AN EXAMINATION OF CROSS CONTAMINATION RATES BETWEEN COMMON KITCHEN SURFACES, HANDS, AND PRODUCE

by

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ABSTRACT OF THE THESIS

An Examination of Cross Contamination Rates Between Common Kitchen Surfaces, Hands, and Produce

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Fresh cut produce consumption has increased recently due to increase consumer demand and availability. Such products may pose a risk of foodborne disease because the product is frequently consumed raw. This thesis addresses three specific concerns related to fresh cut produce safety: (i) quantification of the cross contamination rates between fresh cut produce and hands (ii) quantification of the cross contamination rates between a variety of fresh produce and surface types using scenarios that differ by cross contamination direction, surface type, produce type, and post inoculation drying time and (iii) quantification of the transfer rates of *Escherichia coli* O157:H7 between a single inoculated lettuce leaf to non-inoculated lettuce leaves under various washing times. These studies were carried out using a non-pathogenic surrogate (*Enterobacter aerogenes*) or cocktails of *E. coli* O157:H7 or *Salmonella*. Fresh cut produce types used were cantaloupe, carrots, celery, romaine lettuce or watermelon. Surface types used were ceramic, stainless steel, glass, and plastic. When gloved hands were contaminated with *E. coli* O157:H7 about 30% transferred to carrots, and 10% and 3% to celery and cantaloupe respectively. When carrots and celery were contaminated, about 1% of those bacteria transferred to gloved hands, while inoculated cantaloupe only transferred about 0.3%. Dry food contact surfaces transferred almost 100% of the bacteria present to

carrots and watermelon, but transfer from dry surfaces to celery or lettuce were much more variable. Wash water will become contaminated with 90-99% of bacteria originally present on the lettuce leaves, regardless of washing time and each non-inoculated lettuce piece will become contaminated with ~1% of the *E. coli* O157:H7 originally on the inoculated lettuce. The key observations are that direction of transfer, and moisture play a large role in determining transfer rates, and that a simple wash with tap water may not be sufficient to significantly reduce the microbial load on lettuce, and may result in contamination spread to previously uncontaminated leaves. Understanding the transfer rates to and from fresh cut produce and during washing arising from this work will allow for better risk assessment and management of microbial food safety risks in the home.

Dedication

Laryssa

For challenging me to become more than what I was, and accepting me for who I became.

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List of Abbreviations

BSA-Bismuth Sulfite Agar

BSAR- Bismuth Sulfite Agar with Rifampicin

CDC- Center for Disease Control and Prevention

CFU- colony forming unit

FDA- US Food and Drug Administration

HACCP- Hazard Analysis and Critical Control Point

MC-MacConkey Agar

MC-na- MacConkey Agar with Nalidixic Acid

RTE- Ready to eat

SMACR- Sorbitol MacConkey agar with rifampicin

TSA-Tryptic Soy Agar

TSAR- Tryptic Soy Agar with Rifampicin

TSBR-Tryptic Soy Broth with Rifampicin

USDA- US Department of Agriculture

Preface

This thesis is a compilation of three studies, funded by a grant from the US Food and Drug Administration (FDA), that aims to assess the risks posed by consumer handling practices using laboratory-based fresh cut produce cross contamination studies. These three studies are a collaboration between three land grant institutions; Rutgers University, Department of Food Science; University of Florida, Center for Citrus Research and Education; and University of California Davis, Department of Food Science and Technology. The grant's primary investigator is Michelle Danyluk, Ph.D. from University Florida. Linda Harris Ph.D. from UC Davis, and Donald Schaffner Ph,D. from Rutgers University, serve as co-primary investigators on the project. All three investigators have extension appointments from their respective universities, and each has supervised data collection at their respective locations, and contributed to overall experimental design and data analysis.

Chapter I is the literature review, and serves to lay out the central issues that connect the topics introduced in Chapters II, III, and IV. The thesis is written in manuscript style, such that each chapter after the literature review represents a draft of a separate submission to the peer reviewed literature. Chapter II focuses on quantifying cross contamination between fresh-cut-produce and hands. Dane Jensen, at Rutgers University (NJ), performed the experiments, data analysis, and wrote the first draft of the manuscript. The produce used for these experiments was purchased in New Jersey from local markets. Chapter III focuses on quantifying

cross contamination rates between fresh cut produce and common kitchen surfaces. Loretta Friedrich, a laboratory technician at University of Florida (FL), collected the data, and Dane Jensen, at Rutgers University (NJ), performed the data analysis and wrote the first draft of the manuscript. The produce used in these experiments was purchased in Florida from local markets. Chapter IV focuses on quantifying cross contamination rates between fresh cut lettuce pieces during washing. Loretta Friedrich collected the data at University of Florida (FL), and Dane Jensen, at Rutgers University (NJ), performed the data analysis and wrote the first draft of the manuscript. The produce used in these experiments was purchased in Florida from local markets.

The results described in this thesis will assist policy makers, extension educators across the country, and aid consumers in managing the risks posed by handling fresh cut produce in the home.

Chapter I – Literature Review

I.1 Fresh Cut Produce

I.1.a Popularity of Fresh Produce

The American diet is continuously changing, and current trends focus on nutritious foods, high in vitamins and minerals, and low in added fats or simple sugars (*19*, *20*, *49*, *77*). This focus has paved the way for fresh produce to become a staple in many diets. The demand has increased to such a great degree, that produce is being shipped over greater distances as Americans seek out new varieties of both domestic and imported produce (*22*, *77*). Currently, fresh cut, minimally processed fruits and vegetables are a $15 billion dollar per year business, and account for roughly 15% of produce sales in North America(*60*). Furthermore, increasing consumption of fresh produce has increased importation of fresh produce to meet off-season demand (*19*, *50*). While these imports fill a gap in the market, outbreaks from a single exporter can quickly become multinational (*50*, *72*).

Technology has evolved to deliver precut produce in convenient packaging. Nutritionally, these products are a healthier alternative to fried or baked snacks, however, an unintentional consequence has been an increased risk of foodborne illness, regardless of the produce being shipped in plastic bag or clam shell containers, which many consumers may consider safer than loose produce (*22*, *77*). Increased outbreaks with produce have been linked partially to increased consumption of minimally processed vegetable, however the increased number of

outbreaks is due to many complicating factors, which include, but are not limited to improved detection and increase foreign produce imports (*20*, *22*, *29*, *30*).

I.1.b Risk that comes with fresh cut produce

Between 1996 and 2008, top produce involved in outbreaks were tomatoes (14 outbreaks), lettuce (22 outbreaks), and cantaloupe (9 outbreaks, 13 total for all melons) (*22*). Risk of food pathogen consumption is higher in fresh cut produce compared to many other foods primarily because the product is minimally processed (*19*, *22*, *29*). The FDA designates fresh cut fruits and vegetables as produce that have been minimally processed (e.g., no lethality step), and altered in form, by peeling, slicing, chopping, shredding, coring, or trimming, with or without washing or other treatment, prior to being packaged for use by the consumer. Fresh cut produce does not require additional preparation, processing, or cooking before consumption, with the possible exception of washing or the addition of salad dressing, seasoning, or other accompaniments. Due to the limited number of microbial reduction interventions, fresh produce will often carry the microbes the produce was exposed to while growing in the field, during processing, at the retailer, and/or in the home kitchen (*8*, *22*, *30*, *35*). Produce is commonly exposed to pathogens from the soil they grow in or via contaminated wash and/or irrigation water (*19*, *22*, *99*). Often, less effective or more expensive control strategies, such as the use of sanitizing chemicals, irradiation, or biocontrol agents, are used to control contamination (*22*, *29*, *61*). Thermal inactivation steps will compromise the products "fresh-cut" status, and therefore are not typically used. Due to the high nutrient content and high water activity, pathogens will not only survive, but also

grow on fresh produce, and temperature control remains the strongest deterrent of pathogen growth (*22*, *50*). Temperature abuse, by a rise in temperature of 5 or 10°C will allow many mesophylic foodborne pathogens to grow (*22*, *50*, *73*, *91*).

