concentrations for the different factors and they were compared with the F value obtained for the 5% rejection region. A p-value was then calculated to determine if significant difference occurred among the datasets.

Confidence in statistical inferences obtained from the analysis of variance depends on the degree to which the datasets under consideration satisfy the assumptions needed to run the ANOVA tests. When conducting analysis of variance, the dependent variable is assumed to be normally distributed, have equal variances in each group under consideration and the

datasets have no outliers since analysis of variance is especially sensitive to outliers. For the analysis presented in this study, when the dependent variable was transformed to satisfy the normality test, no outliers were present.

Normality Test

The normality test was conducted using the statistical packages in the R-Language (R Core Team, 2013). If the tests show that the dependent variable is not normally distributed, one can transform the dependent variable to improve or correct the situation. Transformations typically involve replacing a variable Y with Yλ. Common forms of transformations can be $Log_{10}(Y)$, √Y, 1/Y, Y^2, etc. The most common transformation for microbiological datasets is the log_{10} (Y) in which Y is the microbial count.

Homogeneity of Variance

This condition is required when analysis of variance is conducted between one or more groups. This insures that datasets samples of the groups come from the same or similar populations.

Outliers Test

The ANOVA test is very sensitive to outlier values in the datasets. In this study, these tests were conducted using the statistical packages in the R-Language (R Core Team, 2013).

Chapter 4

Results

Before Cleaning and Disinfection

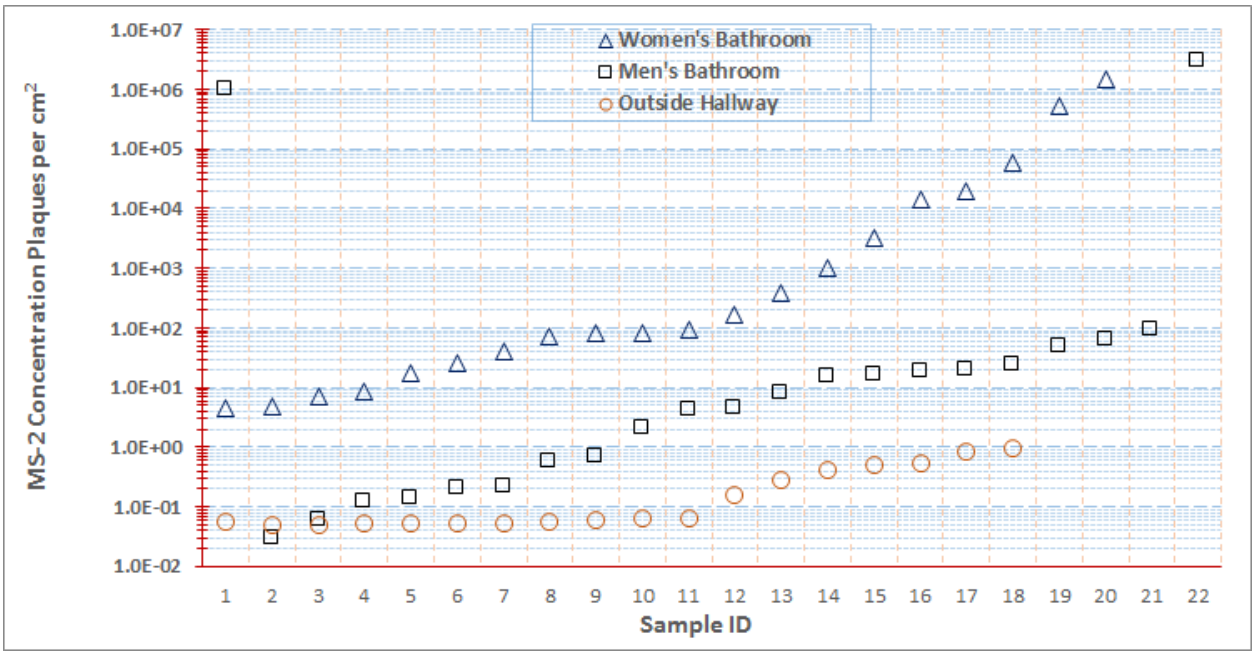
Table 2 shows the arithmetic and geometric averages for the amount of MS2 recovered from then women's restroom, men's restroom and hallway before cleaning and disinfection (control). Figure 1 shows the distribution of virus concertation in each sample from lowest to highest value. There were higher rates of recovery from sites within the women's restroom when compared to the men's restroom. The virus was demonstrated to move from the restrooms into the hallway outside. The sample sites with the most phage recovered within the women's restroom were the inside stall handle and the restroom exit, in the men's restroom they were the inside stall lock and countertop and in the hallway they were waiting room 2 chairs and the hallway door handle.

Table 2. MS2 Arithmetic and Geometric Mean per cm^2 on Fomites in the Restrooms and Outside Hallway before Cleaning and Disinfection (Control).

