ASSESSMENT OF MICROBIAL TRANSFER EFFICIENCY AND ANTIMICROBIAL EFFICACY FOR A SILVER EMBEDDED ANTIMICROBIAL COATING

by

Bryce Richard Kirkpatrick

A Thesis Submitted to the Faculty of the

DEPARTMENT OF AGRICULTURAL AND BIOSYSTEMS ENGINEERING

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2018

STATEMENT BY AUTHOR

The thesis titled ***Assessment of Microbial Transfer Efficiency and Antimicrobial Efficacy for a Silver Embedded Antimicrobial Coating*** by **Bryce Richard Kirkpatrick** 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 acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may 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: Bryce Richard Kirkpatrick

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Date: 8/10/18

Donald Slack
Professor of Biosystems Engineering

ACKNOWLEDGMENTS

I would like to thank first and foremost my loving wife and parents who made all of my academic accomplishments possible.

Additionally I would like to thank my support group in the lab: Dr. Luisa Ikner, Aidan Foster, Dr. David Williams, Jessica Rosansky, Erica Stark, and Dr. Huruy Zerzghi. Their assistance and guidance with this study was crucial to its completion.

I would also like to thank Dr. Gerba and Dr. Ikner for their willingness to take me on as an inexperienced student and guide him through the trials and tribulations of microbiology.

Special thanks are also extended to Dava Jondall. Without her, I would have missed many deadlines and graduated much later than needed.

Lastly, I want to thank Dr. Slack. He has guided me through this whole process and has been crucial to not only my success in graduation but my knowledge as an engineer as well.

Dedication

I dedicate this thesis to my wife, my father, and my mother. They bore the brunt of my less than cordial attitudes, late nights, and costs associated with my completion of this research. I sincerely appreciate their never-ending patience and support throughout this journey. I hope to be able to repay all that you have given me one day.

Table of Contents

List of Tables

Table of Figures

Abstract

Hospital acquired infections (HAIs) are becoming an issue of increasing importance within the medical field. A recent report from the United States Centers for Disease Control and Prevention (CDC) states that over 700,000 HAIs occurred during 2011, with approximately 75,000 deaths attributed or associated with these infections (CDC, 2016). Historically, inadequate disinfection has been implicated in HAI outbreaks (Reiss et al. 2000). This has stimulated a demand for passive disinfection methods that eliminate the need for frequent human intervention to reduce microbial levels on surfaces.

This study examined the antimicrobial efficacy and transfer capabilities for a silver embedded slow release coating. The coating was designed to be used on high touch surfaces where at-risk hospital patients may likely be exposed to pathogens. The efficacy and transfer of two types of bacteria and a one virus were studied using a silver embedded coating and compared to non-coated stainless steel slides. *Enterobacter aerogenes* (ATCC 13048), *Staphylococcus aureus* (ATTC 6538P), and MS2 15597 B-1 bacteriophage were the study organisms. An inoculum was placed onto each carrier. The carriers were either harvested directly (i.e. controls), or subjected to finger-touch transfers after 0, 1, 4, or 8 hours after drying.

The maximum observed reduction due to the antimicrobial properties of the coating for *E. aerogenes* was 4.49 $\log_{10}$, compared to 1.65 $\log_{10}$ for the non-coated carrier controls. The average transfer efficiencies of *E. aerogenes* to finger pads for both carrier types was 10%. The maximum reduction of *S. aureus* was 1.59 $\log_{10}$ for the silver-coated carriers, relative to 1.49 $\log_{10}$ for non-coated controls. For *S. aureus*, the average transfer efficiencies were 13% and 36% for silver-coated and non-coated respectively, with no statistical significance observed ($p=0.10$). MS2 bacteriophage levels were not significantly reduced; however, the mean transfer efficiency to the finger pads from the non-coated control carriers was slightly greater at 10%, compared to only 6% for the silver-embedded carrier

The efficacy and transfer efficiency values obtained were utilized to conduct a Quantitative Microbial Risk Assessment (QMRA) for both *Staphylococcus aureus* and *Enterobacter aerogenes*. *Klebsiella pneumoniae* was chosen as the representative model organism for *E. aerogenes* in the study. For the given exposure assessment scenario and parameters, the infection risk for *K. pneumoniae* following transfer of the organism to finger pads from non-treated stainless steel control carriers was 3 orders of magnitude greater than that following finger pad contamination from silver embedded carriers. Methicillin-resistant *Staphylococcus aureus* (MRSA) was chosen as a representative for *Staphylococcus aureus* (ATTC 6538p). For this risk scenario, the coated vs non-coated surface was the determining factor that made the risk of infection of MRSA acceptable vs not acceptable for a week long hospital stay.

1. Introduction

1.1 Statement of the problem

Hospital acquired infections (HAIs) can result in increased morbidity and mortality rates for patients, and are costly for hospitals. In the hospital setting, pathogens are most frequently transmitted by way of direct contact (e.g. person-to-person), contaminated surfaces (i.e. fomites) that have not been adequately disinfected using standard liquid formulations (e.g. chlorine-based compounds) may also serve as a reservoir for infection. Commercially-available passive self-disinfecting/self-sanitizing antimicrobial technologies may effectively decrease the rates of pathogen transmission from contaminated fomites in the hospital environment. Such technologies require further evaluation to demonstrate whether a decrease in surface contamination will result in a lower risk of infection for susceptible patients.

1.2 Goals of the study

1.2.1 General objective

The objective of this research was to quantify the overall risk of contracting common nosocomial infections following the contamination of finger pads immediately following direct contact with contaminated surfaces. Two types of surfaces were evaluated: 1) stainless steel typical of that found comprising surfaces present in hospitals (e.g. sinks, handrails) which served as a negative control, and 2)

stainless steel treated with a shrink-wrap material comprised, in part, of a slow-release, silver-based active chemistry designated as the "test" surface.

1.2.2 Specific aims

Specific Aim 1: Determine microbial transfer efficiencies from two contaminated surface types (i.e. stainless steel-control and silver embedded shrink-wrapped stainless steel) for one enteric virus surrogate (MS2-15579-B1), one Gram-positive representative bacterium (*Staphylococcus aureus* (ATTC 6538p), and one Gram-negative representative bacterium (*Enterobacter aerogenes* (ATTC 13048).

Specific Aim 2: Assess the log reduction values (LRV) of the three organisms over several exposure periods for both the stainless steel control and silver embedded test surfaces for the three types of organisms, and determine whether reductions were attributed to natural decay or antimicrobial efficacy of the silver-based coating.

Specific Aim 3: Using the data accumulated following completion of Aims 1 and 2, develop a quantitative microbial risk assessment (QMRA) framework for calculating the risk of infection following finger pad contamination via fomite reservoirs for currently-relevant nosocomial pathogens.

2. Literature review

2.1.1 Nosocomial infections

Nosocomial infections (NI), also known as healthcare-associated infections (HAIs), are contracted during medical treatment in a healthcare facility (CDC, 2016). These infections are of growing concern for healthcare providers and patients. The

pathogens causing these infections are often opportunistic and infect patients due to underlying ailments which compromise their immunity, whether it's disease, surgery, or intubation.(Breathnach, 2009).

2.1.2 Incidence

The incidence of nosocomial infections in the U.S. has been on a downward trend recently, but is still of significant concern (CDC, 2016). In 2011, there were an estimated 721,800 nosocomial infections within U.S. acute care hospitals (CDC, 2016). Of these estimated infections, there were approximately 75,000 patient deaths with confirmed HAIs (CDC, 2016).

HAIs are also problematic globally. The incidence of HAIs in 20 separate European pediatric intensive care units (PICU) was 23.6% (Raymond & Aujard, 2000). The mortality rated in a PICU setting in Iran was much higher for patients who contracted NI (40%) than those who did not (14.7%) (Hossein & Alireza, n.d.). In addition to PICUs, long-term care facilities such as nursing homes pose a risk to their patients (Montoya & Mody, 2011). With 1.6 million people residing in US nursing homes, approximately 2 million HAIs occur every year (Montoya & Mody, 2011). This places additional strain on the staff and the residents of these homes.

2.1.3 Mortality rates

For hospital patients, contracting an HAI results in an increased risk of mortality (Glance, Stone, Mukamel, & Dick, 2011; SCOTT, 2010). Patient mortality has been found to increase by six-fold if an HAI is contracted (Glance, Stone, Mukamel, & Dick, 2011). If the transmission of secondary infections can be reduced, patient health

and safety can be significantly increased in the hospital setting (Glance, Stone, Mukamel, & Dick, 2011). One study found that 23% of patients that were carriers (non-harmful colonization) of methicillin-resistant *Staphylococcus aureus* (MRSA) eventually developed symptomatic MRSA infections (Datta & Huang, 2008) within a year of the colonization diagnosis. Further, 21% of these infected patients died from complications associated with MRSA (Datta & Huang, 2008). Another study found that 19% of a group of patients infected with *Acinetobacter baumannii* died directly from the infection, and an additional 25% died due to pre-existing ailments coupled with the infection (Seifert et al. 1995).

2.1.4 Cost

The cost of HAIs can be staggering as hospitals and patients must contend with the extra burden of the secondary diseases that results from these infections. Considering an average of 4.5 HAIs per 100 admissions in U.S. hospitals, the costs are between $35.7 and $45 billion dollars annually (Scott, 2010). Some have speculated a cost analysis of reducing the HAI pathogens on surfaces in a clinical setting. Based on reduction data, it has been demonstrated that treatment of high-touch surfaces using disinfecting wipes can save hospitals a minimum of $51,000 per year for MRSA alone (Dancer, et al., 2009).

2.1.5 Theorized causes of HAIs

There are a multitude of sources that theorize the different origins of HAI pathogens in the clinical setting (Dancer, 2014). HAI pathogens have been found on hospital sinks, bedside surfaces, hospital computers, among other surface types (Dancer,

2014). They have also been isolated from the hands of patients as well as healthcare workers, suggesting their role in aiding the spread of these organisms throughout the clinical setting.

Tracer studies have shown that organisms can travel from a bathroom handle to multiple surfaces in a hospital within relatively short time periods (Pivo et al., 2016). MS2 bacteriophage, an enteric viral surrogate and tracer model, was isolated from all 21 sites sampled, including one 50 feet away from the origin of inoculation, indicating that HAI organisms can spread relatively rapidly through a facility. Therefore, their continuous inactivation on surfaces appears necessary.

Furthermore, these organisms can survive for extended periods of time on high-touch surfaces (Kramer, Schwebke, & Kampf, 2006). Data suggests that *Klesiella* spp. along with certain strains of *Escherichia coli* can survive desiccation for more than a year (Kramer et al., 2006). This further supports the idea that nosocomial pathogens are of considerable danger and may remain so for an extended period of time.

2.2 Nosocomial Infection Prevention Techniques

2.2.1 Disinfection

Disinfection is a common practice in the clinical setting and is practiced in a variety of ways depending on factors such as patient discharge rate, traffic intensity, and spillages (*The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes*, 2007). Surfaces that harbor these pathogens can be classified in two groups: critical and noncritical surfaces (Dancer, 2014). Noncritical surfaces have not been touched or handled often, and include

floors, soft furniture, and ceilings (Dancer, 2014). Critical or high-touch surfaces are touched and/or handled often such as buttons, handrails, computer keyboards, and non-invasive clinical equipment (Dancer, 2014).

While there are no national standards for hand hygiene when it comes to healthcare workers, the CDC recommends that healthcare workers wash their hands before and after interacting with patients in addition to many other scenarios (Boyce & Pittet, 2002). Additionally, the CDC suggests that there is strong evidence that healthcare worker hand hygiene plays a large roll in both the HCWs and their patient's health (Boyce & Pittet, 2002). Although the CDC has developed a broad set of hand washing protocol recommendations for healthcare workers, hospitals enforce protocols of their own (Boyce & Pittet, 2002). The most common procedures for hand hygiene are as follows (Mathur, 2011):

1. Remove jewelry.
2. Rinse hands in warm water.
3. Lather hands thoroughly with soap, covering all parts of the hands and wrists.
4. Rinse thoroughly with warm water and turn sink off with elbow or wrist.
5. Pat dry with a paper towel or dry with forced air.
6. Sanitize hands with alcohol rub if available; rub over hands thoroughly until dry.

Compliance is another issue of concern with regards to handwashing. HCWs do not always follow the established protocols. Compliance rates have been documented ranging from 3%, and to 100% across multiple hospitals (Musu et al. 2017 & Chavali et al. 2014). Compliance is especially important since the carriage of harmful bacteria on HCWs hands has been highly associated with secondary nosocomial infections (Boyce & Pittet, 2002).

Most of the current cleaning protocols utilize manual methods with detergents (i.e. soaps) and/or disinfectants such as sodium hypochlorite or hydrogen peroxide (Dancer, 2014). The process typically involves the use of equipment such as wipes, washcloths, brushes, mops, brooms, etc. (The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes, 2007).

Cleaning and disinfection methods in the clinical setting are important to both the healthcare provider and patients. The methodology of cleaning a clinical setting depends upon patient turnover, intensity of traffic from people, risk of infection, and surface characteristics (Dancer, 2014). Certain areas such as transplant wards, intensive care units, and operating rooms are considered high risk and are therefore subject to more stringent cleaning regimens (Dancer, 2014). There is a need for written specification of cleaning requirements in addition to the ability of infection control personnel to evaluate and publish their findings on cleaning and decontamination technologies (Dancer, 2014).

The cleaning personnel themselves are often a topic of discussion in the effectiveness of preventing HAIs (The national specifications for cleanliness in the

NHS: a framework for setting and measuring performance outcomes, 2007). Most of these types of employees do not enjoy the same opportunities for advancement that other careers provide (The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes, 2007). These factors combined with language barriers and inadequate training can make common cleaning practices insufficient for the proper disinfection of surfaces (The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes, 2007).