I.1.c Lettuce & leafy greens

Leafy greens are considered the second most dangerous fresh produce, being second only to sprouts (alfalfa, bean, or clover) (*22*, *56*). Sprouts are considered to be of the greatest risk due to the methods in which sprouts are grown, which promote bacteria growth (*56*). These conditions include, but are not limited to high water activity, long exposure time, exposure to warm temperatures, and high nutrients content (*56*). Lettuce is not subject to the same risk conditions as sprouts, however, lettuce is not as dense as other produce (especially when cut), and the large volumes of entrapped air make temperature control of the leaves problematic. Furthermore, because lettuce leaves are often layered, initial washes in the fields will not remove any pathogens buried deeper in the head. Furthermore, the surface structures on the roots and leaves of the lettuce plant create an environment in which pathogens can be protected from washes (*80*). An opening includes the stomata on the leaf, or cuts and bruises from processing. In addition to providing ideal attachment sites that are protected, these sites often have higher nutrient concentrations (*22*, *30*, *80*, *100*). Finally, since all lettuce leaves are potentially eaten without removal of any external rind, as in the case of melons, this also raises the risk. These risk factors leave lettuce and leafy greens especially susceptible to serving as a vehicle for foodborne disease transmission.

Lettuce and leafy greens have been implicated as the carrier of pathogens in many outbreaks (*20*, *22*, *30*, *35*, *49*, *59*, *78*). Despite these outbreaks, leafy green consumption has increased 9% between 1995 and 2005, while reported outbreaks have increased 38.6% during the same time (*22*). Contamination can occur from many points during the processing and handling (*8*, *22*, *30*, *35*). This includes risks arising from irrigation, soil, harvesting, packaging, storage, and in the home kitchen (*8*, *22*, *30*, *35*, *73*). Pathogens, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella*, *Campylobacter*, *Yersinia enterocolitica*, and *Salmonella spp.*, have been linked to leafy greens (*8*, *30*, *35*, *59*).

Smith et al. (2003) found that 88% of unwashed lettuce samples had greater than 2 log CFU of aerobic, mesophilic microbes per g of unwashed lettuce (65% had greater than >3 log CFU/g), which were similar to the findings of Vijayakumar et al. (2002) (*78*, *97*). All samples had a total coliform count <100 CFU/g (*78*). Soriano et al. (2000) found a range of 3-7.8 log CFU aerobic, mesophilic microbes per gram of untreated lettuce in homes (*81*). However, many pathogens, especially *E. coli* O157:H7, display a preference, and at times better survival, to cut portions of lettuce (*33*, *88*).

I.1.d Melon

Between 1996 and 2008, 13 out of 82 (15.9%) produce outbreaks were linked to melons (*89*). These outbreaks were linked to 507 illnesses and two deaths. Of the 13 melon outbreaks, cantaloupe was involved in 10 of them. Uncut melons are generally held at room temperature at food markets and in the home, but cut melons need to be refrigerated due the risk of bacterial survival, and rapid bacterial

growth under temperature abuse conditions (*5*, *10*, *22*, *89*, *91*). Melons can attract and hold bacteria on their rind due to their growth in direct contact with soil, and their rough netted exterior (*18*, *22*, *91*). While the rind is typically not eaten, the process of cutting the melon can push bacteria inside to the flesh(*91*). Furthermore, the stem scar, where the melon is separated from the vine, is another potential source of contamination (*31*, *89*). Some melon producers attempt to reduce soil contact by growing the melon on "cups" or plastic covered beds to reduce soil contamination, however, this only eliminates one potential point of cross contamination to the melons (*19*, *22*, *89*, *99*).

I.1.e Carrots and celery

In 2005, the average American per capita consumption of carrots was 8.3 pounds, while the per capita consumption of celery was 7 pounds (*22*, *48*). While outbreaks with fresh carrots and fresh celery are rare, they still pose a health risk due to multiple factors that create microbial control points for other types of produce. First and foremost, carrots and celery as snacks are traditionally consumed raw, without any microbial inactivation step. Furthermore, they are grown in, or in close proximity to the soil. While celery is almost never eaten peeled, carrots can be eaten with or without the peel. Raw consumption, lack of peeling, and close proximity to soil all increase the chances that a produce item may cause illness in humans (*8*, *22*, *30*, *35*, *49*, *73*, *99*).

I.2 Risk analysis & cross contamination

Risk analysis allows data to be used to "describe the likelihood and magnitude of a specific hazard" (*73*). Cross contamination rates measure the amount of a substance

(in this case bacteria), transferred between two surfaces. This data can be used in quantitative risk and generate risk-analysis calculations to provide policy makers with a deeper understanding of the all the potential risks posed by a contaminated food product (*73*, *93*). In addition to cross contamination, the risk posed by a product-pathogen pair varies depending on the virulence of pathogen(s) involved, susceptibility of the target consumers, probability of presence, and quantity of pathogen consumed (*14*, *49*, *73*). Predicting microbial behavior and transmission between handling practices and contamination of fresh cut produce can be difficult because a large number of contamination scenarios exist (*64*). Produce outbreaks and their ultimate causes are generally harder to trace because they are more complex and difficult to investigate (*22*, *93*). Multiple fields, more points of contamination, and variance in vendor handling make tracing the origin and cause of an outbreak difficult. In addition, accusing the wrong producer or vendor of an outbreak can cause severe economic repercussions and damage the reputation of otherwise safe producers, as evidenced by the *E. coli* O104:H4 sprout outbreak in Europe, during the summer of 2011(*92*).

By aiming to create a thorough and accurate quantification of cross contamination rates, food safety analysts can provide risk analysis in both home and food service kitchens (*16*). Studies that estimate transfer rates between kitchens utensils, raw meat, cutting boards, fresh produce, and hands have been done, but often they try to focus a specific object or action (*16*, *50*, *55*, *93*). Patterns that apply to a broad set of scenarios are most useful in creating formulas to assess the risk of cross contamination.

I.2.a Immuno Compromised Individuals

Understanding the target population, or the population most likely to consume the contaminated product, is an integral part of risk analysis (*14*, *29*, *49*, *73*). The highest "at risk" populations are generally considered to be the immunocompromised, young, and elderly (*15*, *46*, *49*). Immunocompromised individuals include those who may be pregnant, HIV positive, malnourished, have diabetes, are in organ failure, are organ transplant recipients, alcoholics, and cancer patients (*49*). These at risk populations comprise almost 20% of the U.S. population. High-risk food includes unpasteurized dairy products, fresh produce, undercooked or raw meats, and eggs. The CDC recommends that immunocompromised individuals follow a low-microbial diet to reduce their risk of infection(*49*).