Statistic	Women's Bathroom	Men's Bathroom	Outside Hallway
Average	1.07E+05	1.77E+05	2.41E-01
Geo Mean	3.75E+02	8.57E+00	1.26E-01
St Dev	3.52E+05	6.43E+05	2.94E-01
Count	20	22	18

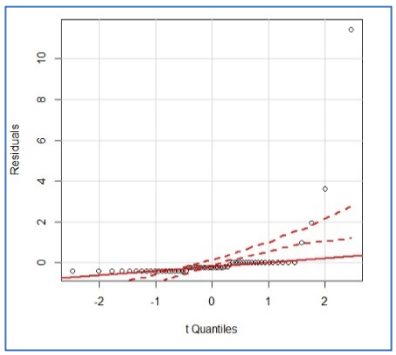
MS-2 Plaque Forming Unit (PFU)/ cm^2

Figure 1. MS2 per cm^2 for Women's Restroom, Men's Restroom and Outside Hallway for Study from Lowest to Highest value for Control

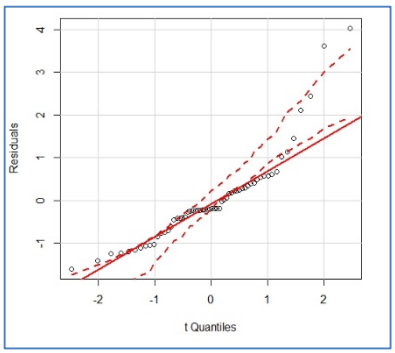


Before an ANOVA could be performed the data needed to be normally distributed. If the tests show that the dependent variable is not normally distributed the data can be normalized to the distribution of the dataset (See Material and Methods). Figure 2 shows that the best fit for normality, variance homogeneity and the absence of outliers. Figure 2c fits the requirement of normality the best for the data set. The solid line in the graph has to fall between the dashed lines for the best fit of the data. Thus the data set was using $\lambda = -0.1006$ for all of the ANOVA tests (see Material and Methods). The analysis showed that the amount of phage recovered on fomites between the restrooms and outside hallway were statistically significant ($p<0.001$).

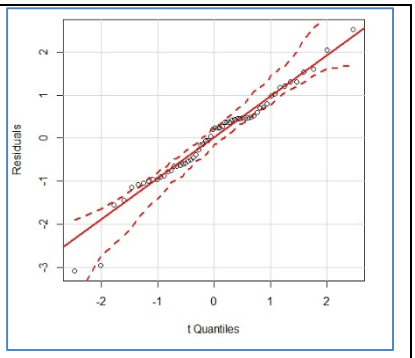
Figure 2. Transformation of MS2 Concentrations for ANOVA Testing: a) Measured MS2 on the Fomites; b) Log Transformation; c) Raising the MS2 Concentrations to the Power of -0.1006



a) No Transformation



b) Log_{10} Transformation



c) λ= - 0.1006 Transformation

After Cleaning and Disinfection

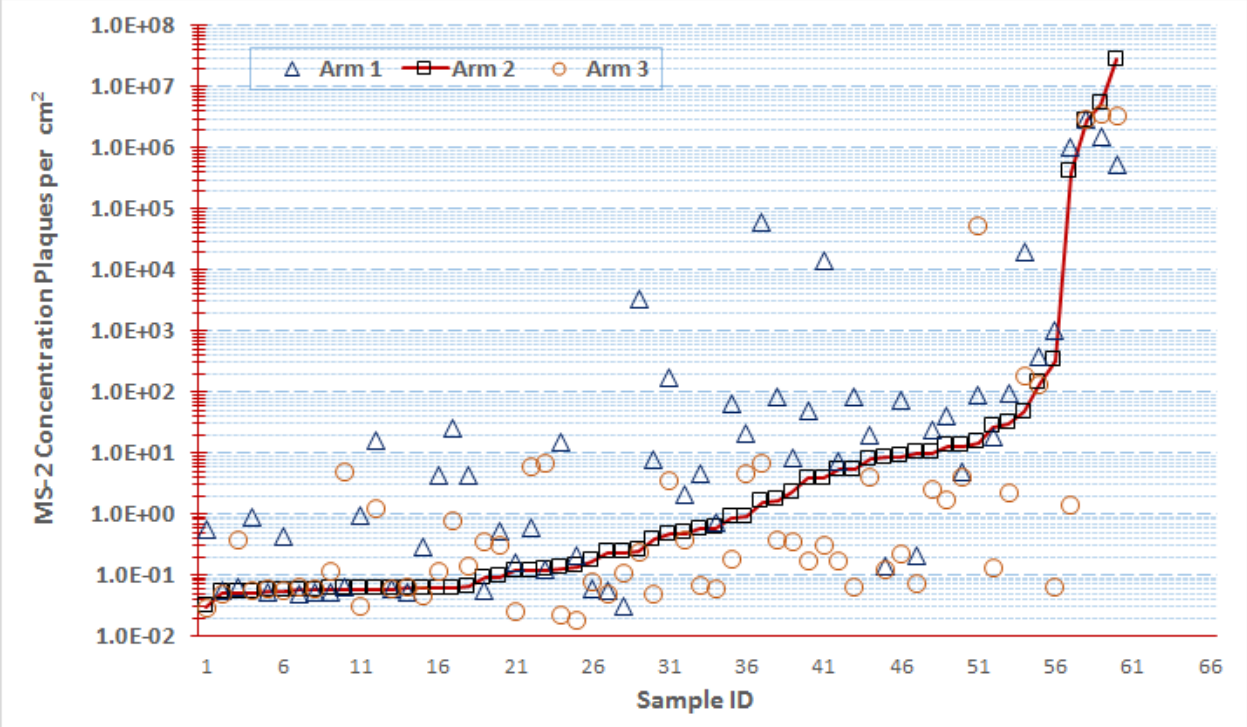
Table 3 summarizes the impact of using the existing cleaning and disinfection protocol (Pre-Intervention) on virus recovery from the fomites and the inclusion of the disinfecting wipe (Post-Intervention). Cleaning with disinfection as currently practiced resulted in an 81% reduction in virus recovery from the fomite. Inclusion of the disinfecting wipe resulted in a 91% reduction in virus recovery from the fomites. The reduction in the virus was more statistically significant when using the disinfectant wipe ($p \leq 0.001$) than using the current disinfecting procedures ($p \leq 0.051$). Figure 3 shows the distribution of MS2 on the fomites from lowest to highest concentration for current cleaning and disinfecting procedures (Pre-Intervention) compared to before cleaning and disinfection (Control) and the impact of the inclusion of the disinfecting wipe (Post-Intervention). Note that the control values are generally above the pre-intervention values and the pre-intervention values are generally above the post-intervention