2.2.3 Antimicrobial surfaces

Inherently antimicrobial surfaces have been gaining popularity in recent years for their use in the prevention of HAIs (Dancer, 2014). There are two types of antimicrobial surfaces typically used in the hospital setting. The first type has surface properties designed to prevent the adhesion of microbes onto surfaces, thus preventing the spread of disease (Page et al. 2009). These materials are effective in preventing the colonization of surfaces by microbes; however, they are not effective at inactivating microorganisms (Page et al. 2009). The second type of surface is comprised of active ingredients employed as coatings, or embedded in the product that inactivates microbes upon contact or over time (Page et al. 2009).

2.2.4 The use of silver as an antimicrobial agent

Historically, the element silver has been known for its antimicrobial properties (Page et al. 2009). Records have shown that both the Greeks and the Romans

utilized silver coins as well as containers to make potable water (Page et al. 2009). In large enough quantities, silver works effectively as a biocide (Page et al. 2009). It is theorized that antimicrobial mechanism of silver occurs when silver cations bind to thiol groups on proteins, disrupting the molecules, and inactivating the microbes (Page et al. 2009). In recent years, companies have increasingly employed silver's antimicrobial properties in coatings designed to prevent surface colonization and contamination.

2.3 Quantitative Microbial Risk Assessment

Quantitative microbial risk assessment (QMRA) is a tool utilized to quantify risk of infection from pathogenic microbes. A formal QMRA is performed in four major steps: hazard identification, dose response, exposure assessment, and risk characterization (Haas, Rose, & Gerba, 2014). The first step, hazard identification, involves researching the pathogen of interest thoroughly to identify its life cycle, infection routes, growth/living conditions, and symptoms after infection (Joan, 2015). The dose response of a QMRA allows for the quantitative modeling aspect of QMRA to come to fruition. Organisms are classified into two dose response models, exponential and Beta-Poisson (Haas, Rose, & Gerba, 2014). The exponential model utilizes a value denoted as "k" that represents the independent and identical probability of organisms to reach and infect a host in the appropriate site in addition to the dose of the organism denoted as "d" to create a probability of infection (C. Haas, n.d.). The Beta-Poisson utilizes the dose at which 50% of the population is affected, denoted as "N_{50}," which is derived from a value unique to each organism

known as "α" (Haas, 2017). From the α, N_{50}, and the dose, the Beta-Poisson model produces a probability of infection akin to the exponential model (C. Haas, 2017). The third step to model in the QMRA process is the exposure assessment. The exposure assessment is performed to characterize human behavior in order to determine probabilistic exposure to organisms (Shibata, n.d.). This involves two steps. The first involves researching the pathways for organisms to reach the area of interest, and the second is the estimation of human exposure to the microbes (Shibata, n.d.). This entails researching trends on how often people are in proximity to exposed areas, how often they make contact with a dose of the organism, and how often the organism is exposed via the appropriate route to an individual (Shibata, n.d.). The last portion of the risk assessment, risk characterization, incorporates the three previous steps in order to obtain probabilistic values for risk of infection (Gurian, 2015). The U.S. Environmental Protection Agency (USEPA) uses Monte Carlo simulations as their standard for risk characterization (Voltaggio, n.d.). Monte Carlo simulations stochastosize variables such as dose to create and account for uncertainty rather than utilizing point variable values (Voltaggio, 2016). This is accomplished via computerized random number generation algorithms (Voltaggio, 2016). With the aid of computer aided Monte Carlo simulations, the QMRA will provide a probability distribution for infection (Voltaggio, 2016). Additionally, from values for risk of morbidity, the risk of mortality can also be quantified using values typically collected from the dose response (Voltaggio, 2016).

2.3.1 Risk

QMRA is used to influence and determine a wide range of governmental policy ("Risk Assessment Guidelines," n.d.). The USEPA utilizes QMRA data to influence regulations developed to protect the populace ("Risk Assessment Guidelines," n.d.). However, there are multiple facets that must be considered once a QMRA is performed. According to the World Health Organization, a risk is considered acceptable when:

It is below a predetermined probability (Fewtrell, Bartram, 2001).

It is below an already utilized or tolerated level (Fewtrell, Bartram, 2001).

It is below a fraction of total disease load in an area or community (Fewtrell, Bartram, 2001).

The cost benefit of reducing the risk is not more than the cost saved this includes the cost of suffering (Fewtrell, Bartram, 2001).

There are other areas where the opportunity costs could benefit more pressing public health issues (Fewtrell, Bartram, 2001).

Public health professionals approve the risk as acceptable (Fewtrell, Bartram, 2001).

The general populace/public openly accept the risk, and when politicians say that the risk is acceptable (Fewtrell, Bartram, 2001).

The US EPA defines the acceptable yearly probabilistic microbial risk as 1 in 10,000 people (Hunter, Fewtrell, n.d.). This is usually applied to activities such as drinking water, eating food, or swimming where facilities are required to provide safe products or environments for people to consume and occupy, respectively (Hunter, Fewtrell, n.d.)

2.4. QMRA framework for the current study

This experiment utilized QMRA to model the risk of morbidity for two common HAI pathogens: *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus*. The experimental portion of the study focused on the use of a silver-embedded antimicrobial shrink wrap material to potentially decrease the transfer of microbes to human finger pads under a standard set of environmental conditions. These pathogens tested were used in a surrogate-wise fashion for the selection organisms to employ in the QMRA model that are major contributors to the HAIs across multiple developed countries (Page et al. 2009).

2.4.1 Test Organisms and their QMRA counterparts

Table 1: Represents the test organisms for this study, the organisms they are intended to represent for the QMRA study and the reason the test organism and QMRA organisms are matched together.

2.4.1.1 MRSA

Efficacy/Transfer Test Organism	**Model Organisms Employed for QMRA model**	**Selection Rationale**
Enterobacter aerogenes (ATTC 13048)	*Klebsiella pnuemoniae*	Gram negative, enteric bacteria
Staphylococcus aureus (ATTC 6538p)	Methicillin-resistant *Staphylococcus aureus*	Same species, medically relevant HAI
MS2 15597-B1	Not performed	Not performed

Table 1: Listing of the test organisms used in the efficacy/transfer efficiency studies, and the corresponding microbial hazard organisms employed in the QMRA with justification/rationale.

MRSA is an opportunistic HAI pathogen that causes a variety of ailments and ("MRSA Tracking," n.d.). This opportunistic pathogen can be carried asymptomatically, usually in the nose ("MRSA Tracking," n.d.). Estimates indicate that up to 2% of people in the US are colonized with MRSA ("MRSA Tracking," n.d.), making the pathogen both common and readily spread. MRSA is resistant to beta lactam antibiotics, making it difficult to treat with most conventional methods ("MRSA Tracking," n.d.). The most common current treatment for MRSA heavily relies the use of vancomyocin ("MRSA Tracking," n.d.).

2.4.1.2 *Klebsiella*

Klebsiella pneumoniae is an opportunistic, enteric bacterial pathogen that is found naturally in the human gut ("*Klebsiella pneumoniae* in Healthcare Settings" n.d.). *Klebsiella* is often introduced to the environment via human stool ("*Klebsiella pneumoniae* in Healthcare Settings" n.d.). Like MRSA, *Klebsiella* has multiple infection routes and causes a variety of ailments ("Kle*bsiella pneumoniae* in Healthcare Settings" n.d.). Some of the more common *Klebsiella* infections occur as blood stream infections or pneumonia, and are traditionally treated with antibiotics ("*Klebsiella pneumoniae* in Healthcare Settings" n.d.). However, there has been an increasing occurrence of antibiotic-resistant *Klebsiella* strains associated with a class of antibiotics known as carbapenems ("*Klebsiella pneumoniae* in Healthcare Settings" n.d.).

3. Present Study

3.1 Materials and Methods

3.1.1 Fomite-to-Finger pad Transfer Study: Pre-experimental considerations and general information

All transfers trials were performed at a "low" relative humidity range of 15-32% (Lopez, 2013) and at a temperature range of 21-26 degrees ^{o}C. To ensure that no residual antimicrobials were present on the test subject's hands, the subject stopped the use of antimicrobial soaps and/or cleaning agents for 4 weeks prior to the first transfer. Dey-Engley (DE) broth (Catalog # 281910, Thermo Fisher Scientific 168 Third Avenue Waltham, MA USA 02451) was used throughout this experiment as a quench for the antimicrobial properties of the silver embedded coating as well as the stainless steel controls.

Each control and test carrier (two-inch by two-inch by one-eighth inch) was inoculated with 10 µL of 10^{8} colony forming units per milliliter (CFU/ml) or plaque forming units per ml (PFU/ml) to achieve a total inoculation of 10^{6} organisms per carrier. An organic soil load was prepared for addition to the test culture comprised of 5% w/v bovine serum albumin (BSA) (catalogue # BP671-1, Thermo Fisher Scientific 168 Third Avenue Waltham, MA USA 02451), 140 µL of 5% w/v yeast extract (YE) (catalogue # H26769, Alfa Aesar 2 Radcliff Rd Tewksbury, MA 01876), 400 µL of 0.4% w/v bovine mucin (catalogue # ICN 15574280, Fisher Scientific 168 Third Avenue Waltham, MA USA 02451), and 1360 µL 0.01M phosphate buffer solution (PBS) (catalogue # P3813, Sigma-Aldrich 3050 Spruce St. St. Louis, MO 63103). This soil load was then filter-sterilized through a 0.2 µm filter (catalogue #4562, Pall Corporation, 25 Harbor Park Drive, Port Washington, NY). One mL of the

prepared soil load was added to nine mL of the 10^8 CFU/mL and PFU/mL stock solutions, resulting in a 10% soil load by volume.

Existing literature suggests that standard finger pad pressure (during organism transfer) for transfer studies should be approximately 1 kg/cm^2 (Lopez, 2013). In order to achieve the appropriate pressure, the subject's finger pad area was assessed. It was assumed the subject's finger pads created a uniform pressure during transfer. A white board and dry erase marker were used to establish a fingerprint negative via finger pad contact with a solid marked area on the board. Each finger pad was approximated as a semicircle located superior to a rectangle. Two measurements were recorded to quantify the finger pad area, the radius of the semi-circle and the lateral side of the finger pad. Figure 1 represents these dimensions below.

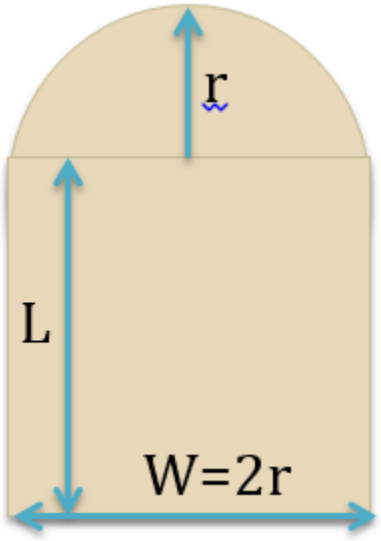

Figure 1: Breakdown of finger pad measurements for pressure calculations.

From the corresponding finger pad contact area, the appropriate applied transfer weight could be calculated to achieve the 1 kg/cm^2 surface area.

Finger	Height (cm)	Tip radius(cm)	Pad area (cm^2)	Weight needed to achieve 1kg/cm^2 (kg)
A-(Thumb)	2.30	0.60	3.04	3.04
B-(Index)	2.20	0.55	2.66	2.66
C-(Middle)	2.45	0.60	3.22	3.22
D-(Ring)	1.85	0.45	1.82	1.82
E-(Pinky)	1.80	0.35	1.36	1.36

Table 2: Measurements of finger pad and calculations for force needed to achieve required pressure of 1kg/cm^2

3.2.1 Carrier preparation

The non-treated stainless steel carriers (no antimicrobial coating applied) were cleaned in order to remove organic contaminants. The slides were put through five individual full submersion washes beginning with 1) a 1% Liquinox solution (Alconox, Inc. 30 Glenn Street, Suite 309 White Plains, NY 10603 USA) in deionized water, 2) rinsing with deionized water, 3) treatment with 95% ethanol, 4) rinsing with deionized water, and 5) rinsing with deionized water. The carriers were patted dry with paper towels, layered in an autoclave-safe container, and sterilized at 121 degrees Celsius for 20 minutes

3.3.1 MS2 Bacteriophage 15597-B1 propagation:

To propagate the MS2-15597-B1 bacteriophage, a titration was performed of an existing laboratory stock. Then, 10^6 PFU was inoculated into approximately 50

petri dishes s using the Double Agar Layover method (DAL) with 500 µL of log-phase *E. coli* 15597 as the phage host. For phage harvesting, 5 mL of 0.01 M PBS was added to each petri plate and agitated on a shaker at 165 RPM for 2 hours. The virus suspension was then extracted and combined before a 12 minute centrifugation at 1090 X g. The supernatant was then extracted and filtered through a 0.22 µm filter that was pre-moistened with 3% (pH-7.0) beef extract. A new titration was performed to quantify the bacteriophage in the stock utilizing the same DAL method.

3.3.2 Bacteriophage host preparation:

The host culture of *Escherichia coli* 15597 was prepared by transferring an isolated colony from a tryptic soy agar plate (TSA) (Catalogue # 236920 Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, NJ 07417-1880) into 10 ml of tryptic soy broth (TSB) (Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, NJ 07417-1880) and incubating at 37 degrees Celsius for 22 ±2 hours. At 22 ±2 hours this culture was vortexed (VWR, Radnor Corporate Center Building One, Suite 200 100 Matsonford Road Radnor, PA 19087-8660) for 10 seconds and 1 mL was inoculated into 100 mL of TSB preheated to 37 degrees Celsius in a sterile 200 mL screw-top Erlenmeyer flask. This flask was then shaken at 165 RPM while incubated at 37 degrees Celsius (Ecella E24, New Brunswick Scientific 175 Freshwater Boulevard Enfield Connecticut, 06082 United States) for 4 hours to achieve a log-phase host culture.