I.3 Organism used in these studies

I.3.a *Salmonella* spp.

Salmonella is a gram negative, rod-shaped, motile bacterium (*24*). Its optimal growth temperature is 38°C (*24*, *29*). It can survive on insects, and in raw food, soiled water, factory surfaces, kitchen surfaces, and feces. Most *Salmonella* isolates can form curli, which are proteinaceous fibers involved in cell adhesion to surfaces, cell aggregation, and biofilm formation (*4*, *79*).

In the United States, approximately two to four million cases of salmonellosis are reported every year, but because many milder cases are often not reported or misdiagnosed, the actual reported number of infections may be higher (*15*, *24*). Salmonellosis is ranked one of the most commonly reported food illnesses worldwide (*29*). Symptoms include nausea, vomiting, diarrhea, and fever. Roughly

400 persons die each year with acute salmonellosis, and an estimated 95% of cases of *Salmonella* infection are linked to food (*15*, *24*, *42*).

The reported frequency of salmonellosis appears to be on the rise in the U.S. and in other industrialized nations (*24*). *Salmonella* has been involved in 53 of 97 US produce outbreaks (*22*). In addition to fresh produce, the foods associated with *Salmonella* include raw meat, poultry, eggs, peanut butter, fish, salad dressings, and cocoa (*24*, *73*).

I.3.b *Escherichia coli* O157:H7

Escherichia coli O157:H7 is a gram negative, rod shaped, facultative anaerobe (*24*, *29*). It is part of a group of pathogenic *E. coli* known as Enterohemmorhagic *E. coli* (EHEC) (*24*, *29*, *73*). *E. coli* O157:H7 is generally known to have a higher acid tolerance than most enteric pathogens, which may make it easier for the organism to infect the intestine (*24*, *29*, *73*, *85*). *E. coli* O157:H7 owes its high infectivity in part due to plasmids with genetic information that can be readily exchanged between cells of closely related species (*29*, *73*). *E. coli* O157:H7 became recognized as a major pathogen after the 1993 Jack in the Box ground beef outbreak (*29*). Since then, *E. coli* O157:H7 has been involved in 19 out of 97 US produce outbreaks (*22*).

E. coli O157:H7 infection can cause a mild to severe gastroenteritis. Young children may develop hemolytic-uremic syndrome, which may cause irreversible damage to the kidneys (*24*, *73*). Gastroenteritis is characterized by severe cramping abdominal pain and diarrhea. The diarrhea is initially watery, but can become bloody. *E. coli* O157:H7 releases one or more related potent toxins that cause severe damage to the

lining of the intestine. These toxins, verotoxin (VT) and shiga-like toxin, are similar to the toxin produced by *Shigella dysenteriae (24)*.

In addition to fresh produce, the foods associated with *E. coli* O157:H7 are uncooked meat, especially beef, salads, dried salami, raw milk, unpasteurized juice, and unpasteurized cheese (*24*, *73*). *E. coli* O157:H7 is often carried asymptomatically in intestines of cattle, as these animals lack the receptor for shiga toxin, and are not noticeably harmed by the presence of these bacteria (*22*, *29*, *85*).

I.3.c *Enterobacter aerogenes*

Enterobacter aerogenes is a bacterium with attachment characteristics similar to that of *Salmonella spp*. (*16*, *103*). The food grade strain used in this research has been developed to remove free sugars in dried egg products in order to prevent Maillard browning during storage (B199A Vivolac Cultures, Indianapolis, Ind.) (*103*). This organism is used in some experiments as a surrogate for more dangerous foodborne pathogens.

I.4 Bacterial attachment and detachment

Bacterial attachment is a multifaceted process. Surface charge, moisture, bacterial surface structures, coarseness of the non-bacterial surface, and Van der Waals forces play a role in initial attachment of microbes (*4*, *18*, *82*, *90*, *94*, *101*). These initial attachment mechanisms, which primarily occur within the first thirty minutes of contact, are believed to allow the pathogen to use vegetative matter as a vector between hosts (*2*).

Generally, biotic surfaces have an overall negative electrostatic surface charge that generates a repulsive charge to other negatively charged surfaces (*90*). Increase in

cations, such as calcium and sodium, in an aqueous medium on one of the surfaces can affect the attachment by reducing repulsive forces between the negatively charged biotic surfaces (*18*, *27*). For example, calcium ions promote an increased attachment of *E. coli O157:H7* to lettuce surfaces, but not to cuts or cracks in the lettuce surfaces (*41*). Both hydrophobicity and electrophoretic mobility play a role in attachment. As hydrophobicity decreases, electrostatic attractions and repulsions play a larger role in initial attachment (*94*, *95*). Hydrophobicity has the greatest effect on attachment, and a strong hydrophobicity can increase bacterial adherence to surfaces (*23*, *94*). *Salmonella* and *L. monocytogenes* adhere more strongly to hydrophobic materials, but adherence is not based on hydrophobicity alone (*76*).

The extracellular matrix of *Salmonella spp.* and *Escherichia coli* O157:H7 is composed of proteinaceous and starchy molecule polymers, mainly cellulose and fimbriae, which assist with both the initial and the irreversible attachment (*4*, *82*). Gram-negative bacteria produce lipopolysaccharides that also assist with attachment (*84*). The O-antigen also has hydrophilic properties that affect the attachment of gram-negative bacteria. Lack of an O-antigen can improve adherence to hydrophobic materials (*84*). Flagella, the H-antigen, play an important role by providing mobility for the bacteria to find prime attachment spots and overcome the repulsive forces, but flagella do not act as adsorbents or adhesives, such that they facilitate attachment, but are not directly responsible for its occurrence(*18*). Cell surface polymers with non-polar sites, such as fimbriae, primarily initiate attachment to hydrophobic substrata, while exopolysaccharides and lipopolysaccharides initiate attachment to hydrophilic materials (*18*).

I.5 Hands

Many cases of foodborne illness can be traced back to improper food handling practices in the consumer's home (*6*, *69*). Contamination levels on human hands or in kitchen foods is well researched and documented; however, data that quantifies the transfer rates between the surfaces of produce and hands is generally limited (*16*, *21*, *34*).

In restaurants and at-home kitchens, measures to reduce pathogens should ideally focus on removing the source(s) of infections, and on disrupting person to person transmission, usually with hand washing and the use of gloves (*46*). In most cases, the source of contamination is an ill food handler or contaminated water source. Except for a few examples, such as *Staphylococcus aureus,* foodborne pathogens are considered transient bacteria on hands, and can easily attach and detach to human skin and to the inanimate environments, such as work surfaces or food (*43*). The number of microorganisms on non-damaged portions of skin in the same person can vary from 100–10,000/cm^2 (*43, 51*, *52*, *57*). These organisms are rarely of the same serotype, except in extreme contamination conditions (*43, 51*, *52*, *57*). However, the subungual region, under the nail, may frequently have as high as 5.5 log CFU/site, and the palm as high as 4 log CFU/site (*56*). Gram-negative species of bacteria generally do not colonize a hand. However, McGinley et al (1988) found that 42% of people tested were positive for gram negative bacteria in their subungal region (*52*).