values. A three-way ANOVA indicated that all of the phases of the study were statistically different ($p<0.0015$).

Table 3. MS2 Arithmetic and Geometric Means per cm^2 Determined on all Study Fomites and for All Phases

Statistic	Control	Pre-Intervention	Post-Intervention
Average	1.01E+05	6.01E+05	1.63E+05
Geo Mean	8.51E+00	1.67E+00	7.96E-01
St Dev	4.39E+05	3.63E+06	7.15E+05
Count	60	60	60

Figure 3. MS2 Concentrations from Lowest to Highest Value per cm^2 for all Fomites in the Study and for all Phases. Control is before cleaning/disinfection**.** Pre-Intervention is with cleaning and disinfection and Post-Intervention is with the addition of disinfecting wipes.



Chapter 5

Discussion

Transfer of Virus

Tracer studies are controlled by three variables, independent, dependent and controlled variables. Within the present study the products used for disinfection within the control, pre-intervention and post-intervention components of the study were controlled. During the control sampling period no products were used to reduce the transmission of virus while within the pre-intervention and post-intervention periods the use of Virex II or Virex II and Clorox bleach wipes were implemented to combat the transmission of virus. Throughout the study the restrooms used, sites selected and time of inoculation were all independent variables. These fixed variables did not change during the course of the study. The dependent variables such as the amount of people in the hospital, the amount of people to use the restroom and individual behaviors were unable to be controlled and contribute to the human variability.

The control phase of the study demonstrated a significant amount of spread of the virus surrogate within the restroom and into the hallway. The phage was recovered from 98% (41/42) of the sites within the restroom, the exception being the outside stall door inside the men's restroom. The stall door opens towards the operator when entering and can be opened using a shoe, if the operator used this method it may result in the site experiencing no transfer of virus. The average amount of phage recovered in the men's restroom ranged from 3.57×10^1 to 4.77×10^3 PFU/100 cm^2 while the average recovery in the women's restroom ranged from 4.67×10^2 to 3.67×10^6 PFU/100 cm^2. The virus was isolated in higher concentration from the women's restroom throughout the study, presumably because women are more likely to touch

surfaces within the restroom. They must use the toilet where they may contact any of the four fomites in that area, additionally the toilet is a manual flush requiring contact for flushing. Men typically use the urinal when entering a restroom requiring a minimal amount of contact with any surfaces and having automatic flushing capabilities. Judah et al (2009) reported that 31% of men and 65% of women wash their hands after using a public restroom. This factor can also attribute to higher concentrations of virus transfer for women because of the additional surfaces that must be touched when washing of the hands. The phage was recovered from 38% (7/18) of the sites within the hallway, with a range of recovery from 10.5 to 732 PFU/ 100 cm^2. The extensive degree of phage spread in the restroom and its subsequent transmission into the hallway demonstrates the ability of viruses to be transmitted from the restroom into other regions of the hospital.

The pre-intervention was designed to assess the current product and protocols used by the cleaning staff. The same individual cleaned the restrooms throughout the experiment to reduce differences in cleaning practices. The use of the Virex II spray resulted in an 81% ($P \leq 0.051$) reduction in virus concentration recovered from the fomites in the restroom. Use of the product reduced the total sites from which virus was recovered to 95% (40/42) in the restroom and had no change on the total sites recovered from the hallway totaling 38% (7/18). Virex II requires a contact time of 10 minutes where the site is visibly wet. In many cases the restrooms were cleaned for a total of 10 minutes, making it near impossible to have all sites experience the required 10-minute contact time. The contact time of the product can have implications on the reduction of microorganisms through use of the Virex II, reduction may increase through proper use of the product.