3.4.1 Preparation of bacterial cultures

Enterobacter aerogenes (ATTC 13048) and *Staphylococcus aureus* (ATTC 6538p) stocks were prepared in the same manner. Isolated bacterial colonies were selected from pure culture streak plates (TSA) and placed into 10 mL of sterile TSB (within a sterile 15 mL conical tube) (Fisher Scientific 168 Third Avenue Waltham, MA USA 02451). The cultures were incubated at 37 degrees Celsius for 22 ±2 hours to achieve a stationary phase cultures of each bacteria. The cultures were then vortexed (VWR, Radnor Corporate Center Building One, Suite 200 100 Matsonford Road Radnor, PA 19087-8660) to evenly distribute cells, and centrifuged at 3600 RPM for 10 minutes. The pellet was re-suspended in 10 mL of sterile PBS to wash the cells. The centrifugation and resuspension was performed a total of 3 times for each organism.

Upon the last centrifugation, wash and resuspension cycle, each culture was transferred to a sterile, borosilicate glass test tube. Utilizing a spectrophotometer (DR 3900, Hach Company P.O. Box 389 Loveland, Colorado 80539-0389) that was calibrated using sterile PBS in a borosilicate glass test tube, the optical density at 600 nm was obtained for each culture. This OD_{600} allowed for the quantification of organism in CFU to be obtained for each culture. The cultures were then diluted using sterile PBS until the OD_{600} was within the range of 0.115 to 0.135, which corresponds directly to a concentration of 10^8 CFU/mL. Note that multiple OD_{600} values were obtained and averaged for each culture to ensure that the values were accurate. The 10^8 CFU/mL culture was then used to inoculate 10^6 CFU on each carrier.

3.5 Hand Sanitization:

3.5.1 Pre transfer:

Hands were washed thoroughly for 45 seconds with warm tap water and a non-antimicrobial handsoap (i.e. Softsoap® Handsoap, Colgate-Palmolive Company, Colgate-Palmolive Company 300 Park Avenue 11th Floor New York, NY 10022-7499). Special care was taken to ensure that the subject's finger pads and fingernails were meticulously scrubbed during this time. The hands were then dried using unused, clean paper towels. The hands were sanitized by were spraying three times with 70% ethanol and rubbed for 15 seconds, ensuring that the finger pads were rubbed together continuously to ensure even distribution of the ethanol. These steps were performed immediately prior to conducting the transfers.

3.6.1 Post transfer:

Immediately following the transfers, the subject's fingers were submerged in a 10% bleach solution for a total of 1 minute. The subject rubbed their finger pads together while in the bleach bath to remove as much organism as possible. The hands were then washed in warm tap water for a minimum of 15 seconds. Next, the hands were washed twice in a 10% Povidone-iodine topical solution (Betadine, 201 Tresser Boulevard Stamford, CT 06901-3431) for 30 seconds each wash, with rinsing between each wash. Then, the subject's hands were sprayed with 70% ethanol and rubbed for 30 seconds. The ethanol was supplied to the hands as needed throughout this time to prevent drying before the 30 seconds had expired. The last step in the post-transfer sanitization was a 45 second hand wash with Softsoap® (Colgate-Palmolive Company, Colgate-Palmolive Company 300 Park

Avenue 11th Floor New York, NY 10022-7499) followed by drying with paper towels.

3.7.1 Carrier inoculation:

Prior to inoculation, each carrier was transferred aseptically to sterile petri plates and covered to prevent unwanted contamination. Each carrier was inoculated with 10 µL of the prepared 10^8 solution (10^6 CFU or PFU per carrier). The 10 µL droplet was dispensed as consistently as possible on the center of each carrier with an Eppendorf® Reference® micropipette (Sigma-Aldrich 3050 Spruce St. St. Louis, MO 63103). The droplet was inoculated on as small an area as possible, ensuring that unnecessary contact area was avoided. The petri plates were then stacked onto an incubating tray no more than two plates high, and placed into an incubator at 37 degrees Celsius until visibly dry.

Four time points were tested for the transfer of each organism, all of which were initiated upon drying for all slides. The first time point, which will be referred to as time point 0, was sampled upon drying of the inoculum. The second time point, time point 1, was sampled one hour after inoculum drying. The third and fourth time points denoted as time point 4 and 8 were both sampled 4 and 8 hours after inoculum drying, respectively.

Sample type (Performed for Coated and Non-Coated)	Number of Tests replicates Per Time Point	Number of Replicates Per Test	Contact Times (hours)
Non-touched Control Carrier (control)	3	2	0, 1, 4, 8

Post Transfer Finger Harvest	5	2	0, 1, 4, 8
Post Transfer Carrier Harvest	5	2	0, 1, 4, 8

Table 3: Experimental parameters for the study

3.8.1 Transfer trials

The carrier-to-hand transfers were performed upon the completion of each contact time. Five of each type of carriers were used for transfer at each time point. Each carrier was assigned to a finger on the subject's right hand. Upon removal from the incubator, a carrier was placed on an Explorer Pro mass balance model # EP4102DC (OHAUS CORPORATION, 7 Campus Drive, Suite 310 Parsippany, NJ 07054 USA) and tared with the lid off. A flame was used in the testing area throughout this procedure to prevent settling of contaminants on the exposed carrier. Starting with the thumb of the right hand, the subject pressed down onto the carrier at the predetermined mass (reference section 3.1.1) to achieve 1 kg/cm^2 for 10 seconds while ensuring that the dried inoculum was centered on the finger pad. At the end of the 10-second transfer interval, the carrier was placed in 20 mL of DE neutralizer in a stomacher bag (Stomacher Bag 400, Seward Laboratory Systems Inc. One Suffolk Square 1601 Veterans Memorial Highway Suite 315 Islandia, NY 11749)

The subject then immediately bathed the finger in 5 mL of phosphate buffer solution (PBS) contained within a two-inch petri plate (Catalogue # 25384-090, VWR Radnor Corporate Center Building One, Suite 200 100 Matsonford Road Radnor, PA 19087-8660) for a total of 10 seconds. During this wash, special care was taken to rub the finger pad on the bottom of the petri plate in an attempt to remove a larger percentage of the transferred organisms. This volume was then

transferred into 15 mL of sterile DE in a sterile conical and vortexed for 5 seconds. The carriers were placed into individual stomacher bags (Thermo Fisher Scientific 168 Third Avenue Waltham, MA USA 02451) and agitated in a stomacher machine on a "normal" setting for 60 seconds. Once the stomacher cycle was complete, the DE in each stomacher bag was transferred aseptically into a sterile 50 mL conical.

3.9 Reference carriers

Three carriers were placed in individual stomacher bags with 20 mL of DE each and stomached in a stomacher machine on the "normal" setting for 60 seconds, at which time the DE was transferred into a sterile 50 mL conical tube. These tests were performed in order to evaluate the natural die-off of microbes on non-coated stainless steel and the antimicrobial efficacy of the coating.

3.10 Plating of harvested bacteria

Tenfold serial dilutions were made by transferring 100 µL of DE from each respective reference, carrier post-transfer, and finger transfer suspension volumes into sterile tubes containing 900 µL of DE broth.

The dilutions for each time point per organism were plated aseptically using standard spread plating technique. Selective media was utilized for both organisms. For *Staphylococcus aureus* (ATTC 6538p), TSA supplemented with 10% (w/v) sodium chloride was used. For *Enterobacter aerogenes* (ATTC 13048) eosin methyl blue agar (EMB) (catalogue # E-5149, Milipore Sigma 109 Woodbine Downs Blvd. Unit 5, Etobicoke Ontario M9W 6Y1 Canada) was utilized. Duplicates of each dilution were plated.

Spread plates containing 1 mL volumes were allowed to dry with the media exposed in a laminar flow hood. Once the entirety of the one mL inoculum had taken to the media the petri lids were secured back in place. Additionally, two five mL plates were performed to quantify a 10 mL concentration of the stock solutions. Pour plating was performed for these volumes by mixing 5 mL of test suspension with 15 mL of the respective selective molten agar for each respective organism. The plates were incubated 36 hours and counted.

3.11 MS2 Transfer

The media used to perform the DAL assay for enumeration of MS2 plaque-forming units were top agar and TSA. The top agar consisted of 8 grams of agar (catalogue # 214010, Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, NJ 07417-1880) and 30 grams of TSB (catalogue # 211771, Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, NJ 07417-1880) per each liter of deionized water. The top agar components were stirred vigorously and heated until boiling, then dispensed by 5 mL aliquots into borosilicate glass test tubes before steam-sterilizing. Prior to use in each experiment, the top agar tubes were liquefied using either a hot plate or a specially-programmed autoclave cycle (5 minute fast exhaust), then held at 55 degrees Celsius in a water bath until use.

To perform the DAL assay, 500 µL of log phase *E.coli* 15597 was added to the molten top agar. Then, a volume of harvested DE suspension per each carrier was added to each tube depending on the dilution. The tube was then rotated back and forth to mix, and the contents poured onto a TSA bottom agar plate. The plate was

rotated in a circular motion to ensure even distribution across the plate. The plates were then incubated for 12 to 18 hours at 37 degrees Celsius, and the visible plaques counted.

3.12 Microbial background identification

Per each test organism, normal flora morphologies (background organisms) which were able to grow on the experiment's chosen selective and differential media were elucidated. For each test organism, the subject's hands were sanitized and an assay of their finger wash was obtained for no transfer. This procedure was the same as the finger wash for a transfer run except the subject did not touch anything before the wash.

3.13 Quantifying microbial counts

Microbial counts were obtained per experimental run for each of the following:

- Non-touched stainless steel and silver-embedded carrier controls at Time Zero (immediately after drying), and per study time point (i.e. 1 hour, 4 hours, and 8 hours) in replicates of three
- Stainless steel and silver-embedded carriers subjected to finger pad transfers at Time Zero (immediately after drying), and per study time point (i.e. 1 hour, 4 hours, and 8 hours) in replicates of five
- Finger pads after performing transfers of the study organisms in replicates of five

Mean CFU or PFU per carrier or per finger pad was calculated using Equation 1 below:

Equation 1: $(Average\ count\ on\ duplicate\ plates)\ \times\ 20mL\ \times\ (\frac{1}{10^{dilution\ value}})$

Arithmetic as well as geometric means were calculated for each set of carriers or finger pads values per the equation below:

Equation 2: $Geometric\ Mean = Antilog(\frac{Log_{10}(X_1)+Log_{10}(X_2)+\cdots Log_{10}(X_R)}{R})$

Where X_i is the mean CFU or PFU per fingerpad or carrier individually (see equation 1) and R is the number of duplicate plates.

The geometric means are utilized to get a fair representation for realistic real world reductions while the arithmetic means are utilized for the risk assessment so that values tend to be higher and as such are more of a worst-case scenario. These are the scenarios that are of interest in QMRA.

Microbial transfer efficiencies were then calculated using Equation 3 shown below:

Equation 3: $Percent\ Transfer\ Efficiency =$

$$\frac{Geomean(All\ five\ touch\ CFU\ or\ PFU\ values)*100}{Geomean(All\ five\ touch\ CFU\ or\ PFU\ values)+Geomean(All\ five\ post\ transfer\ carrier\ CFU\ or\ PFU\ values)}$$

The reduction in test organisms due to environmental factors or efficacy of the silver-embedded antimicrobial were calculated separately to delineate the source of observed decreases. The reduction of the microbes due to environmental

factors and the antimicrobial efficacy of the coating were performed using equation 4 and 5 respectively. These equations can be seen below:

Equation 4: $Environmental\ reduction = Log_{10}(GEOMEAN(SS_{time\ 0,C1\ to\ c3}) - Log_{10}(GEOMEAN(SS_{Contact\ time.C1\ to\ C3}))$

$SS_{time\ 0,C1\ to\ C3}$ represents the three non-touched, non-coated stainless steel carriers that were quantified immediately after the inoculum dried. The $SS_{Contact\ time,C1\ to\ C3}$ variable represents the three non-coated stainless steel carriers that were left untouched, harvested at the contact time of interest i.e. one, four, or eight hours.

Equation 5: $Silver\ embedded\ coating\ reductions = Log_{10}(SS_{Contact\ time\ X,C1} + SS_{Contact\ time\ X,C2} + SS_{Contact\ time\ X,C3})^3 - Log_{10}(CS_{Contact\ time\ X,C1} + CS_{contact\ time\ X,C2} + CS_{contact\ time\ X,C3})^3$

$SS_{Contact\ time\ X,C1}$, $SS_{Contact\ time\ X,C2}$, and $SS_{Contact\ time\ X,C3}$, represent the three non-coated stainless steel carriers that were not touched and quantified upon contact time X equals zero, one, four, or eight hours . The $CS_{Contact\ time\ X,C1}$, $CS_{Contact\ time\ X,C2}$, and $CS_{Contact\ time\ X,C3}$, variables represent the three coated carriers that were left untouched, harvested at the contact time of X equals zero, one, four, or eight hours.