I.5.a Washing rates

Risk of illness from temperature-abused foods is greatly increased if the food handlers follow poor hand hygiene practices (*37*, *38*, *43, 52*). Green et al. observed

that food workers only performed a full handwash (removed gloves, placed hands in running water, used soap, and dried hands) 27% of the time during food preparation activities (*37*, *38*). Restaurant workers were more likely to wash hands and use gloves appropriately when not involved in multiple tasks, i.e., when employees were not busy (*37*). These researchers also noted that handwashing and glove use was more common in chain establishments, in comparison to non-corporate restaurants (*37*, *38*). In a survey of 16 local food service operations compliance with FDA food code recommendations during production phase was only 7% overall in restaurants, and that hands were washed frequently before beginning preparation, but not when changing tasks, touching cloths, or before handling different kinds of foods (*86*). These same researchers report zero compliance with FDA food code recommendations for hand washing, due to a combination of incomplete lathering of hands, limited time under running water, and non-hygienic means of drying hands (*86*). A notable concern the researchers found is that food service workers rarely complied with handwashing between raw and RTE foods. However, 72% of food handlers did wash hands before entering food preparation area. In related research these investigators noticed improve handwashing regimes in assisted living facilities, schools, and childcare facilities when compared to restaurants (*86*). Guzewich and Ross (1999) determined that, between 1975-1998, 66 of 81 outbreaks had sufficient evidence to track the course of infections to food workers (*40*). Furthermore, an estimated 26% of consumers do not wash surfaces during the preparation of ready to eat, raw, or cooked foods,

although an estimated 60% reported that they "always wash surfaces before food preparation" (*44*).

I.6 Kitchen Surfaces

Samples of common kitchen objects used in the preparation of food, produce, and cleaning fabrics were examined microscopically in order to reveal that used dishrags and used sponges, especially if damp, displayed many types of bacteria and advanced biofilm formation (*68*). This is not surprising, as investigators have noted that bacterial colonization of both biological and non-biological surfaces generally (not just in the kitchen environment) are a key aspect of pathogenesis and microbial ecology (*66*). Researchers have also demonstrated that kitchen surfaces, including water spigots and the meal's preparers' hands, become quickly contaminated after preparing a meal or when handling raw foods (*16*, *34*).

I.7. Introduction to the thesis

The research in this thesis will: quantify cross contamination between fresh-cut-produce and hands; quantifying cross contamination rates between fresh cut produce and common kitchen surfaces; and quantifying cross contamination rates between fresh cut lettuce pieces during washing. The results described below will assist policy makers and extension educators across the country aid consumers in managing the risks posed by handling fresh cut produce in the home.

Chapter II- Quantifying Bacterial Cross Contamination Rates Between Fresh Cut Produce and Hands

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Key Words: Cross Contamination, *Salmonella*, *Escherichia coli*, Produce

II.1 Abstract

Fresh cut produce consumption has increased over the past few decades due to increase consumer demand and increase availability off-season. Such products may pose a risk of foodborne disease due to the product frequently being consumed raw, often with minimal or ineffective washings beforehand, and no lethal inactivation steps during preparation. Because fresh cut produce is not cooked prior to consumption, cross contamination by hands and other kitchen surfaces is a serious concern. This study aims to quantify the cross contamination rates between fresh cut produce and hands. This study was carried out using a non-pathogenic surrogate (*Enterobacter aerogenes*) or a cocktail of *E. coli* O157:H7 strains. Each participant handled 25g of fresh cut produce in two different scenarios. In the first scenario the participant's gloved hands were inoculated with 6 log CFU/hand bacteria, and then the participant handled 25g of fresh produce. In the second scenario, the participant handled five pieces of 25g of inoculated fresh produce (6 log CFU/25g). The glove juice method was used to quantify bacterial counts on hands, while produce samples where diluted with buffer and stomached. *E. aerogenes* were enumerated on MacConkey agar with 50mg/g nalidixic acid added, while *E. coli* O157:H7 were enumerated on MacConkey agar with 2mL/mL rifampicin stock . About 30% of *E. aerogenes* inoculated on to gloved hands were transferred to carrots and celery, while only 18% transferred to cantaloupe. When inoculated carrots or cantaloupe contained *E. aerogenes*, about 1% were transferred to gloved hands, while inoculated celery only transferred about 0.3% of *E. aerogenes* to gloved hands. When gloved hands were contaminated with *E. coli* O157:H7, about 30% of those bacteria transferred to carrots, 10% celery, and 3% to cantaloupe. When carrots and celery were inoculated

with *E. coli* O157:H7, about 1% of those bacteria were transferred to gloved hands, while inoculated cantaloupe only transferred about 0.3%. The key observations are that direction of transfer, and moisture play a large role in determining transfer rates. Understanding transfer rates to and from fresh cut produce will allow for better risk assessment and management of microbial food safety risks in the home.

II.2 Introduction

Fresh cut produce has become an increasingly popular snack over the past two decades as Americans have become more conscious of their personal nutrition (*77*). Efforts to increase fruit and vegetable intake have led food companies to develop fresh cut produce alternatives to traditional snack-foods that are baked or fried (*13*). Aside from the nutritional standpoint, fresh cut produce has also gained popularity due to it becoming more accessible over recent years, in part due to the rise of ready-to-eat fresh cut produce in prepackaged convenience containers at super markets, convenience stores, and even vending machines (*13*, *77*). The FDA defines fresh cut produce as "fruits and vegetables that have been minimally processed (e.g. no lethal inactivation step), but may be altered in form, by peeling, slicing, chopping, shredding, coring, or trimming, with or without washing or other treatment, prior to being packaged for use by the consumer or a retail establishment" (*39*). Fresh produce is an increasingly common vector of foodborne disease worldwide (*15*, *46*, *49*, *77*). In 2011 fruits and nuts (24% of outbreaks), and vine-stalk vegetables (23% of outbreaks), were the top commodities linked to foodborne disease outbreaks (*49*, *53*). Reported outbreaks associated with fresh produce have increased from 1% to 12% from 1970 to 1990's (*77*). This may be due in part to improvements in pathogen detection, and the use of CDC's PulseNet.

Risk of food pathogen consumption is higher in fresh cut produce, than when compared to traditional snacks, because the product is minimally processed and contains no inactivation steps. Instead, less effective or more expensive control strategies, such as the use of sanitizing chemicals, irradiation, or biocontrol agents,

are used to control contamination, but these methods may not be used because they are unpopular or unwanted by the consumers who buy fresh cut product (*61*). Produce can be exposed to pathogens from the soil they grow in or via contaminated wash water and/or irrigation water (*99*).

Hands have been cited as major vectors of pathogens in food preparation (*16*, *40*, *46*). Handwashing rates vary depending on workload, education about handwashing, and people affected by frequent or infrequent washings (*9*, *16*, *37*, *38*, *40*, *46*, *65*, *70*, *86*). Reij et al. (2004) highlighted that numerous sources of contamination of hands and produce during home preparation, and suggested that more research on cross contamination in the kitchen is needed (*70*).