The post-intervention component included Clorox bleach germicidal wipes to be used as a disinfectant intervention paired with the Virex II spray currently used by the cleaning staff. When used together the products resulted in a 91% reduction in virus concentration recovered from the fomites. The reduction in virus was more statistically significant when using the disinfectant wipe ($P \leq 0.001$) than when using only the Virex II for disinfection ($P \leq 0.051$). Additionally, the number of sites from which virus was recovered was reduced to 85% (36/45) in the restroom but increased to 44% (8/18) from the hallway. Transmission into the hallway is the most susceptible component of the study to human variability. Depend variables such as number of people who use the restroom during the four-hour period, number of people present on the floor or in the hospital on the sampling day and individual tendencies during and after a restroom trip all contribute to overall transmission.

These public restrooms were also used by hospital staff and of the total sampling periods virus was recovered 83% (5/6) of the time from the hallway door handle. This door required a code for entry and the code was only known by hospital staff. The transmission of virus within a hospital in not exclusive to patients, hospital staff are a source of transmission as well. Other sites of concern were the nonporous countertops associated with the waiting rooms and nurse's stations throughout the floor, a total of three were selected for sampling. Virus was recovered from the countertops a total of 28% (5/18) of the time. Nearly all patients must see a nurse at one of these stations before visiting a doctor and hand contact with the counter is common, resulting in a prevalent site for the transmission of virus to patients.

Chapter 6

Conclusions

1) In a short period of time viruses are readily moved from a single fomite to multiple fomites within the restroom and into the hallway.
2) Fomites of concern within the women's restroom are identified as the stall door, faucet handles and exit handle.
3) Fomites of concern with the men's restroom are stall door, counter top and soap dispenser.
4) Fomites of concern within the hallway are the hallway door handle, waiting room counter tops and waiting room chairs.
5) The use of a hygiene intervention is effective at reducing the spread of a viral surrogate in a hospital.

Chapter 7

Appendix

Table 4: Total bacteria HPC[c] (CFU/100 cm^2) and MS2[d] Coliphage (PFU/100 cm^2) found on Surfaces Inside Restrooms Without Cleaning Products (Control)

Surface	Trial 1 MS-2 Tracer[a]				Trial 2 MS-2 Tracer[b]			
	Men's Restroom		Women's Restroom		Men's Restroom		Women's Restroom	
	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]
Restroom Entrance[a]	2.95E+01	1.30E+08	9.00E+01	6.83E+07	4.35E+02	4.50E+07	2.80E+01	2.36E+07
Outside Stall	3.47E+02	1.62E+03	6.16E+02	2.02E+06	1.09E+03	≥ 6.0	2.75E+03	9.95E+04
Inside Stall	1.34E+03	1.89E+03	3.78E+02	5.96E+06	4.96E+03	2.12E+01	2.12E+03	1.37E+06
Inside Stall Lock	6.90E+02	6.51E+03	3.00E+01	3.82E+04	2.01E+03	3.00E+00	6.10E+01	9.11E+03
Toilet Handle	8.06E+02	6.10E+00	1.44E+03	8.42E+03	3.05E+01	2.14E+02	1.35E+02	8.85E+02
Urinal Handle	1.83E+03	7.15E+01	N/A	N/A	1.20E+03	4.36E+02	N/A	N/A
Counter top	6.80E+03	9.51E+03	3.87E+03	4.08E+03	1.16E+04	2.10E+01	6.60E+03	8.31E+02
Soap Dispenser	1.60E+02	4.91E+03	3.00E+02	2.49E+03	1.55E+02	7.92E+02	1.65E+02	7.35E+03
Sink Faucet/ Handle	1.95E+03	2.42E+03	4.03E+02	7.26E+02	7.29E+03	1.52E+03	5.27E+03	8.43E+03
Paper Towel Dispenser	3.97E+02	5.76E+01	1.36E+03	4.90E+02	2.66E+03	1.38E+01	6.00E+01	4.44E+02
Restroom Exit	6.08E+02	2.01E+03	1.92E+02	3.31E+05	2.75E+03	2.02E+02	3.42E+02	8.19E+03

[a] Restroom Entrance seeded with an MS-2 concentration of 4.5 x 10^10
[b] Restroom Entrance seeded with an MS-2 concentration of 1.59 x 10^9
[c] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2
[d] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Table 5: Total Bacteria HPC[a] (CFU/100 cm2) and MS2[b] Coliphage (PFU/100 cm2) found on Surfaces Outside Restrooms Without Cleaning Products (Control)

Surface	Trial 1 MS-2 Tracer		Trial 2 MS-2 Tracer	
	HPC[a]	MS-2[b]	HPC[a]	MS-2[b]
Elevator Push Buttons	7.32E+02	≥ 6.1	1.05E+03	≥ 5.5
Door to Surgery Physician offices	3.52E+02	≥ 6.2	1.01E+03	≥ 6.2
Waiting room 1 chairs	2.41E+03	2.85E+01	2.58E+03	≥ 4.8
Waiting room 1 check in pen	2.95E+01	≥ 5.3	6.20E+01	≥ 5.1
Waiting room 1 Counter top	1.07E+03	8.58E+01	1.65E+03	≥ 5.3
Waiting room 2 Chairs	7.41E+03	4.24E+01	2.45E+03	5.40E+01
Waiting room 2 Counter top	1.83E+02	≥ 5.4	9.00E+02	1.56E+01
Hallway Door Handle	5.67E+02	9.52E+01	2.98E+03	5.12E+01
Nurses station 1 Counter top	1.03E+03	≥ 5.7	1.50E+03	≥ 5.2