3.14 Risk assessment

3.14.1 Hazard Identification: *Klebsiella pneumoniae* as a representative Gram-negative nosocomial pathogen of concern

Since *Enterobacter aerogenes* is not a large concern for HAI a gram negative virulent bacteria needed to be chosen for the QMRA. *Klebsiella pneumoniae,* a gram negative, enteric pathogen was chosen to model in the QMRA. Members of the Enterobacteriaceae family, such as *Klebsiella pneumonia,* have become of increasing concern in the hospital environment, particularly in sinks located in patient rooms (Dancer, 2014). Studies have shown that bacteria in this taxonomic family can form biofilms in the elbow of sink drainpipes capable of advancing upwards at a rate of approximately 2.5 cm (1 inch) per day under ideal conditions (Kotay, et al., 2017). *Klebsiella pneumoniae* can also grow and outcompete other bacteria under low nutrient conditions on cleaning supplies such as sanitation brushes (Herruzo et al., 2017). Kotay et al. (2017) performed an experiment to identify the amount of bacteria spread via dispersion of water droplets and aerosols. They found that aerosols do not constitute a large amount of microbiological deposition on high touch areas such as sink handles; however, they did find that larger water droplets dispersed bacteria from the drain to the sink basin in concentrations between 5-100 CFU/cm^2.

3.14.2 Dose response

For the comparative risk assessment the probability of a patient contracting *Klebsiella pneumoniae* and developing pneumonia due to exposures via sink drains. Utilizing the range of 5-100 CFU/cm^2 of *Klebsiella pneumoniae* in sink basins (Kotay, Chai, Guilford, Barry, & Mathers, 2017) a possible dose can be simulated for a patient using the hospital room faucet. To simulate this dose, a normal distribution

was created using R software with 10,000 values with an average of 53 CFU/cm^2 and a standard deviation of 15 CFU/cm^2. In this scenario, several assumptions have been made:

The sink drain was colonized with carbapenem-resistant *Klebsiella pneumoniae*

The counters and sink adjacent to the drain are insufficiently cleaned once daily. This would be due to incorrect implementation of cleaning protocols which has historically been implicated in outbreaks among patients (Reiss et al. 2000)

The *Klebsiella pneumoniae* is not significantly reduced by cleaning supplies since it can grow in a biofilm. (Bridier et al. 2011).

Three individual doses of *Klebsiella pneumonia* within a 7 hour period are equal to a single point risk exposure.

3.15.3 Exposure Assessment

Utilizing the assumptions described, the sink basin and the counter are wiped with a moistened sponge by a hospital sanitation worker after the water has been run in the sink, dispersing the *Klebsiella pneumoniae* biofilm throughout the sink basin. Assuming that the sink basin is approximately 9" deep by 12" wide by 6" deep ("Just Manufacturing," n.d.), the total wiping surface area of the sink is 360 in^2 or 2322.58 cm^2. The minimum mechanical transfer of bacteria to the sponge or rag can be estimated as a minimum of 48%, since studies have shown sponge sampling recovery rates at this value (Krauter et al., 2012). The sanitation worker then rubs the handles of the sink with the contaminated sponge. The transfer from the sponge to the sink handle was extrapolated from Rossi et al. 2013 as an average of 0.71%.

Following deposition of the hazardous organism, two side-by-side patient exposure scenarios were considered - one where the patient's hands come into direct contact with a faucet handle coated with silver embedded shrink wrap, and the second during which the patient is exposed in a similar fashion to a non-treated handle with no protective shrink wrap. The patient is exposed upon deposition (T=0), and at three different subsequent time points of 1 hour, 4 hours, and 8 hours. Assuming that *Klebsiella pneumoniae* populations demonstrate similar environmental reductions as those observed for *Enterobacter aerogenes* on non-coated stainless steel, then reductions of 69%, 91%, and 98% would occur within 1, 4, and 8 hours after deposition via the sink drain. Additionally if the handles were coated with silver embedded shrink-wrap, the reductions would be 99.7% within the first hour and approximately 99.99% after four hours and 8 hours.

The simulation considers that *Klebsiella pneumoniae* transferred to a patient via the hands while touching a contaminated faucet handle while turning the faucet off. Polls have shown that 98% of Americans wash their hands at least 3-4 times a day ("2009 National Clean Hands Report Card® Survey Findings" n.d.). Therefore, a cumulative daily dose was developed based on a patient washing their hands 3 times daily at one, four, and eight hours after deposition of *Klebsiella pneumoniae* by cleaning personnel. The transfer percentage of *Klebsiella pneumoniae* to the hand from the sink handle is estimated at 10% from the data obtained in this study, and 33.97% from the hand to the lip (Rusin et al. 2002), assuming that gram negative transfer rates are similar. Figure 2 below shows a flow diagram for the risk scenario

for *Klebsiella pneumoniae*.

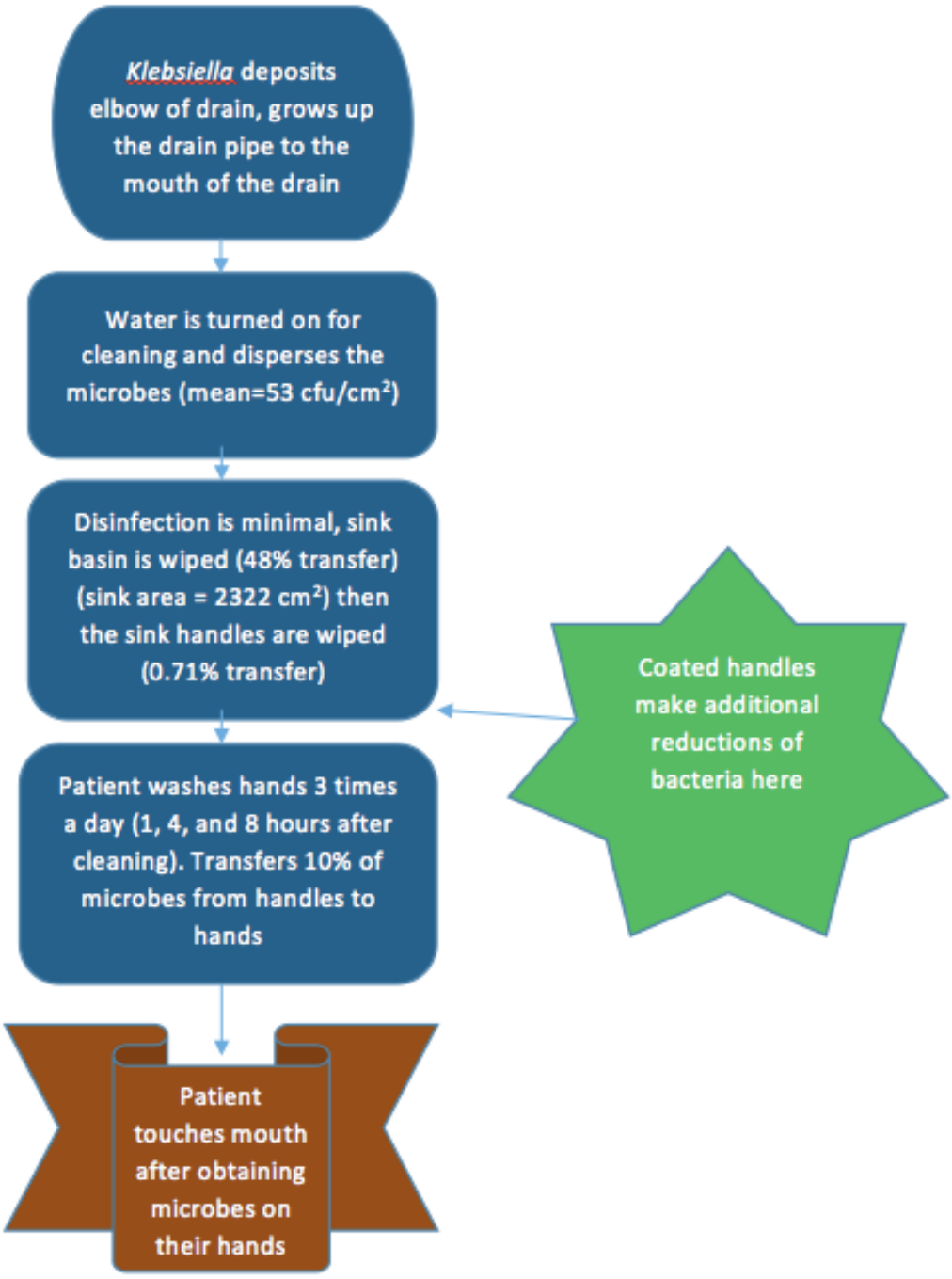

Figure 2 outlines the QMRA scenario for the risk of infection for Klebsiella pneumoniae.

The dose response model for *Klebsiella pneumoniae* uses the exponential dose response (equation 6) equation with a k value of 2.3e-7 for (Harb & Hong, 2017).

Equation 6: $P(r) = 1 - e^{(-k*dose)}$

Where P(r) is the probability of the risk of infection given a single dose, denoted above with the parameter k defined from the literature ("4. Dose Response," n.d.). The parameter k is the probability of infection for a dose of a single organism ("4. Dose Response," n.d.).

A cumulative yearly probability of infection for multiple doses throughout a hospital stay is calculated using equation 7 below:

Equation 7: $P(r)_{yearly} = 1 - (1 - P(r)_{daily})^{exposure\ days\ per\ year}$

The patient is assumed to have a one week stay.

3.15 Methicillin-resistant *Staphylococcus aureus* (MRSA) QMRA

3.15.1 Hazard ID

MRSA was chosen as the model organism for this QMRA because it is problematic in the medical field and its population dynamics are akin to the test organism, *Staphylococcus aureus* (ATTC 6538p). Their similarities arise due to the fact that they are both the same species. The primary difference between the two is that MRSA expresses antibiotic resistant genes. This QMRA focuses on patients self-infecting themselves in hospitals, causing secondary skin infections. Though these effects will not be explored here, it is important to keep in mind that these infections in turn could cause additional spread of the pathogen within the patient and throughout the hospital.

3.15.2 QMRA Scenario and dose response framework

The QMRA framework developed for MRSA in this study designates a patient's bed rail as the environmental reservoir for transmission in the hospital setting. Kurashige et al. (2016) found that the range of MRSA was between 0 to 1,620 CFU per bed rail with a mean of 159 CFU. For the QMRA, MRSA levels present on two surface types (i.e. non-treated bedrails and bedrails treated with the silver embedded shrink wrap) were considered. The initial deposition of MRSA onto the bedrails is simulated as a right skewed beta distribution randomly generated with 10,000 data points. For this distribution the majority of the data points are centered around 160 CFU with a maximum of approximately 1,500 CFU.

3.15.3 Exposure Assessment

The risk of infection by MRSA was evaluated at four different time points for the coated and non-coated bedrails: 1) immediately after MRSA deposition, assuming a dried surface; 2) one hour post-deposition; 3) four hours post-deposition, and 8 hours after the MRSA deposition. For the simulation, the hard, nonporous bedrails of hospitals beds are assumed to demonstrate the same fomite-to finger pad transfer efficiency those observed for *Staphylococcus. aureus* (ATTC 6538p) from stainless steel in the study described herein. Therefore, when the patient touches the non-coated bedrail, the transfer of MRSA to the finger pad is 36%, while the coated surface transfer efficiency is 27%. The study assumes that the patient touches the bed rail four times daily, once after the initial deposition of MRSA then at one, four, and eight hours after deposition. The dose to the compromised portion of the skin can be estimated Utilizing a hand to skin transfer efficiency of 35% (Kurashige et al. 2016) the dose to the compromised portion of the skin can be

estimated, and therefore the probability of infection. This probability will be calculated using Equation 5 with a k value of 7.64e-8 (Tamrakar, 2013). The cumulative risk is also calculated utilizing Equation 6 assuming the patient stays seven days in the hospital. The process diagram for the MRSA risk scenario is depicted in Figure 3 below.

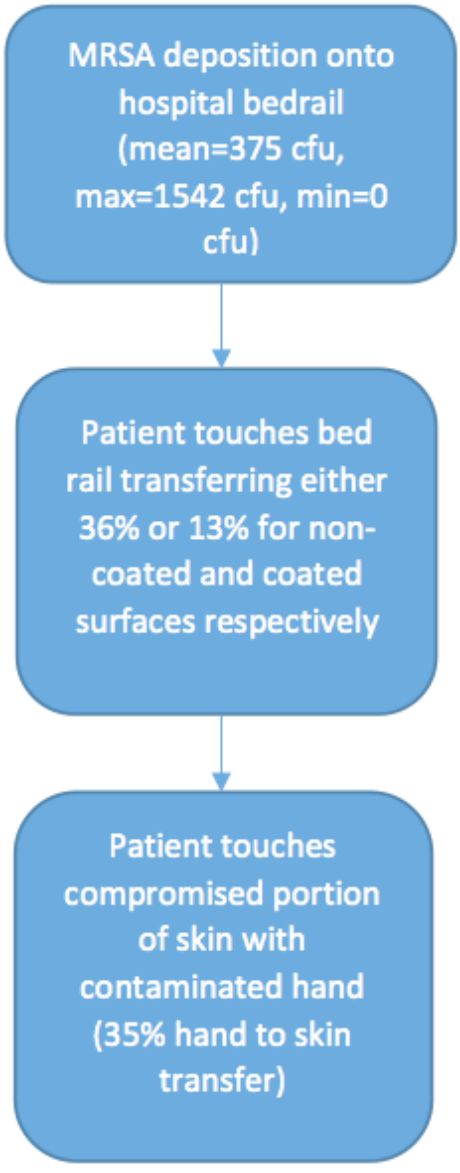

Figure 3 above depicts the QMRA scenario for hypothetical MRSA exposure.

4. Results and Discussion

4.1 Transfer and Efficacy Study

4.1.1 Enterobacter aerogenes

The stainless steel and silver embedded carrier counts (non-touched and touched) and finger pad counts for the *Enterobacter aerogenes* (ATTC 13048) transfer study are listed in Tables 4-11 below. All four experimental time points could not be performed in a single day; therefore, an additional Time Zero time point was performed for the one hour contact time.