Two separate studies, Chen et al. (2001) and Gorman et al. (2002), demonstrated that many surfaces in a kitchen quickly become contaminated after preparation of just a single meal (*16*, *34*). Chen et al. (2001) also demonstrated that a single hand wash, after preparing a risky food product, such as raw chicken, might not be enough to eliminate the risk of spreading pathogens to other parts of a meal (*16*). Bacterial levels on human hands, on kitchen surfaces, and on food have been documented, however the rates at which the bacteria readily move between these surfaces is minimal (*16*, *21*, *34*). Many cases of foodborne illness can be traced back to improper food handling practices in the consumer's home, and therefore, having a deeper understanding the risk that fresh cut produce poses to a consumer's kitchen will help create policies and information for consumers that will reduce these instances of improper handling (*6*, *69*).

This study aims to find a quantifiable relationship between hands and fresh cut produce. Two scenarios are tested, one in which the hands are the source of contamination, and one in which the produce is the source of contamination. Determining a quantifiable relationship between handling practices and contamination of fresh cut produce can be difficult because a large number of contamination scenarios exist (*64*). In this study, we aim to quantify the transfer rates during the handling of produce. Quantification of cross contamination rates provides a more thorough and accurate risk analysis in both home and food service kitchens (*16*). Data of transfer rates are useful for risk analysis, in ways such as being used in formulas to assess the risk of cross contamination (*93*).

II.3 Materials and Methods

II.3.a Bacterial strain and growth conditions.

The methods used were based in part on previous studies in our lab (*16*, *54*, *75*). Nalidixic-acid resistant *Enterobacter aerogenes* was used as a surrogate for a cocktail of five rifampicin resistant *Salmonella* strains (*16*, *54*, *75*, *103*). The *Salmonella* strains (Tbl. II.3.1) used were from produce or produce-related commodities outbreaks. Their sources and designations included the following: Enteritidis (ATCC BAA-1045, raw almonds), Agona (LJH 517, Alfalfa sprouts), Gaminara (F2712, Orange juice), Michigan (LJH 521, Clinical isolate cantaloupe outbreak), and Montevideo (G4639, Clinical isolate tomato outbreak). All *Salmonella* strains were adapted to grow in the presence of 80 mg/g rifampin (Thermo Fisher Scientific, Waltham, MA), through stepwise exposure (Parnell, 2005).

Table II.3.a.1

***Salmonella* Serotype**	**Origin (Source)**	**Designation**
Enteritidis	2000-2001 Almond Outbreak	ATCC BAA-1045
Agona	Alfalfa sprouts	LJH 517
Gaminara	Orange Juice 1995	F2712
Michigan	Cantaloupe	LJH 521
Montevideo	Human-Tomato Outbreak	G4639

A five strain cocktail of *E. coli* O157:H7 was used (Tbl. II.3.2). Their designation and sources included: Odwalla outbreak (223), human isolate from a cantaloupe outbreak (F658), human isolate from a lettuce outbreak (H1730), human isolate from an Alfalfa Sprouts outbreak (F4546), and human isolate from a spinach outbreak (EC4042)**.** All *E. coli* O157:H7 strains were adapted to grow in the presence of 80 mg/g rifampin (Thermo FisherScientific, Waltham, MA), through stepwise exposure (*62*).

Table II.3.a.2

***Escherichia coli* Serotype**	**Origin (Source)**	**Designation**
O157:H7	Lettuce (human-feces)	H1730
O157:H7	Alfalfa sprout (human-feces)	F4546
O157:H7	1996 Odwalla (unpasteurized apple juice)	Odwalla strain # 223
O157:H7	Cantaloupe	CDC 658
O157:H7	Spinach	EC4042

II.3.b Produce.

Carrots and celery were purchased pre-cut from a local supermarket. Cantaloupe was bought whole from the same supermarket, and then washed and cut shortly

before use. The cantaloupe was not bought in a precut form, due to many of the fresh cut cantaloupe pieces being of poor quality or non-uniform in shape. All produce samples were allowed to reach room temperature before inoculation. Sampling ensured that rifampicin resistant *Salmonella,* rifampicin resistant *E. coli* O157:H7, and nalidixic acid resistant *E. aerogenes* were not present on surfaces, hands, or produce prior to the studies.

II.3.c Preparation inoculation solution from bacterial cultures.

The bacteria were grown overnight at 37°C in tryptic soy broth containing 50mg/g nalidixic acid for *E. aerogene*s or 2mL/mL rifampicin stock for *Salmonella* and *E. coli*. Rifampicin stock was made by adding 1 g rifampicin to 20mL methanol. The rifampicin-methanol solution is then pushed through a sterile, 0.2 μm filter (MILLEX®HA Filter Unit, Millipore, Billerica, MA). Cells were harvested by centrifugation (Micro 12, Fisher Scientific) at 1.8 x *g* for 5min, and then washed three times in phosphate buffer saline (PBS; 0.1M, pH 7.2). The cell pellets were re-suspended in PBS after the final wash to form a solution ~8 log CFU/mL. 1mL of the solution was serially diluted twice in 9mL PBS to create a 6 log CFU/mL solution. This solution was considered the hand inoculation solution. The inoculation solution for the produce was prepared by diluting the harvested cell suspension once with 9mL PBS, then taking 5mL of the diluted suspension and adding it to 500mL PBS, yielding a solution of ~10^7CFU/mL. Experiments done in the lab determined that a produce sample would have a total concentration of bacteria 1 log less than the dip inoculation solution bacterial count per mL (data not shown).

After the transfer scenarios were completed, the samples collected were serially diluted with PBS, and plated onto appropriate agar. For the scenarios involving *E. aerogenes*, BBL™ MacConkey agar with 50mg/g nalidixic acid added was used to enumerate (MC-na). For the scenarios involving the *E. coli* O157:H7 cocktail, BBL™ MacConkey agar with 2mL/mL rifampicin stock was used to enumerate (MC-R). For scenarios involving *Salmonella* spp., Difco™ Bismuth Sulfite Agar and Difco™ Tryptic Soy Agar, both with 2mL/mL rifampicin stock, were used to enumerate (BSA-R & TSA-R).

II.3.d Participants.

Volunteers were asked to participate in the handling of the cut produce. The participant was rejected if any open cuts or wounds were present on their hand, if the participant was ill or self-identified as immunocompromised, or if they were uncomfortable with any aspect of the study. Before and after each scenario, and before leaving the lab, the participants were instructed to wash their hands and use hand sanitizer. The participants were given very basic instructions on how to pick up the produce. The participants were told to pick up 25g produce sample at a time, in a way that felt natural to them. Then, they were told to put the produce into a sampling bag, if the handled produce sample needed to be quantified, or waste bag, the produce sample was no longer needed.

II.3.e Preparing the produce.

A knife and cutting board were sterilized using 70% ethanol. Produce were cut into 25g groups. The groups consisted of the following: one 25g piece for cantaloupe;

two 12.5g pieces for celery; and three 8.3g pieces for carrots**.** The carrots and celery were trimmed to obtain pieces of a similar size. The cantaloupe rind was washed with tap water, dried with paper towels, and cut into 25g pieces.

II.3.f Transfer of bacteria to produce coming into contact with inoculated gloved hand (gloved hands as the source of contamination).

An inoculation solution for the gloved hands was prepared by serial diluting the harvested bacteria suspension twice with 9mL PBS. The participant donned gloves (High Five long cuff, nitrile examination gloves, High Five Products INC., Chicago, IL), and 0.5mL inoculation solution was dispensed onto each gloved hand. Participant then rubbed their hands together to evenly coat the gloves. The gloves were then allowed to dry for roughly 1 min (until no visible moisture remained). This method reliably resulted in $\sim 10^6$CFU/hand.