[a] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2

[b] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Table 6: Total Bacteria HPC[c] (CFU/100 cm^2) and MS2[d] Coliphage (PFU/100 cm^2) found on Surfaces Inside Restrooms with Existing Hospital Products (Pre-Intervention)

Surface	Trial 1 MS-2 Tracer[a]				Trial 2 MS-2 Tracer[b]			
	Men's Restroom		Women's Restroom		Men's Restroom		Women's Restroom	
	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]
Restroom Entrance[a]	2.24E+02	1.81E+07	1.24E+02	1.25E+09	9.74E+02	1.24E+08	3.10E+01	2.34E+08
Outside Stall	2.95E+02	1.68E+01	1.62E+04	3.20E+04	5.31E+02	5.80E+00	8.85E+02	4.68E+03
Inside Stall	1.16E+02	1.38E+01	1.06E+03	3.88E+02	2.38E+03	7.87E+02	4.27E+02	1.57E+02
Inside Stall Lock	6.20E+02	2.32E+01	1.68E+02	1.49E+03	7.04E+02	8.55E+01	1.83E+02	1.38E+04
Toilet Handle	3.10E+01	3.05E+00	6.30E+01	1.33E+03	1.58E+02	≥ 5.8	1.58E+02	2.32E+01
Urinal Handle	2.70E+03	≥ 6.5	N/A	N/A	1.05E+04	8.64E+01	N/A	N/A
Counter top	2.12E+03	9.79E+02	1.74E+03	2.24E+02	1.27E+03	3.04E+03	8.40E+02	1.29E+03
Soap Dispenser	8.41E+02	3.78E+01	9.30E+01	8.60E+02	2.28E+02	3.81E+02	6.10E+01	6.10E+00
Sink Faucet/ Handle	8.40E+02	1.30E+01	1.71E+03	5.36E+02	1.98E+03	1.10E+04	1.48E+03	5.33E+02
Paper Towel Dispenser	6.10E+01	8.28E+02	1.12E+02	5.70E+01	3.84E+02	1.18E+01	4.88E+02	1.30E+03
Restroom Exit	2.82E+02	4.80E+01	9.90E+02	1.65E+02	1.86E+02	6.38E+01	1.33E+02	2.48E+01

[a] Restroom Entrance seeded with an MS-2 concentration of 9.9 x 10^10
[b] Restroom Entrance seeded with an MS-2 concentration of 4.2 x 10^10

[c] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2

[d] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Table 7: Total Bacteria HPC[a] (CFU/100 cm2) and MS2[b] Coliphage (PFU/100 cm2) found on Surfaces Outside Restrooms with Existing Hospital Products (Pre-Intervention)

Surface	Trial 1 MS-2 Tracer		Trial 2 MS-2 Tracer	
	HPC[a]	MS-2[b]	HPC[a]	MS-2[b]
Elevator Push Buttons	3.60E+02	9.00E+00	1.32E+03	≥ 5.9
Door to Surgery Physician offices	4.32E+02	≥ 5.2	4.43E+02	≥ 5.8
Waiting room 1 chairs	1.73E+03	5.70E+00	2.24E+03	≥ 6.0
Waiting room 1 check in pen	6.10E+01	6.00E+00	1.86E+02	≥ 5.5
Waiting room 1 Counter top	1.20E+02	≥ 5.7	7.20E+02	≥ 5.2
Waiting room 2 Chairs	5.77E+03	3.00E+00	4.83E+03	≥ 5.5
Waiting room 2 Counter top	6.49E+02	1.18E+01	3.00E+02	≥ 5.2
Hallway Door Handle	4.88E+02	9.30E+00	1.28E+03	≥ 5.8
Nurses station 1 Counter top	≥ 5.4	≥ 5.8	4.12E+03	2.30E+01

[a] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2

[b] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Table 8: Total Bacteria HPC[c] (CFU/100 cm^2) and MS2[d] Coliphage (PFU/100 cm^2) found on Surfaces Inside Restrooms using Intervention Product (Post-Intervention)