Non-Coated Stainless Steel			
0 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	8.50E+04		
Untouched Control 2	5.67E+04	6.24E+04	6.40E+04
Untouched Control 3	5.03E+04		
Post Transfer Carrier Harvest-A	3.98E+04		
Post Transfer Carrier Harvest-B	2.32E+04		
Post Transfer Carrier Harvest-C	3.08E+04	3.13E+04	3.17E+04
Post Transfer Carrier Harvest-D	3.13E+04		
Post Transfer Carrier Harvest-E	3.36E+04		
Post Transfer Finger Pad Harvest-A	3.05E+03	1.02E+03	1.51E+03
Post Transfer Finger Pad Harvest-B	2.54E+03		

Post Transfer Finger Pad Harvest-C	1.30E+03		
Post Transfer Finger Pad Harvest-D	3.80E+02		
Post Transfer Finger Pad Harvest-E	2.94E+02		

Table 4: Non-coated stainless steel: CFU of Enterobacter aerogenes (ATTC 13048) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0 hours).

Non-Coated Stainless Steel			
0 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	3.67E+04		
Untouched Control 2	2.52E+04	3.86E+04	4.13E+04
Untouched Control 3	6.21E+04		
1 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	9.40E+03		
Untouched Control 2	1.24E+04	1.19E+04	1.20E+04
Untouched Control 3	1.43E+04		
Post Transfer Carrier Harvest-A	1.30E+03		
Post Transfer Carrier Harvest-B	1.36E+03		
Post Transfer Carrier Harvest-C	1.00E+03	9.69E+02	1.01E+03
Post Transfer Carrier Harvest-D	7.10E+02		
Post Transfer Carrier Harvest-E	6.80E+02		

Post Transfer Finger Pad Harvest-A	8.80E+02		
Post Transfer Finger Pad Harvest-B	4.90E+02		
Post Transfer Finger Pad Harvest-C	3.70E+02	4.71E+02	5.06E+02
Post Transfer Finger Pad Harvest-D	2.90E+02		
Post Transfer Finger Pad Harvest-E	5.00E+02		

Table 5 Non-coated stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1 Hour)

Non-Coated Stainless Steel			
4 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	6.80E+03		
Untouched Control 2	3.10E+03	5.64E+03	6.13E+03
Untouched Control 3	8.50E+03		
Post Transfer Carrier Harvest-A	4.80E+02		
Post Transfer Carrier Harvest-B	2.90E+03		
Post Transfer Carrier Harvest-C	1.33E+03	9.46E+02	1.21E+03
Post Transfer Carrier Harvest-D	9.10E+02		
Post Transfer Carrier Harvest-E	4.50E+02		
Post Transfer Finger Pad Harvest-A	4.00E+00		
Post Transfer Finger Pad Harvest-B	2.80E+01	1.80E+01	2.20E+01
Post Transfer Finger Pad Harvest-C	3.20E+01		

Post Transfer Finger Pad Harvest-D	2.60E+01		
Post Transfer Finger Pad Harvest-E	2.00E+01		

Table 6: Non-coated stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4 hours).

Non-Coated Stainless Steel			
8 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	3.23E+03		
Untouched Control 2	1.27E+03	1.38E+03	1.71E+03
Untouched Control 3	6.40E+02		
Post Transfer Carrier Harvest-A	9.94E+02		
Post Transfer Carrier Harvest-B	3.30E+02		
Post Transfer Carrier Harvest-C	1.72E+02	3.62E+02	4.33E+02
Post Transfer Carrier Harvest-D	3.02E+02		
Post Transfer Carrier Harvest-E	3.66E+02		
Post Transfer Finger Pad Harvest-A	2.20E+01		
Post Transfer Finger Pad Harvest-B	1.80E+01		
Post Transfer Finger Pad Harvest-C	1.40E+01	1.35E+01	1.44E+01
Post Transfer Finger Pad Harvest-D	1.00E+01		
Post Transfer Finger Pad Harvest-E	8.00E+00		

Table 7: Non-coated stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8 hours).

Silver Embedded Carriers			
0 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	8.80E+04		
Untouched Control 2	5.10E+04	6.89E+04	7.07E+04
Untouched Control 3	7.30E+04		
Post Transfer Carrier Harvest-A	8.80E+04		
Post Transfer Carrier Harvest-B	3.50E+04		
Post Transfer Carrier Harvest-C	4.17E+04	4.78E+04	5.07E+04
Post Transfer Carrier Harvest-D	3.93E+04		
Post Transfer Carrier Harvest-E	4.96E+04		
Post Transfer Finger Pad Harvest-A	2.10E+03		
Post Transfer Finger Pad Harvest-B	2.39E+03		
Post Transfer Finger Pad Harvest-C	1.87E+03	7.91E+02	1.37E+03
Post Transfer Finger Pad Harvest-D	3.94E+02		
Post Transfer Finger Pad Harvest-E	8.40E+01		

Table 8: Silver embedded coating stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0)

Silver Embedded Carriers			
0 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**

Untouched Control 1	7.00E+03		
Untouched Control 2	1.47E+04	9.71E+03	1.02E+04
Untouched Control 3	8.90E+03		
1 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	6.24E+02		
Untouched Control 2	4.00E+00	1.26E+02	4.76E+02
Untouched Control 3	8.00E+02		
Post Transfer Carrier Harvest-A	6.52E+02		
Post Transfer Carrier Harvest-B	2.70E+02		
Post Transfer Carrier Harvest-C	4.78E+02	5.58E+02	6.02E+02
Post Transfer Carrier Harvest-D	7.42E+02		
Post Transfer Carrier Harvest-E	8.70E+02		
Post Transfer Finger Pad Harvest-A	4.82E+02		
Post Transfer Finger Pad Harvest-B	9.00E+01		
Post Transfer Finger Pad Harvest-C	1.80E+01	1.28E+02	2.04E+02
Post Transfer Finger Pad Harvest-D	2.70E+02		
Post Transfer Finger Pad Harvest-E	1.62E+02		

Table 9: Silver embedded coating stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1).

Silver Embedded Carriers

4 hour	CFU per Carrier	Geometric mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	<2.00E+00		
Untouched Control 2	<2.00E+00	2.00E+00	2.00E+00
Untouched Control 3	<2.00E+00		
Post Transfer Carrier Harvest-A	2.34E+02		
Post Transfer Carrier Harvest-B	<2.00E+00		
Post Transfer Carrier Harvest-C	<2.00E+00	5.18E+00	4.84E+01
Post Transfer Carrier Harvest-D	<2.00E+00		
Post Transfer Carrier Harvest-E	<2.00E+00		
Post Transfer Finger Pad Harvest-A	<2.00E+00		
Post Transfer Finger Pad Harvest-B	<2.00E+00		
Post Transfer Finger Pad Harvest-C	<2.00E+00	2.64E+00	3.20E+00
Post Transfer Finger Pad Harvest-D	<2.00E+00		
Post Transfer Finger Pad Harvest-E	8.00E+00		

Table 10: Silver embedded coating stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4). Note that the values represented by a less than value indicate the occurrence of a detection limit.

Silver Embedded Carriers			
8 hour	**CFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.80E+01	5.24E+00	8.00E+00

Untouched Control 2	4.00E+00		
Untouched Control 3	<2.00E+00		
Post Transfer Carrier Harvest-A	<2.00E+00		
Post Transfer Carrier Harvest-B	<2.00E+00		
Post Transfer Carrier Harvest-C	<2.00E+00	2.00E+00	2.00E+00
Post Transfer Carrier Harvest-D	<2.00E+00		
Post Transfer Carrier Harvest-E	<2.00E+00		
Post Transfer Finger Pad Harvest-A	2.00E+00		
Post Transfer Finger Pad Harvest-B	2.00E+00		
Post Transfer Finger Pad Harvest-C	2.00E+00	2.30E+00	2.40E+00
Post Transfer Finger Pad Harvest-D	2.00E+00		
Post Transfer Finger Pad Harvest-E	4.00E+00		

Table 11: Silver embedded coating stainless steel: CFUs of Enterobacter aerogenes (ATTC 13048) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8.) Note that the values represented by a less than value indicate the occurrence of a detection limit.

***Enterobacter:* Transfer Efficiencies based off Geometric Means**		
Contact time (hours)	**Stainless steel transfer efficiency**	**Silver Coating transfer efficiency**
0	3%	2%
1	33%	19%
4	2%	34%

8	4%	53%
Average	10%	10%

Table 12: Transfer efficiencies based on geometric means for non-coated and coated carriers per time point, and mean transfer efficiency over the 8 hour study period. The transfer efficiencies where a detection limit (DL) value (2 CFU) was used were not included in the average calculations.

***Enterobacter*: Transfer Efficiencies based off Arithmetic Means**		
Contact time (hours)	**Stainless steel transfer efficiency (%)**	**Silver Coating transfer efficiency (%)**
0 1 4 8	5 33 2 3	3 25 6 (D.L.) 55 (D.L.)
Average	11	14

Table 13: Transfer efficiencies based on arithmetic means for non-coated and coated carriers per time point, and mean transfer efficiency over the 8 hour study period. The transfer efficiencies where a detection limit (DL) value (2 CFU) was used were not included in the average calculations.

Tables 12 and 13 show that on average, non-coated stainless steel and coated surfaces do not show a relatively large difference in the transfer efficiency of *Enterobacter aerogenes*. This was expected as both surfaces were essentially non-porous and transfer occurred under the same environmental conditions.

Exposure Time	Reductions due to environmental stressors and natural die-off		Reductions due to the antimicrobial efficacy of coating		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction
0 hour	N.D.	N.D.	-0.04	-11%	-0.04	-11%
1 hour	0.51	69%	1.97	98.9%	2.49	99.7%
4 hour	1.04	91%	3.45	99.96%	4.49	99.997%
8 hour	1.65	98%	2.42	99.6%	4.08	99.992%

Table 14: Reductions of E. aerogenes *(ATTC 13048) due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings based on geometric means.*

Exposure Time	Reductions due to environmental stressors and natural die-off		Reductions due to the antimicrobial efficacy of coating		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction
0 hour	N.D.	N.D.	-0.04	-0.10	-0.04	-10.4%
1 hour	0.54	71%	1.40	96%	1.94	98.848%
4 hour	1.02	90%	3.49	99.967%	4.51	99.997%
8 hour	1.57	97%	2.33	99.533%	3.90	99.988%

Table 15: Reductions of E. aerogenes (ATTC 13048) due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings based on arithmetic means.

The *Enterobacter* reductions in Table 14 show a combined reduction due to both environmental die-off and antimicrobial efficacy of 99.7%, 99.997%, and 99.992% at one-, four-, and eight-hour dry contact times respectively for the coated carriers. This contrasts with reductions of 69%, 91%, and 98% for the one-, four-, and eight-hour dry contact times, respectively, on non-coated stainless steel. Table 15, which is based on arithmetic means from the experimental data shows similar results with only slight variations.

4.1.2 *Staphylococcus aureus*

The stainless steel and silver embedded carrier counts (non-touched and touched) and finger pad counts for the *Staphylococcus aureus* (ATTC 6538p) transfer study are listed in Tables 16-23 below. All four experimental time points could not be performed in a single day; therefore, an additional Time Zero time points were performed per each study date to obtain baseline CFUs per carrier values.

Non-Coated Stainless Steel			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	6.50E+04		
Untouched Control 2	9.10E+04	9.14E+04	9.50E+04
Untouched Control 3	1.29E+05		
Post Transfer Carrier Harvest-A	4.71E+04		
Post Transfer Carrier Harvest-B	3.26E+04	4.67E+04	4.81E+04

Post Transfer Carrier Harvest-C	5.25E+04		
Post Transfer Carrier Harvest-D	6.84E+04		
Post Transfer Carrier Harvest-E	4.01E+04		
Post Transfer Finger Pad Harvest-A	3.21E+04		
Post Transfer Finger Pad Harvest-B	4.33E+04		
Post Transfer Finger Pad Harvest-C	2.97E+04	3.44E+04	3.47E+04
Post Transfer Finger Pad Harvest-D	3.12E+04		
Post Transfer Finger Pad Harvest-E	3.72E+04		

Table 16: Non-coated stainless steel: CFU of Staphylococcus aureus *(ATTC 6538p) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0 hours).*

Non-Coated Stainless Steel			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	4.89E+05		
Untouched Control 2	6.52E+05	5.54E+05	5.58E+05
Untouched Control 3	5.33E+05		
1 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	3.15E+05		
Untouched Control 2	4.94E+05	3.91E+05	3.98E+05
Untouched Control 3	3.84E+05		

Post Transfer Carrier Harvest-A	3.45E+05		
Post Transfer Carrier Harvest-B	2.68E+05		
Post Transfer Carrier Harvest-C	3.19E+05	3.61E+05	3.69E+05
Post Transfer Carrier Harvest-D	4.85E+05		
Post Transfer Carrier Harvest-E	4.29E+05		
Post Transfer Finger Pad Harvest-A	7.00E+04		
Post Transfer Finger Pad Harvest-B	8.80E+04		
Post Transfer Finger Pad Harvest-C	3.72E+04	3.19E+04	4.44E+04
Post Transfer Finger Pad Harvest-D	1.93E+04		
Post Transfer Finger Pad Harvest-E	7.50E+03		

Table 17: Non-coated stainless steel: CFU of Staphylococcus aureus *(ATTC 6538p) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1 hour).*

Non-Coated Stainless Steel			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	2.43E+05		
Untouched Control 2	1.23E+05	2.12E+05	2.29E+05
Untouched Control 3	3.20E+05		
4 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	1.40E+05	1.78E+05	1.81E+05

Untouched Control 2	1.99E+05		
Untouched Control 3	2.04E+05		
Post Transfer Carrier Harvest-A	1.19E+05		
Post Transfer Carrier Harvest-B	1.62E+05		
Post Transfer Carrier Harvest-C	6.15E+04	1.24E+05	1.31E+05
Post Transfer Carrier Harvest-D	1.55E+05		
Post Transfer Carrier Harvest-E	1.57E+05		
Post Transfer Finger Pad Harvest-A	2.43E+04		
Post Transfer Finger Pad Harvest-B	2.06E+04		
Post Transfer Finger Pad Harvest-C	4.85E+03	8.04E+03	1.16E+04
Post Transfer Finger Pad Harvest-D	5.55E+03		
Post Transfer Finger Pad Harvest-E	2.49E+03		

Table 18: Non-coated stainless steel: CFUs of Staphylococcus (ATTC 6538p) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4 hours).