The bacterial count on the participant's non-dominant hand was measured using the glove juice method (*16*, *63*, *75*). A short cuff, nitrile glove (Fisher brand, short cuffed, nitrile examinations gloves) was filled with 20mL PBS. The investigator put this glove on over the non-dominant gloved hand of the participant. The participant's non-dominant hand was massaged for 30s-1min to loosen bacteria. The resulting volume recovered was recorded. The recovered buffer was serially diluted, spread plated onto MC-na for *E. aerogenes* or MC-R for *E. coli* O157:H7, and incubated at 37°C for 24hrs. The resulting counts from the non-dominant hand were used as the number of bacteria on the contaminated source.

With the dominant hand, the participant picked up a 25g sample of produce, and placed the handled produce into a filter bag. Then, 50 mL of PBS was added to the

filter bag, and the bag was stomached for 2 minutes. The resulting suspension was serially diluted and plated on the appropriate agar. The counts recovered from the stomached suspension were used as the number of bacteria transferred to the previously uncontaminated produce.

II.3.g Transfer of bacteria from inoculated produce coming into contact with gloved hand (produce as the source of contamination).

The inoculation solution for the produce was prepared by diluting the harvested cell suspension once with 9mL PBS, then adding 5mL of the diluted suspension to 500mL PBS, yielding a solution of ~10^7CFU/mL. The cut produce and the inoculation solution were put into a sealable plastic bag. The bag was then gently shaken and turned by the investigator for 10 min to ensure that each piece of produce was evenly exposed to the solution. After 10 min the produce was removed from the solution and placed onto wire racks to dry for 20 min. Despite the 20 min drying period, the produce had the same moist appearance as when freshly cut. No wilting or change in color was observed during and after drying.

Next, a 25g sample of produce and 225mL PBS was placed into a filter bag, and stomached for 2 minutes. The resulting suspension was serially diluted and plated. This process was repeated four times with different sets of 25g pieces in order to obtain an average count on the contaminated produce. Because volunteers handled 125g of produce per study, the average counts were multiplied by five in order to obtain the number of bacteria the volunteer was exposed to from the contaminated produce.

The participant donned gloves (High Five long cuff, nitrile examination gloves) and picked up 125g of the produce, or five 25g groups, from the wire rack, and placed it into a bowl several feet away from the wire rack. The five 25g sets of produce was chosen for two reasons: first, it provided a consistently measurable result, and second, also approximated the average serving a person would normally eat or handle in a single sitting. The bacterial count on the participant's non-dominant hand was measured using the glove juice method (*16*, *63*, *75*). The non-dominant hand was measured in order to acquire counts on the dominant hand, without affecting the microbial levels. The volunteer was instructed to use the dominant hand to handle the produce. The recovered volume from the glove juice method was recorded. The resulting solutions were serially diluted and plated onto appropriate agar. The counts recovered were the counts on the previously uncontaminated source and the bowl of handled produce was discarded.

II.3.h Data Analysis.

The bacterial counts on both the contaminated surface and the previously uncontaminated surface were determined. The formula for transfer rate (%) is $\%TC = (P_G/P_C) \times 100$, which was used in Chen et al. (2001) and Montville and Schaffner (2003), was used to determine the transfer rate (%) (*16*, *54*).

Formula definitions:

TC = the transfer coefficient

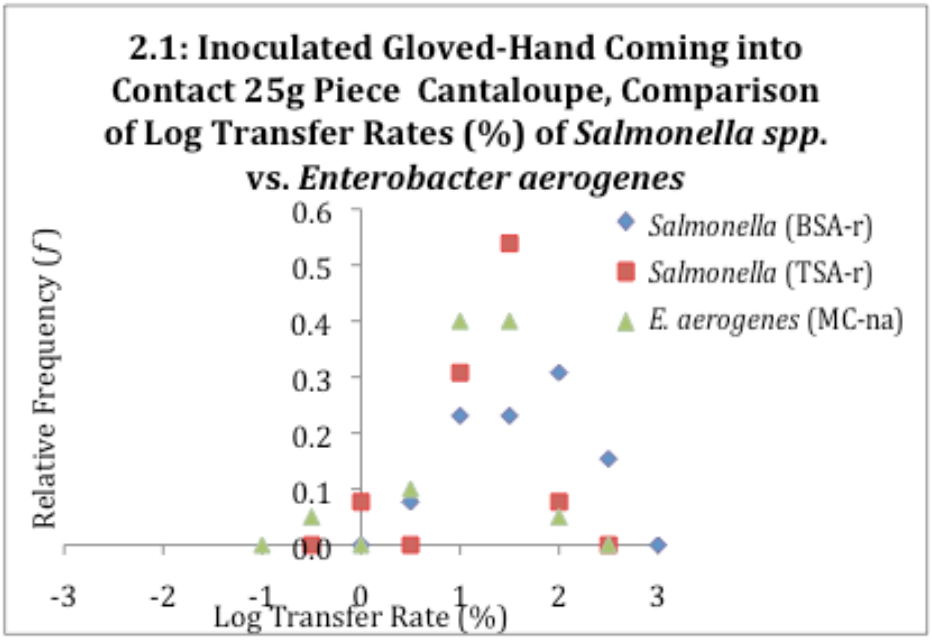
P_G = the count from the previously uncontaminated source

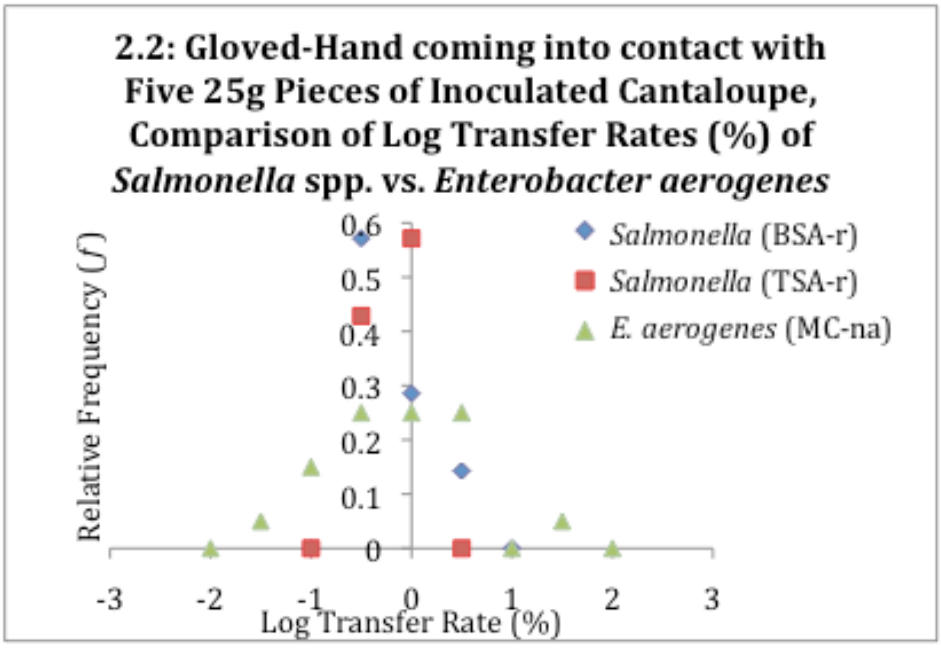
P_C = the counts from the inoculated source (or source of contamination).