Surface	Trial 1 MS-2 Tracer[a]				Trial 2 MS-2 Tracer[b]			
	Men's Restroom		Women's Restroom		Men's Restroom		Women's Restroom	
	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]	HPC[c]	MS-2[d]
Restroom Entrance[a]	6.56E+02	6.30E+01	3.29E+03	1.45E+08	3.30E+02	1.28E+08	≥ 6.0	1.64E+08
Outside Stall	5.32E+02	7.50E+00	2.70E+02	≥ 6.5	5.70E+01	1.24E+02	6.00E+01	1.80E+04
Inside Stall	1.40E+03	1.85E+00	4.10E+02	3.10E+01	4.65E+02	3.97E+02	5.80E+01	6.96E+02
Inside Stall Lock	1.63E+02	1.05E+01	4.20E+02	5.10E+06	4.48E+02	1.83E+01	1.59E+02	1.34E+04
Toilet Handle	3.77E+02	≥ 5.9	3.00E+01	6.50E+00	≥ 6.2	3.38E+02	≥ 6.1	1.79E+02
Urinal Handle	2.45E+03	1.37E+01	N/A	N/A	9.74E+02	≥ 5.8	N/A	N/A
Counter top	8.70E+02	7.35E+00	4.65E+03	3.47E+01	6.48E+02	2.23E+02	5.60E+01	1.65E+02
Soap Dispenser	2.10E+03	≥ 5.0	6.93E+02	2.21E+01	1.00E+02	1.68E+01	7.67E+02	7.91E+01
Sink Faucet/ Handle	3.03E+03	2.20E+00	3.60E+03	≥ 6.5	2.88E+03	2.48E+02	9.59E+04	1.74E+01
Paper Towel Dispenser	4.50E+02	1.20E+01	2.41E+03	≥ 6.7	1.57E+03	6.08E+02	1.80E+02	4.02E+02
Restroom Exit	1.40E+03	3.69E+01	1.30E+02	3.80E+01	3.54E+02	4.58E+02	1.83E+02	2.34E+01

[a] Restroom Entrance seeded with an MS-2 concentration of 1.21 x 10^11
[b] Restroom Entrance seeded with an MS-2 concentration of 1.6 x 10^10
[c] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2
[d] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Table 9: Total Bacteria HPC[a] (CFU/100 cm2) and MS2[b] Coliphage (PFU/100 cm2) found on Surfaces Outside Restrooms Using Intervention Product (Post-Intervention)

Surface	Trial 1 MS-2 Tracer		Trial 2 MS-2 Tracer	
	HPC[a]	MS-2[b]	HPC[a]	MS-2[b]
Elevator Push Buttons	1.74E+03	3.58E+01	5.23E+02	≥ 6.0
Door to Surgery Physician offices	8.96E+02	3.72E+01	2.24E+02	4.88E+02
Waiting room 1 chairs	1.08E+03	≥ 6.2	2.75E+02	≥ 4.6
Waiting room 1 check in pen	3.10E+01	≥ 6.2	2.44E+02	≥ 6.0
Waiting room 1 Counter top	6.18E+02	≥ 5.9	3.00E+02	≥ 5.5
Waiting room 2 Chairs	3.39E+03	2.85E+00	6.27E+02	≥ 5.5
Waiting room 2 Counter top	3.16E+03	2.60E+00	4.68E+02	≥ 5.0
Hallway Door Handle	3.71E+02	3.00E+01	2.02E+03	3.05E+00
Nurses station 1 Counter top	2.97E+03	1.16E+01	1.45E+02	≥ 5.0

[a] Heterotrophic Plate Count Colony Forming Unit (CFU)/100 cm^2

[b] MS-2 Plaque Forming Unit (PFU)/100 cm^2

Literature cited

Ansari, S. A., Springthorpe, V. S., Sattar, S. A., Rivard, S., & Rahman, M. (1991). Potential role of hands in the spread of respiratory viral infections: studies with human parainfluenza virus 3 and rhinovirus 14. *Journal of Clinical Microbiology*, *29*(10), 2115-2119.

Aitken, C., & Jeffries, D. J. (2001). Nosocomial spread of viral disease. *Clinical Microbiology Reviews*, *14*(3), 528-546.

Barker, J., Stevens, D., & Bloomfield, S. F. (2001). Spread and prevention of some common viral infections in community facilities and domestic homes. *Journal of Applied Microbiology*, *91*(1), 7-21.

Barker, J. (2001). The role of viruses in gastrointestinal disease in the home. *Journal of Infection*, *43*(1), 42-44.

Barker, J., & Jones, M. V. (2005). The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. *Journal of Applied Microbiology*, *99*(2), 339-347.

Beamer, P. I., Plotkin, K. R., Gerba, C. P., Sifuentes, L. Y., Koenig, D. W., & Reynolds, K. A. (2015). Modeling of human viruses on hands and risk of infection in an office workplace using micro-activity data. *Journal of Occupational and Environmental Hygiene*, *12*(4), 266-275.

Berendt, A. E., Turnbull, L., Spady, D., Rennie, R., & Forgie, S. E. (2011). Three swipes and you're out: How many swipes are needed to decontaminate plastic with disposable wipes?. *American Journal of Infection Control*, *39*(5), 442-443.

Boone, S. A., & Gerba, C. P. (2007). Significance of fomites in the spread of respiratory and enteric viral disease. *Applied and Environmental Microbiology*, *73*(6), 1687-1696.

Boone, S. A., & Gerba, C. P. (2005). The occurrence of influenza A virus on household and day care center fomites. *Journal of Infection*, *51*(2), 103-109.

Cosgrove, S. E., Qi, Y., Kaye, K. S., Harbarth, S., Karchmer, A. W., & Carmeli, Y. (2005). The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infection Control & Hospital Epidemiology*, *26*(02), 166-174.

Dancer, S. J. (2009). The role of environmental cleaning in the control of hospital-acquired infection. *Journal of Hospital Infection*, *73*(4), 378-385.

England, B. L. (1982). Detection of viruses on fomites. *Methods in environmental virology. Marcel Dekker, Inc., New York, NY*, 179-220.