Non-Coated Stainless Steel			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	4.43E+05		
Untouched Control 2	4.69E+05	4.12E+05	4.16E+05
Untouched Control 3	3.37E+05		

8 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	1.54E+04		
Untouched Control 2	1.95E+04	1.48E+04	1.52E+04
Untouched Control 3	1.07E+04		
Post Transfer Carrier Harvest-A	1.69E+04		
Post Transfer Carrier Harvest-B	2.80E+04		
Post Transfer Carrier Harvest-C	3.00E+04	2.32E+04	2.37E+04
Post Transfer Carrier Harvest-D	2.30E+04		
Post Transfer Carrier Harvest-E	2.08E+04		
Post Transfer Finger Pad Harvest-A	1.33E+03		
Post Transfer Finger Pad Harvest-B	2.01E+03		
Post Transfer Finger Pad Harvest-C	8.80E+02	9.00E+02	1.15E+03
Post Transfer Finger Pad Harvest-D	1.32E+03		
Post Transfer Finger Pad Harvest-E	1.90E+02		

Table 19: Non-coated stainless steel: CFU of Staphylococcus aureus (ATTC 6538p) quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8 hours).

Silver Embedded Carrier			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	8.50E+03	2.69E+04	4.92E+04

Untouched Control 2	1.90E+04		
Untouched Control 3	1.20E+05		
Post Transfer Carrier Harvest-A	2.26E+04		
Post Transfer Carrier Harvest-B	8.60E+04		
Post Transfer Carrier Harvest-C	7.40E+04	5.64E+04	6.17E+04
Post Transfer Carrier Harvest-D	6.40E+04		
Post Transfer Carrier Harvest-E	6.20E+04		
Post Transfer Finger Pad Harvest-A	1.36E+04		
Post Transfer Finger Pad Harvest-B	2.75E+04		
Post Transfer Finger Pad Harvest-C	2.84E+04	1.58E+04	1.79E+04
Post Transfer Finger Pad Harvest-D	1.30E+04		
Post Transfer Finger Pad Harvest-E	7.20E+03		

Table 20: Silver embedded coating stainless steel: CFU of Staphylococcus aureus (ATTC 6538p) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0 hours).

Silver Embedded Carrier			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	3.80E+05		
Untouched Control 2	5.36E+05	4.42E+05	4.47E+05
Untouched Control 3	4.24E+05		

1 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	3.19E+05		
Untouched Control 2	1.81E+05	2.56E+05	2.63E+05
Untouched Control 3	2.89E+05		
Post Transfer Carrier Harvest-A	2.50E+05		
Post Transfer Carrier Harvest-B	3.28E+05		
Post Transfer Carrier Harvest-C	2.44E+05	2.05E+05	2.27E+05
Post Transfer Carrier Harvest-D	2.34E+05		
Post Transfer Carrier Harvest-E	7.80E+04		
Post Transfer Finger Pad Harvest-A	1.47E+04		
Post Transfer Finger Pad Harvest-B	2.32E+04		
Post Transfer Finger Pad Harvest-C	2.53E+04	9.98E+03	1.45E+04
Post Transfer Finger Pad Harvest-D	1.47E+03		
Post Transfer Finger Pad Harvest-E	7.80E+03		

Table 21: Silver embedded coating stainless steel: CFU of Staphylococcus aureus (ATTC 6538p) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1 hour).

Silver Embedded Carrier			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	2.51E+05	3.11E+05	3.18E+05

Untouched Control 2	2.91E+05		
Untouched Control 3	4.13E+05		
4 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	1.36E+05		
Untouched Control 2	1.39E+05	1.43E+05	1.43E+05
Untouched Control 3	1.54E+05		
Post Transfer Carrier Harvest-A	9.39E+04		
Post Transfer Carrier Harvest-B	7.43E+04		
Post Transfer Carrier Harvest-C	7.53E+04	7.33E+04	7.42E+04
Post Transfer Carrier Harvest-D	5.80E+04		
Post Transfer Carrier Harvest-E	6.93E+04		
Post Transfer Finger Pad Harvest-A	8.60E+03		
Post Transfer Finger Pad Harvest-B	5.09E+03		
Post Transfer Finger Pad Harvest-C	4.52E+03	3.83E+03	4.50E+03
Post Transfer Finger Pad Harvest-D	2.79E+03		

Post Transfer Finger Pad Harvest-E	1.50E+03		

Table 22: Silver embedded coating stainless steel: CFU of Staphylococcus aureus (ATTC 6538p) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4 hours).

Silver Embedded Carrier			
0 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	3.46E+05		
Untouched Control 2	2.22E+05	2.47E+05	2.55E+05
Untouched Control 3	1.96E+05		
8 hour	CFU per Carrier	Geometric Mean (CFU)	Arithmetic mean (CFU)
Untouched Control 1	1.21E+04		
Untouched Control 2	8.70E+03	1.07E+04	1.08E+04
Untouched Control 3	1.15E+04		
Post Transfer Carrier Harvest-A	1.75E+04		
Post Transfer Carrier Harvest-B	1.36E+04		
Post Transfer Carrier Harvest-C	2.76E+04	1.79E+04	1.91E+04
Post Transfer Carrier Harvest-D	1.05E+04		
Post Transfer Carrier Harvest-E	2.65E+04		
Post Transfer Finger Pad Harvest-A	8.20E+03		
Post Transfer Finger Pad Harvest-B	7.70E+03	4.86E+03	5.36E+03

Post Transfer Finger Pad Harvest-C	4.90E+03		
Post Transfer Finger Pad Harvest-D	2.50E+03		
Post Transfer Finger Pad Harvest-E	3.50E+03		

Table 23: Silver embedded coating stainless steel: CFUs of Staphylococcus aureus (ATTC 6538p) quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8 hours).

The *Staphylococcus aureus* (ATTC 6538p) transfer efficiency values calculated for the study time points are listed in Table 24 and 25.

***Staphylococcus*: Transfer Efficiencies based off Geometric Means**		
Contact time (hours)	**Stainless steel transfer efficiency (%)**	**Silver Coating transfer efficiency (%)**
0	42	22
1	92	5
4	6	5
8	4	21
Average	36	13

Table 24: Mean transfer efficiencies based off geometric mean data values for non-coated and coated carriers per time point and overall mean transfer efficiency over the 8 hour study period

***Staphylococcus*: Transfer Efficiencies based off Arithmetic Means**		
Contact time (hours)	**Stainless steel transfer efficiency (%)**	**Silver Coating transfer efficiency (%)**
0	42	23
1	89	6

4	8	6
8	5	22
Average	36	14

Table 25: Mean transfer efficiencies based off arithmetic data values for non-coated and coated carriers per time point, and overall transfer mean transfer efficiency over the 8 hour study period.

There appears to be a relatively large variation within the transfer efficiencies for both the coated and non-coated surface. The largest transfer variations for the non-coated surface ranges from a minimum of 4% to a maximum of 92% with mean of 36%. The coated surface varied slightly less with a minimum of 5% from the geometric mean based values and a maximum of 23% from the arithmetic based values and an average transfer of 13% and 14% respectively. A t-test was performed to determine if the two averages were significantly different and no significance was found. This was due to the large variability in the data.

The *S. aureus* reductions attributed to both environmental stressors and the antimicrobial efficacy of the silver-coated carriers for *Staphylococcus aureus* (ATTC 6538p) are shown in Table 26 and 27.

Exposure Time	Reductions due to environmental stressors and natural die-off		Reductions due to the antimicrobial efficacy of coating		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent
0 hour	N.D.	N.D.	0.10	21%	0.10	21%
1 hour	0.15	29%	0.18	35%	0.34	54%
4 hour	0.08	16%	0.10	20.0%	0.17	32.7%
8 hour	1.45	96.4%	0.14	27.8%	1.59	97.4%

Table 26: Reductions of S. aureus (ATTC 6538p) due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings. These calculations are based on geometric means from the data.

Exposure Time	Reductions due to environmental stressors and natural die-off		Reductions due to the antimicrobial efficacy of coating		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction
0 hour	N.D.	N.D.	0.10	20%	0.10	20%
1 hour	0.15	29%	0.18	34%	0.33	53%
4 hour	0.10	21%	0.10	21.0%	0.20	37.5%
8 hour	1.44	96.3%	0.15	29.2%	1.59	97.4%

Table 27: Reductions of S. aureus (ATTC 6538p) due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings. These calculations are based on arithmetic means from the data.

Based on the geometric means from the data (Table 26), a total reduction (i.e. natural die-off plus the antimicrobial efficacy of the silver coating) of 97.4% during the eight-hour contact time was achieved for *S. aureus*. However, the reductions observed for the prior contact times do not follow a clear trend. This is likely due to inconsistencies in the product from two different rounds of shipments. It is possible that one shipment was used from an older material and therefore the silver ions were allowed to oxidize further than its counterpart. The one-hour post dry contact time shows a larger reduction at 54% than the four-hour post dry contact time at 32.7%. This could be due to a variety of different factors. It is possible that on the day of the four-hour contact time that environmental conditions within the drying incubator made the inoculum dry faster than the one-hour post dry contact time thus reducing overall contact time and microbial reductions. It is also possible that there are variations in the product from the one and four-hour time points since these trials were run on separate shipments from the sponsor company. The table representing reductions based on arithmetic means shows similar results with small variations.

4.1.3 MS2 Bacteriophage 15597-B1

The raw data values for the MS2 transfer trials are displayed in Tables 28 through 35 below. Like the *Staphylococcus* and *Enterobacter* studies, not all transfer trials were conducted on the same day; therefore, additional non-touch control carriers were harvested for the one hour and four hour post dry contact time in order to quantify reductions.

Non-Coated Stainless Steel Carrier			
0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	4.08E+05		
Untouched Control 2	6.60E+05	4.90E+05	5.02E+05
Untouched Control 3	4.37E+05		
Post Transfer Carrier Harvest-A	2.96E+05		
Post Transfer Carrier Harvest-B	7.60E+05		
Post Transfer Carrier Harvest-C	3.80E+05	4.71E+05	5.20E+05
Post Transfer Carrier Harvest-D	8.40E+05		
Post Transfer Carrier Harvest-E	3.22E+05		
Post Transfer Finger Pad Harvest-A	2.61E+05		
Post Transfer Finger Pad Harvest-B	2.28E+05		
Post Transfer Finger Pad Harvest-C	2.39E+05	1.26E+05	1.66E+05
Post Transfer Finger Pad Harvest-D	3.23E+04		
Post Transfer Finger Pad Harvest-E	6.80E+04		

Table 28: Non-coated stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0 hours).

Non-Coated Stainless Steel Carrier

0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.60E+06		
Untouched Control 2	1.16E+06	1.31E+06	1.32E+06
Untouched Control 3	1.21E+06		
1 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	2.30E+06		
Untouched Control 2	1.50E+06	1.61E+06	1.67E+06
Untouched Control 3	1.20E+06		
Post Transfer Carrier Harvest-A	9.10E+05		
Post Transfer Carrier Harvest-B	3.36E+05		
Post Transfer Carrier Harvest-C	1.09E+06	9.01E+05	1.08E+06
Post Transfer Carrier Harvest-D	1.51E+06		
Post Transfer Carrier Harvest-E	1.54E+06		
Post Transfer Finger Pad Harvest-A	1.90E+05		
Post Transfer Finger Pad Harvest-B	1.40E+05		
Post Transfer Finger Pad Harvest-C	1.03E+05	1.19E+05	1.24E+05
Post Transfer Finger Pad Harvest-D	9.30E+04		

Post Transfer Finger Pad Harvest-E	9.50E+04		

Table 29: Non-coated stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1 hour).

Non-Coated Stainless Steel Carrier			
0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.09E+04		
Untouched Control 2	1.74E+04	2.08E+04	2.53E+04
Untouched Control 3	4.76E+04		
4 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	3.01E+04		
Untouched Control 2	2.19E+04	2.19E+04	2.27E+04
Untouched Control 3	1.60E+04		
Post Transfer Carrier Harvest-A	8.30E+03		
Post Transfer Carrier Harvest-B	3.30E+03		
Post Transfer Carrier Harvest-C	1.50E+03	3.43E+03	4.06E+03
Post Transfer Carrier Harvest-D	4.80E+03		
Post Transfer Carrier Harvest-E	2.40E+03		

Post Transfer Finger Pad Harvest-A	2.50E+02		
Post Transfer Finger Pad Harvest-B	5.00E+01		
Post Transfer Finger Pad Harvest-C	1.20E+02	9.03E+01	1.10E+02
Post Transfer Finger Pad Harvest-D	8.00E+01		
Post Transfer Finger Pad Harvest-E	5.00E+01		

Table 30: Non-coated stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4 hours).