Each scenario was grouped by the bacteria used, (cocktail of rifampicin resistant-*E. coli* O157:H7 or nalidixic acid-resistant *E. aerogenes*), the direction of bacterial transfer (hands to produce or produce to hands), and produce used (celery, cantaloupe, or carrot). This resulted in 12 individual cross contamination scenarios.

Data were compiled, log transformed, and used to create histograms, using Microsoft Excel (Microsoft, Redmond, Washington), and the data were then assembled into graphs using sigma plot (Systat Software Inc., Chicago, IL). The number of times a particular transfer rate occurred within a target data set (i.e. it's frequency) was plotted on the y-axis to visualize variability in log percent transfer rates during the different transfer events. The x-axis in these histograms is log percent transfer, as previous research in our lab has indicated that this transformation generally produces normally distributed data (*74*). The x-axis bin width used to create these histograms was either 0.25 or 0.5 log percent transfer as past experience in our lab indicates that this is generally satisfactory. Optimal bin size is determined by multiple factors, but generally the fewer observations available, the larger the bin needs to be to visualize meaningful trends. The bin size used in this chapter is 0.50 log percent.

II.4 Results.





Figures 2.1 and 2.2 compare *E. aerogenes* and the cocktail of five serotypes of *Salmonella* being studied, in terms of attachment and detachment, in order to tell if *E. aerogenes* is an acceptable surrogate. Previous studies demonstrated that *E. aerogenes* would be a safe alternative to pathogenic strains of *Salmonella (16, 54,*

75). This part of the study involved performing two separate cross contamination studies, one using the rifampicin resistant *Salmonella* cocktail, and the other using *Enterobacter aerogenes.* In both scenarios, cantaloupe was the model produce, and both produce to hands, and hands to produce directionalities were explored. The data were compared in order to determine if *E. aerogenes* was a suitable surrogate for *Salmonella* in this study. Once *E. aerogenes* was determined to be an appropriate surrogate, the scenarios involving celery and carrots were performed only using E. *aerogenes*.

Figure 2.1 demonstrates *E. aerogenes* has about a 10% transfer from the inoculated hands to 25g cantaloupe scenario, while the *Salmonella* cocktail has a 30% transfer. Figure 2.2 shows that both *E. aerogenes* and a cocktail of *Salmonella* commonly have about a 1% transfer between five 25g pieces of inoculated cantaloupe (125g) to gloved hands. The histograms, and further statistical analysis of the data, suggest that *E. aerogenes* is an acceptable surrogate for the *Salmonella* cocktail when used in this particular experiment and set of conditions ($p>0.05$ for both scenarios, data not shown).

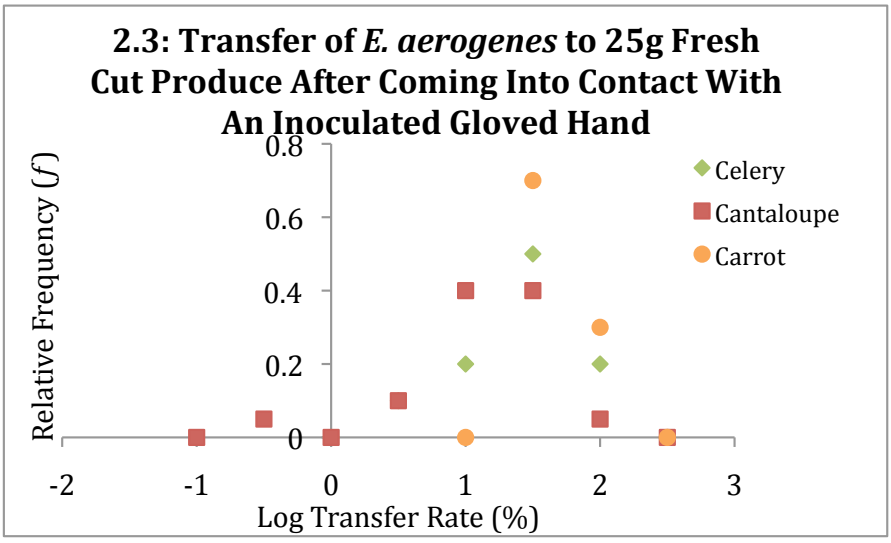


Figure 2.3 compares scenarios in which gloved hands were inoculated with E. *aerogenes*, and then handled 25g of produce (carrots, celery, cantaloupe). The figure indicates that both carrots and celery have a slightly higher %transfer (~30%) than cantaloupe (~18%).

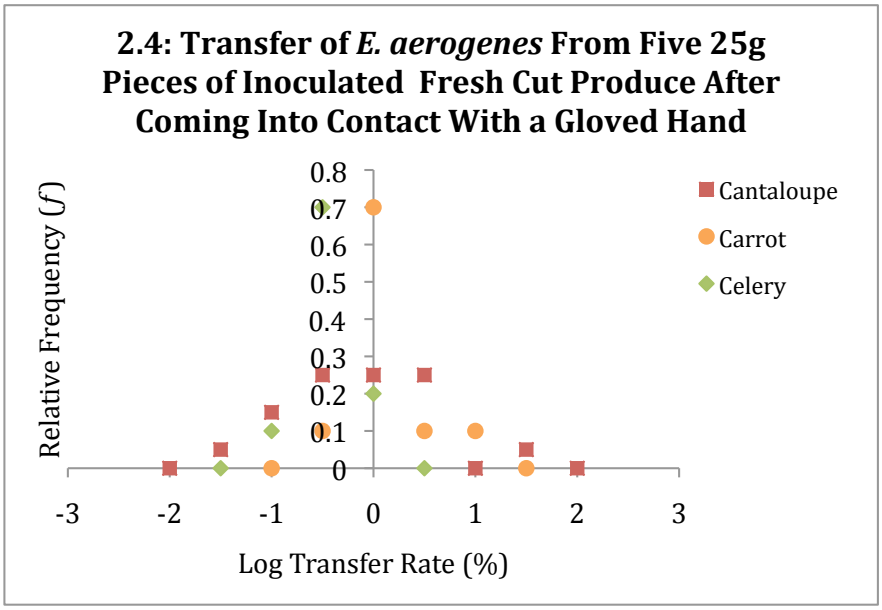


Figure 2.4 compares scenarios in which 125g of produce, inoculated with *E. aerogenes,* were handled by a gloved hand. The data suggest that 125g of inoculated

carrots and 125g of inoculated cantaloupe will transfer similar percentage of bacteria to the gloved hand, approximately 1%. The data also suggest that 125g of inoculated celery will transfer a lower percentage, approximately 0.3%.