Ferguson, A., Biddle, D., Coleman, S., Bursac, Z., & Johnson, W. (2009). In-vitro soil adherence for dermal exposure using a controlled mechanical chamber. *Journal of Applied Sciences Research*, *5*(2), 232-243.

Flores, G. E., Bates, S. T., Knights, D., Lauber, C. L., Stombaugh, J., Knight, R., & Fierer, N. (2011). Microbial biogeography of public restroom surfaces. *PLoS One*, *6*(11), e28132.

Ganime, A. C., Carvalho-Costa, F. A., Mendonça, M. C. L., Vieira, C. B., Santos, M., Costa Filho, R., & Leite, J. P. G. (2012). Group A rotavirus detection on environmental surfaces in a hospital intensive care unit. *American Journal of Infection Control*, *40*(6), 544-547.

Gibbons, S. M., Schwartz, T., Fouquier, J., Mitchell, M., Sangwan, N., Gilbert, J. A., & Kelley, S. T. (2015). Ecological succession and viability of human-associated microbiota on restroom surfaces. *Applied and Environmental Microbiology*, *81*(2), 765-773.

Johnson, D., Lynch, R., Marshall, C., Mead, K., & Hirst, D. (2013). Aerosol generation by modern flush toilets. *Aerosol Science and Technology*, *47*(9), 1047-1057.

Johnson, D. L., Mead, K. R., Lynch, R. A., & Hirst, D. V. (2013). Lifting the lid on toilet plume aerosol: a literature review with suggestions for future research. *American Journal of Infection Control*, *41*(3), 254-258.

Judah, G., Aunger, R., Schmidt, W. P., Michie, S., Granger, S., & Curtis, V. (2009). Experimental pretesting of hand-washing interventions in a natural setting. *American Journal of Public Health*, *99*(S2), S405-S411.

Julian, T. R., Leckie, J. O., & Boehm, A. B. (2010). Virus transfer between fingerpads and fomites. *Journal of Applied Microbiology*, *109*(6), 1868-1874.

Kelley, S. T., Theisen, U., Angenent, L. T., Amand, A. S., & Pace, N. R. (2004). Molecular analysis of shower curtain biofilm microbes. *Applied and Environmental Microbiology*, *70*(7), 4187-4192.

Kelley, S. T., & Gilbert, J. A. (2013). Studying the microbiology of the indoor environment. *Genome Biol*, *14*(2), 202.

Klevens, R. M., Edwards, J. R., Richards Jr, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A., & Cardo, D. M. (2007). Estimating health care-associated infections and deaths in US hospitals, 2002. *Public health reports*, 160-166.

Klevens, R. M., Edwards, J. R., Richards Jr, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A., & Cardo, D. M. (2007). Estimating health care-associated infections and deaths in US hospitals, 2002. *Public Health Reports*, 160-166.

Kramer, A., Schwebke, I., & Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*, *6*(1), 1.

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*, 69-76.

Lee, L., Tin, S., & Kelley, S. T. (2007). Culture-independent analysis of bacterial diversity in a child-care facility. *BMC Microbiology*, *7*(1), 1.

Link, L., Lukens, S., & Bush, M. A. (1995). Spherical grip strength in children 3 to 6 years of age. *American Journal of Occupational Therapy*, *49*(4), 318-326.

Lopez, G. U., Kitajima, M., Havas, A., Gerba, C. P., & Reynolds, K. A. (2014). Evaluation of a disinfectant wipe intervention on fomite-to-finger microbial transfer. *Applied and Environmental Microbiology*, *80*(10), 3113-3118.

Love, S. S., Jiang, X., Barrett, E., Farkas, T., & Kelly, S. (2002). A large hotel outbreak of Norwalk-like virus gastroenteritis among three groups of guests and hotel employees in Virginia. *Epidemiology and Infection*, *129*(01), 127-132.

Masago, Y., Shibata, T., & Rose, J. B. (2008). Bacteriophage P22 and Staphylococcus aureus attenuation on nonporous fomites as determined by plate assay and quantitative PCR. *Applied and Environmental Microbiology*, *74*(18), 5838-5840.

Mbithi, J. N., Springthorpe, V. S., & Sattar, S. A. (1991). Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Applied and Environmental Microbiology*, *57*(5), 1394-1399.

McManus, C. J., & Kelley, S. T. (2005). Molecular survey of aeroplane bacterial contamination. *Journal of Applied Microbiology*, *99*(3), 502-508.

Ott, R. L., & Longnecker, M. (2001). Statistical methods and data analysis. *Thomson Learning*.

Panousi, M. N., Williams, G. J., Girdlestone, S., Hiom, S. J., & Maillard, J. Y. (2009). Evaluation of alcohol wipes used during aseptic manufacturing. *Letters in Applied Microbiology*, *48*(5), 648-651.

Prazuck, T., Compte-Nguyen, G., Pelat, C., Sunder, S., & Blanchon, T. (2010). Reducing gastroenteritis occurrences and their consequences in elementary schools with alcohol-based hand sanitizers. *The Pediatric Infectious Disease Journal*, *29*(11), 994-998.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reynolds, K. A., Watt, P. M., Boone, S. A., & Gerba, C. P. (2005). Occurrence of bacteria and biochemical markers on public surfaces. *International Journal of Environmental Health Research*, *15*(3), 225-234.