Non-Coated Stainless Steel Carrier			
8 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.44E+05		
Untouched Control 2	5.40E+04	7.44E+04	8.37E+04
Untouched Control 3	5.30E+04		
Post Transfer Carrier Harvest-A	4.49E+04		
Post Transfer Carrier Harvest-B	4.73E+04		
Post Transfer Carrier Harvest-C	8.10E+04	6.43E+04	6.66E+04
Post Transfer Carrier Harvest-D	7.90E+04		
Post Transfer Carrier Harvest-E	8.06E+04		
Post Transfer Finger Pad Harvest-A	6.28E+03		
Post Transfer Finger Pad Harvest-B	4.23E+03	3.97E+03	4.11E+03

Post Transfer Finger Pad Harvest-C	3.72E+03		
Post Transfer Finger Pad Harvest-D	3.07E+03		
Post Transfer Finger Pad Harvest-E	3.24E+03		

Table 31: Non-coated stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched control carriers; five carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8 hours).

Silver Embedded Carrier			
0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	2.82E+05		
Untouched Control 2	2.65E+05	2.50E+05	2.52E+05
Untouched Control 3	2.09E+05		
Post Transfer Carrier Harvest-A	1.53E+05		
Post Transfer Carrier Harvest-B	2.10E+05		
Post Transfer Carrier Harvest-C	2.56E+05	2.22E+05	2.29E+05
Post Transfer Carrier Harvest-D	2.03E+05		
Post Transfer Carrier Harvest-E	3.24E+05		
Post Transfer Finger Pad Harvest-A	1.30E+04	8.56E+03	9.14E+03
Post Transfer Finger Pad Harvest-B	1.15E+04		

Post Transfer Finger Pad Harvest-C	9.00E+03		
Post Transfer Finger Pad Harvest-D	7.90E+03		
Post Transfer Finger Pad Harvest-E	4.32E+03		

Table 32: Silver embedded coating stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=0 hours).

Silver Embedded Carrier			
0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.85E+06		
Untouched Control 2	1.94E+06	1.76E+06	1.77E+06
Untouched Control 3	1.51E+06		
1 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	2.23E+06		
Untouched Control 2	1.48E+06	1.80E+06	1.83E+06
Untouched Control 3	1.78E+06		
Post Transfer Carrier Harvest-A	1.69E+06	2.17E+06	2.30E+06

Post Transfer Carrier Harvest-B	1.19E+06		
Post Transfer Carrier Harvest-C	2.87E+06		
Post Transfer Carrier Harvest-D	2.67E+06		
Post Transfer Carrier Harvest-E	3.10E+06		
Post Transfer Finger Pad Harvest-A	2.18E+05		
Post Transfer Finger Pad Harvest-B	2.79E+05		
Post Transfer Finger Pad Harvest-C	2.44E+05	1.89E+05	2.00E+05
Post Transfer Finger Pad Harvest-D	1.54E+05		
Post Transfer Finger Pad Harvest-E	1.06E+05		

Table 33: Silver embedded coating stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=1 hour).

Silver Embedded Carrier			
0 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	4.56E+05		
Untouched Control 2	5.43E+05	5.01E+05	5.03E+05
Untouched Control 3	5.09E+05		
4 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	3.50E+05	4.34E+05	4.41E+05

Untouched Control 2	5.39E+05		
Untouched Control 3	4.33E+05		
Post Transfer Carrier Harvest-A	4.13E+05		
Post Transfer Carrier Harvest-B	4.60E+05		
Post Transfer Carrier Harvest-C	4.30E+05	4.80E+05	4.84E+05
Post Transfer Carrier Harvest-D	5.74E+05		
Post Transfer Carrier Harvest-E	5.45E+05		
Post Transfer Finger Pad Harvest-A	5.63E+04		
Post Transfer Finger Pad Harvest-B	5.63E+04		
Post Transfer Finger Pad Harvest-C	2.64E+04	3.48E+04	3.78E+04
Post Transfer Finger Pad Harvest-D	2.14E+04		
Post Transfer Finger Pad Harvest-E	2.87E+04		

Table 34: Silver embedded coating stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=4 hours).

Silver Embedded Carrier			
8 hour	**PFU per Carrier**	**Geometric mean (CFU)**	**Arithmetic mean (CFU)**
Untouched Control 1	1.99E+05		
Untouched Control 2	2.34E+05	2.12E+05	2.12E+05
Untouched Control 3	2.04E+05		

Post Transfer Carrier Harvest-A	2.36E+05		
Post Transfer Carrier Harvest-B	1.79E+05		
Post Transfer Carrier Harvest-C	1.61E+05	1.93E+05	1.96E+05
Post Transfer Carrier Harvest-D	1.65E+05		
Post Transfer Carrier Harvest-E	2.39E+05		
Post Transfer Finger Pad Harvest-A	1.79E+04		
Post Transfer Finger Pad Harvest-B	5.55E+03		
Post Transfer Finger Pad Harvest-C	1.09E+04	1.06E+04	1.15E+04
Post Transfer Finger Pad Harvest-D	8.60E+03		
Post Transfer Finger Pad Harvest-E	1.43E+04		

Table 35: Silver embedded coating stainless steel: PFUs of MS2 bacteriophage 15597-B1 quantified for untouched coated control carriers; five coated carriers post-transfer; and five individual finger pad harvests immediately upon inoculum drying (Time=8 hours).

MS2: Transfer Efficiencies based off Geometric Means		
Contact time (hours)	**Stainless steel transfer efficiency (%)**	**Silver Coating transfer efficiency (%)**
0	21	4
1	12	8
4	3	7
8	6	5
Average	10	6

Table 36: Mean transfer efficiencies of MS2 for non-coated and coated carriers per time point, and overall transfer mean transfer efficiency over the 8 hour study period, all utilizing the geometric mean values from the data

MS2: Transfer Efficiencies based off Arithmetic Means		
Contact time (hours)	**Stainless steel transfer efficiency (%)**	**Silver Coating transfer efficiency (%)**
0	24	4
1	10	8
4	3	7
8	6	6
Average	11	6

Table 37: Mean transfer efficiencies of MS2 for non-coated and coated carriers per time point, and overall transfer mean transfer efficiency over the 8 hour study period, all utilizing the arithmetic mean values from the data

The table above depicting the transfer efficiencies of the non-coated stainless steel and the silver coated carriers shows that the average transfer efficiencies appear to be relatively close to one another. The silver coated carriers appear to demonstrate a more consistent transfer efficiency over the 8-hour time period, whereas the non-coated carriers appear to have more variable transfer efficiencies. This appears to be consistent for both tables.

Exposure Time	Reductions due to environmental stressors (e.g. desiccation) and natural die-off		Reductions due the antimicrobial efficacy of SafeHandles		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction
0 hour	N.D.	N.D.	0.29	49%	0.29	49%
1 hour	-0.09	-23%	-0.05	-12%	-0.14	-38%
4 hour	-0.02	-5%	N.D.	N.D.	N.D.	N.D.
8 hour	0.82	85%	-0.45	-184.6%	0.36	57%

Table 38: Reductions of MS2 bacteriophage 15597-B1 due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings utilizing the geometric mean values from the data

Exposure Time	Reductions due to environmental stressors (e.g. desiccation) and natural die-off		Reductions due the antimicrobial efficacy of SafeHandles		Total reduction due to environmental factors and antimicrobial activity	
	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction	Log_{10} Reduction	Percent Reduction
0 hour	N.D.	N.D.	0.30	50%	0.30	50%
1 hour	-0.10	-26%	-0.04	-10%	-0.13	-38%
4 hour	0.05	10%	N.D.	N.D.	N.D.	N.D.
8 hour	0.78	83%	-0.40	-154%	0.37	58%

Table 39: Reductions of MS2 bacteriophage 15597-B1 due to natural, environmental die-off and to the antimicrobial efficacy of the silver embedded antimicrobial coatings utilizing the arithmetic mean values from the data.

The MS2-15597 reductions were inconsistent, particularly those due to the antimicrobial efficacy of the silver-based coating. The four-hour contact time study was performed on separate days for the coated and non-coated transfer trials; therefore, the original inoculum on each carrier type varied due to variations from the photo-spectrometer (1.965E8 for the uncoated and 7.5E7 for the coated). The MS2 did not appear to be reduced consistently over the 8-hour time period. The negative reduction values indicate that there were more viable phages on the carriers at that contact time than there were at the initial harvest (0 hour). Additionally, for the antimicrobial reductions at one and eight hours, the values are negative as there were more viable phages remaining on the coated carriers than on the non-coated. This indicates that the antimicrobial effects of the silver-treated carriers are less effective against non-enveloped viruses relative to the environmental effects of the non-coated silver.

4.2 QMRA Risk Characterization

4.2.1 *Klebsiella pneumoniae*

The initial point risk of a patient touching a contaminated sink handle is shown in Table 32 below.

Klebsiella pneumoniae	Non-coated handle	Silver-treated handle
Contact time (hours)	Point risk	Point risk

0	5.57E-05	5.57E-05
1	1.79E-06	1.73E-08
4	5.19E-07	5.77E-10
8	1.15E-07	5.77E-10

Table 40: Relative 95th percentile point risks for a patient touching a Klebsiella-contaminated handle at zero, one, four, and eight-hour contact times based off of geometric mean data (reductions as well as transfer efficiencies).

These point risks, aside from the zero-hour contact time, are combined into the scenario described in the methods. This creates a point risk for a single day as well as a weeklong hospital stay. The table demonstrates that there are relatively large differences between the coated and uncoated carriers. At four and eight hours, there are three orders of magnitude difference in the *Klebsiella* population between the coated and non-coated handle populations.

Organism: *Klebsiella pneumoniae*			
Uncoated handle		**Silver-coated handle**	
Daily risk	Weekly risk	Daily risk	Weekly risk
2.42E-06	1.70E-05	1.79E-08	1.26E-07

Table 41: 95th percentile point risk values for a single day and the cumulative risk of infection from Klebsiella pneumoniae for a weeklong hospital stay utilizing the geometric mean values from the data

The data in Table 33 displays the risks of infection given the transfer of *K. pneumoniae* to finger pads from silver-coated and non-coated handles. All values are within the acceptable risk range for infection as defined by the Environmental Protection agency (Paule & Lorna, n.d.), and the cumulative risk for the silver-coated handle for a week-long stay (1.26E-07) is less than the daily risk of an uncoated handle (2.42E-06).

4.2.2 MRSA

For MRSA, the daily point risks as well as a cumulative risk for infection via compromised skin during a weeklong stay are listed in Table 34. Since reductions per contact time were variable and inconsistent for MRSA in the transfer study, point risks were not performed for MRSA.

Organism: MRSA			
Uncoated handle		**Coated handle**	
Daily risk	Weekly risk	Daily risk	Weekly risk
1.95E-05	1.36E-04	5.30E-06	3.71E-05

Table 42: 95th percentile point risk values for a single day and the cumulative risk of infection from MRSA for a weeklong hospital stay utilizing the geometric mean values from the data

The cumulative risk for the non-coated handle represents a risk of approximately 1 infection in 7,350 people, which does not meet the minimum 1 in 10,000 annual risk set by the USEPA (Plipat et al. 2013). Observing the risk for the coated handles, the cumulative risk for a one-week stay is approximately 1 in 12,600. This value is acceptable by USEPA recommendation (Plipat et al. 2013)

An MS2 risk assessment was not performed due to the low levels of efficacy observed for the silver embedded coating against the bacteriophage.

4.3 Overall evaluation of results

The silver embedded antimicrobial coating was most effective against *Enterobacter aerogenes* (ATTC 13048) (4-$\log_{10}$ reduction) followed by *S. aureus* (1-$\log_{10}$) over the 8 hour contact time. The silver-based coating was least effective against MS2 bacteriophage. The observed differences may be attributed to the uptake of the slow-release silver ions by bacteria and the consequential effects on metabolic processes. MS2 is a virus, and therefore not susceptible to silver in the same manner. Additionally, the drying of the surface likely impacted the inactivation of virus. Previous studies performed at the WEST Center (not published) showed much higher reductions under moistened slides than in this study.

4.4 Further research

There are additional potential studies worth of consideration based on the data yielded from the research described herein. For the current study, the inoculum titer target of 10^6 PFU or CFU per carrier was several orders of magnitude greater than the environmental values found for the QMRA, and thus are not realistic with regards to bacterial load found on common-touch surfaces in the hospital setting. However, the high 10^6 CFU/PFU inoculum was especially useful for determining accurate reductions for organisms where the detection limit was hit such as *Enterobacter aerogenes*. According to the data produced from the literature review a more reasonable number would be between 10^2 to 10^3 CFU per surface. Additionally, the inoculum area for this transfer study was small (approximately 2-3 mm) and the organisms likely dried in layers on the surface of the carriers - preventing the exposure of silver ions to the organisms higher in the layers. An

additional transfer study performed at a lower inoculum titer of 10^3 CFU or PFU per carrier would allow for assessment of the effects of inoculum density on microbial reductions. Humidity has been demonstrated as a key variable in the transfer efficiency of microorganisms from hard, nonporous surfaces (Lopez, 2013). The transfer trials should similarly be performed at a high relative humidity (40-65%) (Lopez, 2013) to confirm these findings in the literature. The increased moisture may also serve to solubilize the silver ions and increase the antimicrobial efficacy of the coating. The differences in antimicrobial efficacy and transfer efficiency could significantly affect the probability of infection for various microbes. The use of alternative microbes of concern in hospitals will also further understanding of how silver embedded coatings can help prevent infection in the clinical setting. The use of a Gram-positive bacilli in the transfer study would allow for problem microbes such as *Acinetobacter baumannii* to be simulated in a risk assessment.

Fomites of varying compositions should be also be tested to reflect the types of surfaces most readily found in the clinical setting, including hard plastics, glass, and wood/wood laminate. The antimicrobial capabilities of stainless steel may be higher than those of non-metallic surfaces; therefore, the silver-coated surface could be even more effective than what is represented by the current dataset. For example, the bedrail in the risk assessment could be assumed as plastic material instead of stainless steel and would therefore have the possibility of poorer microbial reductions whereas the coated bedrail reductions would likely be the same. Additionally, the presence of organic matter (i.e. soil load) on a plastic bedrail may

even allow for the replication of organisms such as MRSA, therefore increasing the risk of infection for the patient.

Overall, silver embedded coatings may provide an additional barrier of protection to reduce the probability of infection in the healthcare setting. Embedded antimicrobial materials are low maintenance and self-sanitizing surfaces, and therefore are less susceptible to sanitation errors demonstrated in outbreaks (Kotay et al. 2017).

5. Appendix A

QMRA for *Klebsiella pneumoniae*

```
sink_area=(2*6*9)+(2*6*12)+(9*12)
sink_area_cm=sink_area*(2.54^2)
sink_area_cm
set.seed(665);
bac_density_in_sink=rnorm(10000, mean=53,  sd=15)
bac_load_on_sponge=bac_density_in_sink*sink_area_cm
bac_trans_sink2sponge=0.48
bac_load_on_sponge=bac_density_in_sink*sink_area_cm*bac_tra
ns_sink2sponge
bac_trans_sponge2handle=0.0071

bac_load_on_sink_handle=bac_load_on_sponge*bac_trans_sponge
2handle
 bac_trans_handle2handC=0.14


bac_load_on_hand_coated=((bac_load_on_sink_handle*(1-
.98848))+(bac_load_on_sink_handle*(1-
.99997))+(bac_load_on_sink_handle*(1-
.99988)))*bac_trans_handle2handC
bac_load_in_mouth=bac_load_on_hand_coated*bac_trans_hand2li
p
 iterations=10000
 k=2.76e-7
 for (x in 1:iterations){
 risk=1-exp((-k)*bac_load_in_mouth)}
 sorted=sort(risk)
sorted[9500]
visit_risk=1-(1-sorted)^7
visit_risk[9500]

bac_trans_handle2handSS=0.11
bac_load_on_hand_non_coated=((bac_load_on_sink_handle*(1-
.71))+(bac_load_on_sink_handle*(1-
.90))+(bac_load_on_sink_handle*(1-
.97)))*bac_trans_handle2handSS

bac_load_in_mouth=bac_load_on_hand_non_coated*bac_trans_han
d2lip
  iterations=10000
 k=2.76e-7
 for (x in 1:iterations){
risk=1-exp((-k)*bac_load_in_mouth)}
```

```
 sorted=sort(risk)
 sorted[9500]
visit_risk=1-(1-sorted)^7
 visit_risk[9500]



QMRA for MRSA
set.seed(665);
bac_load_on_rail=(rbeta(10000,2,7)*1680)
 bac_trans_to_hand_noncoated=0.36
bac_trans_to_hand_coated=0.14
k_staph=7.64e-8
bac_on_hand=bac_load_on_rail*bac_trans_to_hand_noncoated
bac_trans_hand_to_skin_breach=0.35
bac_on_skin_breach=bac_on_hand*bac_trans_hand_to_skin_breac
h
iterations=10000
daily_dose_non_coated=bac_on_skin_breach+(bac_on_skin_breac
h*(1-.29))+(bac_on_skin_breach*(1-
.21))+(bac_on_skin_breach*(1-.963))
for (x in 1:iterations){
risk=1-exp((-k_staph)*daily_dose_non_coated)}
sorted=sort(risk)
sorted[9500]
visit_risk=1-(1-sorted)^7
visit_risk[9500]

bac_on_hand=bac_load_on_rail*bac_trans_to_hand_coated
bac_trans_hand_to_skin_breach=0.35
bac_on_skin_breach=bac_on_hand*bac_trans_hand_to_skin_breac
h


daily_dose_coated=(bac_on_skin_breach*(1-
.20))+(bac_on_skin_breach*(1-.53))+(bac_on_skin_breach*(1-
.375))+(bac_on_skin_breach*(1-.974))
for (x in 1:iterations){
risk=1-exp((-k_staph)*daily_dose_coated)}
sorted=sort(risk)
sorted[9500]
visit_risk=1-(1-sorted)^7
visit_risk[9500]
```

6. References

2009 National Clean Hands Report Card® Survey Findings (n.d.). Retrieved from https://www.cleaninginstitute.org/clean_living/092109_summary.aspx

4. Dose Response. (n.d.). Retrieved from http://www.fao.org/docrep/009/a0238e/A0238E04.htm

Boyce, J. M., & Pittet, D. (2002). Guideline for Hand Hygiene in Health-Care Settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Infection Control and Hospital Epidemiology*, *23*(S12), S40. https://doi.org/10.1086/503164

Breathnach, A. S. (2009). Nosocomial infections. *Medicine*, *37*(10), 557–561. https://doi.org/10.1016/j.mpmed.2009.07.008

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: a review. *Biofouling*, *27*(9), 1017–1032. https://doi.org/10.1080/08927014.2011.626899

CDC. (2016, March). HAI Data and Statistics. Retrieved from https://www.cdc.gov/hai/surveillance/index.html

Chavali, S., Menon, V., & Shukla, U. (2014). Hand hygiene compliance among healthcare workers in an accredited tertiary care hospital. *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine*, *18*(10), 689. https://doi.org/10.4103/0972-5229.142179

Dancer, S. J. (2014). Controlling hospital-acquired infection: focus on the role of the

environment and new technologies for decontamination. *Clinical Microbiology Reviews*, *27*(4), 665–690. https://doi.org/10.1128/CMR.00020-14

Dancer, S. J., White, L. F., Lamb, J., Girvan, E. K., & Robertson, C. (2009). Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study. *BMC Medicine*, *7*(1), 28. https://doi.org/10.1186/1741-7015-7-28

Datta, R., & Huang, S. S. (2008). Risk of Infection and Death Due to Methicillin-Resistant Staphylococcus aureus in Long-Term Carriers. *Clinical Infectious Diseases*, *47*(2), 176–181. https://doi.org/10.1086/589241

Fewtrell, L., & Bartram, J. (2001). *Water quality : guidelines, standards, and health : assessment of risk and risk management for water-related infectious disease*.

Glance, L. G., Stone, P. W., Mukamel, D. B., & Dick, A. W. (2011). Increases in Mortality, Length of Stay, and Cost Associated With Hospital-Acquired Infections in Trauma Patients. *Archives of Surgery*, *146*(7), 794–801. https://doi.org/10.1001/archsurg.2011.41

Gurian, P. (2015). Risk Characterization. Retrieved from http://qmrawiki.canr.msu.edu/index.php/Risk_Characterization

Haas, C. (n.d.). Dose response assessment Retrieved from http://qmrawiki.canr.msu.edu/index.php?title=Dose_response_assessment

Haas, C. N., Rose, J. B., & Gerba, C. P. (2014). Quantitative microbial risk assessment.

Harb, M., & Hong, P.-Y. (2017). Molecular-based detection of potentially pathogenic bacteria in membrane bioreactor (MBR) systems treating municipal wastewater: a case study. *Environmental Science and Pollution Research*, *24*(6), 5370–5380. https://doi.org/10.1007/s11356-016-8211-y

Herruzo, R., Ruiz, G., Vizcaino, M. J., Rivas, L., Pérez-Blanco, V., & Sanchez, M. (2017). Microbial competition in environmental nosocomial reservoirs and diffusion capacity of OXA48. *Journal of Preventive Medicine and Hygiene*, *58*(1), E34. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28515629

Hossein, & Alireza. (n.d.). Epidemiology of nosocomial infections in a pediatric intensive care unit (PICU).

Joan, R. (2015). Hazard Identification: General Overview.

Just Manufacturing. (n.d.). Retrieved from https://www.justmfg.com/details/SL-1515-A-GR

Klebsiella pneumoniae in Healthcare Settings (n.d.). Retrieved from https://www.cdc.gov/hai/organisms/klebsiella/klebsiella.html

Kotay, S., Chai, W., Guilford, W., Barry, K., & Mathers, A. J. (2017). Spread from the Sink to the Patient: In Situ Study Using Green Fluorescent Protein (GFP)-Expressing Escherichia coli To Model Bacterial Dispersion from Hand-Washing Sink-Trap Reservoirs. *Applied and Environmental Microbiology*, *83*(8), E03327. Retrieved from https://search.proquest.com/docview/1885754590

Kramer, A., Schwebke, I., & Kampf, G. (2006, January). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*. https://doi.org/10.1186/1471-2334-6-130

Krauter, P. A., Piepel, G. F., Boucher, R., Tezak, M., Amidan, B. G., & Einfeld, W. (2012). False-Negative Rate and Recovery Efficiency Performance of a Validated Sponge Wipe Sampling Method. *Applied and Environmental Microbiology*, *78*(3), 846–854. https://doi.org/10.1128/AEM.07403-11

Kurashige, E. J. O., Oie, S., & Furukawa, H. (2016). Contamination of environmental surfaces by methicillin-resistant Staphylococcus aureus (MRSA) in rooms of inpatients with MRSA-positive body sites. *Brazilian Journal of Microbiology*, *47*(3), 703–705. https://doi.org/10.1016/j.bjm.2016.04.002

Lopez, G. U. (2013). *Transfer of Microorganisms from Fomites to Hands and Risk Assessment of Contaminated and Disinfected Surfaces*. Retrieved from http://arizona.openrepository.com/arizona/handle/10150/272839

Mathur, P. (2011). Hand hygiene: back to the basics of infection control. *The Indian Journal of Medical Research*, *134*(5), 611. https://doi.org/10.4103/0971-5916.90985

Montoya, A., & Mody, L. (2011). Common infections in nursing homes: a review of current issues and challenges. *Aging Health*, *7*(6), 889–899. https://doi.org/10.2217/ahe.11.80

MRSA Tracking. (n.d.). Retrieved from https://www.cdc.gov/mrsa/tracking/index.html

Musu, M., Lai, A., Mereu, N. M., Galletta, M., Campagna, M., Tidore, M., … Coppola, R. C. (2017). Assessing hand hygiene compliance among healthcare workers in six Intensive Care Units. *Journal of Preventive Medicine and Hygiene*, *58*(3), E231. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29123370

Page, K., Wilson, M., & Parkin, I. P. (2009). Antimicrobial surfaces and their potential in reducing the role of the inanimate environment in the incidence of hospital-acquired infections. *Journal of Materials Chemistry*, *19*(23), 3819–3831. https://doi.org/10.1039/b818698g

Paule, H. R., & Lorna, F. (n.d.). *Acceptable risk*. Retrieved from http://www.who.int/water_sanitation_health/dwq/iwachap10.pdf

Pivo, T., Cabrera, D., Lopez, G., Sassi, H. P., & Gerba, C. P. (2016). Movement of Pathogens From Public Restroom to Clinical Areas in a Hospital. *AJIC: American Journal of Infection Control*, *44*(6), S44. https://doi.org/10.1016/j.ajic.2016.04.199

Plipat, N., Spicknall, I. H., Koopman, J. S., & Eisenberg, J. N. S. (2013). The dynamics of methicillin-resistant Staphylococcus aureus exposure in a hospital model and the potential for environmental intervention. *BMC Infectious Diseases*, *13*(1), 595. https://doi.org/10.1186/1471-2334-13-595

Raymond, J., & Aujard, Y. (2000). Nosocomial Infections in Pediatric Patients: A European, Multicenter Prospective Study. *Infection Control and Hospital Epidemiology*, *21*(4), 260–263. https://doi.org/10.1086/501755

Reiss, I., Borkhardt, A., Füssle, R., Sziegoleit, A., & Gortner, L. (2000). Disinfectant contaminated with Klebsiella oxytoca as a source of sepsis in babies. *The Lancet*, *356*(9226), 310. https://doi.org/10.1016/S0140-6736(00)02509-5

Risk Assessment Guidelines. (n.d.). Retrieved from https://www.epa.gov/risk/risk-assessment-guidelines

Rossi, E. M., Scapin, D., & Tondo, E. C. (2013). Survival and transfer of microorganisms from kitchen sponges to surfaces of stainless steel and polyethylene. *Journal of Infection in Developing Countries*, *7*(3), 229. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23493001

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. https://doi.org/10.1046/j.1365-2672.2002.01734.x

Scott, R. D. (2010). *Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention;2010 ASI 4208-83*. Retrieved from https://statistical.proquest.com/statisticalinsight/result/pqpresultpage.previewtitle?docType=PQSI&titleUri=/content/2010/4208-83.xml

Seifert, H., Strate, A., & Pulverer, G. (1995). Nosocomial Bacteremia due to Acinetobacter baumannii:Clinical Features, Epidemiology, and Predictors of Mortality. *Medicine*, *74*(6), 340–349. Retrieved from https://europepmc.org/abstract/med/7500897

Shibata, T. (n.d.). Introduction to Exposure Assessment. Retrieved from http://qmrawiki.canr.msu.edu/images/Introduction.pdf

Tamrakar, S. (2013). Staphylococcus aureus: Dose Response Models. Retrieved from http://qmrawiki.canr.msu.edu/index.php/Staphylococcus_aureus:_Dose_Response_Models

The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes. (2007).

Voltaggio, T. (n.d.). Use of Monte Carlo Simulation in Risk Assessments. Retrieved from https://www.epa.gov/risk/use-monte-carlo-simulation-risk-assessments