Rheinbaben, F. V., Schünemann, S., Gross, T., & Wolff, M. H. (2000). Transmission of viruses via contact in a household setting: experiments using bacteriophage ϕX174 as a model virus. *Journal of Hospital Infection*, *46*(1), 61-66.

Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L., & Nevalainen, A. (2008). Diversity and seasonal dynamics of bacterial community in indoor environment. *BMC Microbiology*, *8*(1), 56.

Rose, L. J., Hodges, L., O'Connell, H., & Noble-Wang, J. (2011). National validation study of a cellulose sponge wipe-processing method for use after sampling Bacillus anthracis spores from surfaces. *Applied and environmental microbiology*, *77*(23), 8355-8359.

Rusin, P., Maxwell, S., & Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*, *93*(4), 585-592.

Rutala, W. A., White, M. S., Gergen, M. F., & Weber, D. J. (2006). Bacterial contamination of keyboards: efficacy and functional impact of disinfectants. *Infection Control*, *27*(04), 372-377.

Sassi, H. P., Sifuentes, L. Y., Koenig, D. W., Nichols, E., Clark-Greuel, J., Wong, L. F., & Reynolds, K. A. (2015). Control of the spread of viruses in a long-term care facility using hygiene protocols. *American Journal of Infection Control*, *43*(7), 702-706.

Sattar, S. A. (2001). Survival of microorganisms on animate and inanimate surfaces and their disinfection. *Disinfection, sterilization and antisepsis: principles and practices in healthcare facilities. Association for Professionals in Infection Control and Epidemiology, Inc., Washington, DC*, 195-205.

Scott, E., & Bloomfield, S. F. (1990). The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Bacteriology*, *68*(3), 271-278.

Seymour, I. J., & Appleton, H. (2001). Foodborne viruses and fresh produce. *Journal of Applied Microbiology*, *91*(5), 759-773.

Sobsey, M. D., & Meschke, J. S. (2003). Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin. *Report for the World Health Organization, Geneva, Switzerland*, *70*.

Sifuentes, L. Y., Koenig, D. W., Phillips, R. L., Reynolds, K. A., & Gerba, C. P. (2014). Use of hygiene protocols to control the spread of viruses in a hotel. *Food and Environmental Virology*, *6*(3), 175-181.

Sperber, S. J., Turner, R. B., Sorrentino, J. V., O'Connor, R. R., Rogers, J., & Gwaltney Jr, J. M. (2000). Effectiveness of pseudoephedrine plus acetaminophen for treatment of symptoms attributed to the paranasal sinuses associated with the common cold. *Archives of Family Medicine*, *9*(10), 979.

Siani, H., Cooper, C., & Maillard, J. Y. (2011). Efficacy of “sporicidal” wipes against Clostridium difficile. *American Journal of Infection Control*, *39*(3), 212-218.

Stebbins, S., Cummings, D. A., Stark, J. H., Vukotich, C., Mitruka, K., Thompson, W., & Dato, V. (2011). Reduction in the incidence of influenza A but not influenza B associated with use of hand sanitizer and cough hygiene in schools: a randomized controlled trial. *The Pediatric Infectious Disease Journal*, *30*(11), 921.

Strauss, J. H., & Sinsheimer, R. L. (1963). Purification and properties of bacteriophage MS2 and of its ribonucleic acid. *Journal of molecular biology*, *7*(1), 43-54.

Tamimi, A. H., Carlino, S., Edmonds, S., & Gerba, C. P. (2014). Impact of an alcohol-based hand sanitizer intervention on the spread of viruses in homes. *Food and Environmental Virology*, *6*(2), 140-144.

Verani, M., Bigazzi, R., & Carducci, A. (2014). Viral contamination of aerosol and surfaces through toilet use in health care and other settings. *American Journal of Infection Control*, *42*(7), 758-762.

Ward, R. L., Bernstein, D. I., Knowlton, D. R., Sherwood, J. R., Young, E. C., Cusack, T. M., & Schiff, G. M. (1991). Prevention of surface-to-human transmission of rotaviruses by treatment with disinfectant spray. *Journal of Clinical Microbiology*, *29*(9).

Warren-Gash, C., Fragaszy, E., & Hayward, A. C. (2013). Hand hygiene to reduce community transmission of influenza and acute respiratory tract infection: a systematic review. *Influenza and Other Respiratory Viruses*, *7*(5), 738-749.

Weemering, M. L. (2001). Survival of enterococci and staphylococci on hospital fabrics and plastic. *AORN Journal*, *73*(2), 515-515.

Williams, G. J., Denyer, S. P., Hosein, I. K., Hill, D. W., & Maillard, J. Y. (2007). The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with Staphylococcus aureus. *Journal of Hospital Infection*, *67*(4), 329-335.

Williams, G. J., Denyer, S. P., Hosein, I. K., Hill, D. W., & Maillard, J. Y. (2009). Limitations of the efficacy of surface disinfection in the healthcare setting. *Infection Control & Hospital Epidemiology*, *30*(06), 570-573.