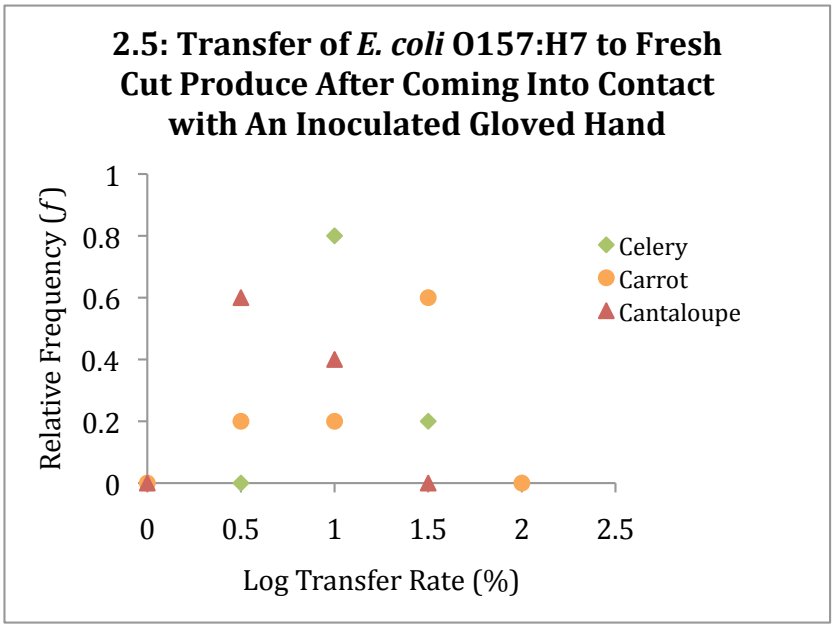


Figure 2.5 compares scenarios in which gloved hands were inoculated with a cocktail of *E. coli* O157:H7, and then the inoculated gloved hand touched 25g of produce (carrots, celery, cantaloupe). The figure indicates that gloved hands transferred the highest percentage of *E. coli* O157:H7 to 25g carrots, approximately 30%. Approximately 10% is transferred to 25g celery, and the lowest %transfer is to 25g cantaloupe, at 3%.

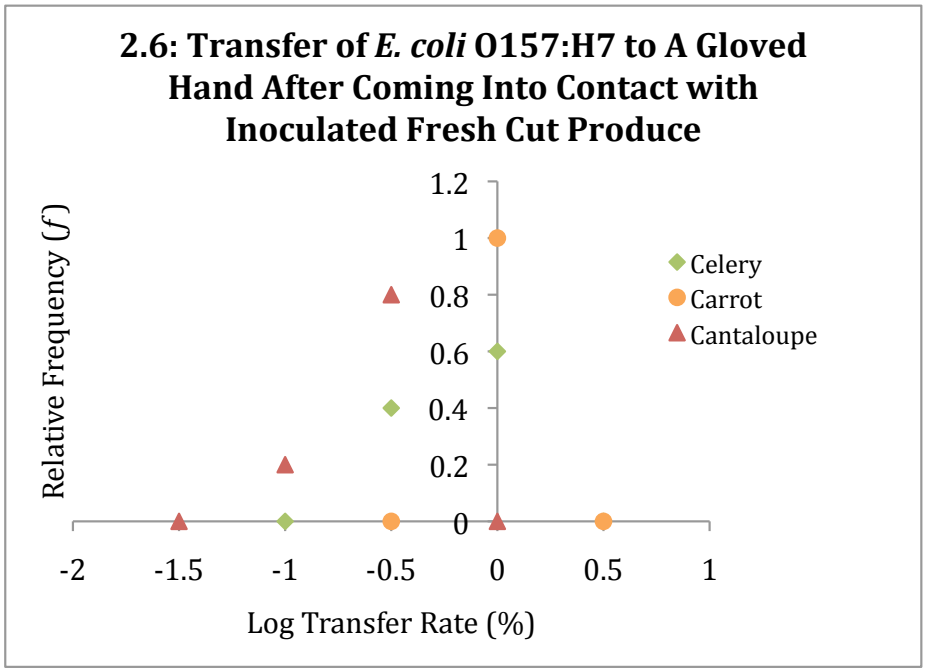


Figure 2.6 compares scenarios in which 125g of produce, inoculated with a cocktail of *E. coli* O157:H7, were handled by a gloved hand. The data suggest that 125g of inoculated carrots and 125g of inoculated celery will have similar transfer of bacteria from the produce to the gloved hand, at approximately 1%. The data also suggest that 125g of inoculated cantaloupe will transfer approximately 0.3%.

II.5 Discussion

Bacterial cell stressors, such as moisture, nutrient availability, and media selectivity, will cause depressed final counts on an agar medium. Bismuth sulfite agar (BSA) is a more selective medium than tryptic soy agar (TSA), and will reduce the growth of injured cells due to its selectivity. This selectivity added to the stress the cells experienced during the cross contamination scenarios. In regards the scenarios that involve the gloved hand being inoculated, the cells also experienced stress from

being on the nitrile gloves. The bacteria experienced low moisture, due to the gloves being allowed to dry before the handling. The bacteria will be surrounded by a lower nutrient concentration on the glove, than if the cells were on the cantaloupe. Taking these three factors into consideration, the BSAR bacterial counts may show increased transfer rates, when compared the TSAR bacterial counts. This is because the glove sample's counts will be depressed by low nutrient concentration, low moisture, and the stress of BSAR, while the cantaloupe samples will only experience stress from the BSAR. This will cause the load to be higher on the cantaloupe, and therefore show a higher transfer rate and possible transfer rates greater than 100%. This effect is not a significant when the direction of transfer is from inoculated melon to gloved hand. This is because the cantaloupes have a high surface moisture and nutrient concentration, and therefore the bacteria will not be stressed on the inoculated source. This may explain the differences between TSAR and BSAR counts in Fig 2.1 and Fig 2.2.

Salmonella enterica appears to use a molecular mechanism to attach to food, particularly on produce, which reduces the effectiveness of washing the produce (*2*, *3*). Cellulose polymers in the extracellular matrix, and the O-antigen facilitate aggressive attachment to plant surfaces. Thin aggregative fimbriae, called tafi, in *Salmonella enterica,* and curli in *Escherichia coli* also contribute to the bacteria's strong attachment to vegetative matter. Furthermore, Beuchat and Scouten (2002) determined that bacteria are difficult to remove once they have become attached to produce (*7*). Barak et al. (2005) put forth that these attachment mechanisms, which primarily occur within the first thirty minutes of contact, allow the pathogen to use

vegetative matter as a vector between hosts (*2*). This research sheds light on our findings for the influence-of-direction-of-transfer pattern seen for *E. aerogenes* and for the *E. coli* O157:H7 cocktail, which shows reduced transfer rates when produce was the source of contamination (~1%), rather than when hands or gloves are as the source of contamination (~18-30%). Suggesting that the bacteria display a preference to stay attached to produce and to readily detach from hands.

A cross contamination study done by Kusumaningrum et al. (2002) measured the transfer rates of *Staphphylococcus aureus* 196E (human isolate, enterotoxin A producer), *Salmonella enteritidis* (phage type 4, chicken product isolate), and *Campylobacter jejuni* (NCTC 81116) from sponges to stainless steel surfaces, and the subsequent transfer of these bacteria from the stainless steel surface to either cucumber slices or roasted chicken fillets (*45*). Their results indicate the transfer rates between stainless steel surfaces and food (cucumber slices, roasted chicken) were not influenced by the type of microorganism when pressure, simulating that of a hand pressing lightly, was applied. Furthermore, their studies demonstrated that, depending on the time of exposure and initial load, pathogens could survive on dry steel surfaces. The pressured applied in these studies ($20g/cm^2$) mimics the amount of pressure applied during food preparation in the kitchen. The results suggest that transfer rates from *Salmonella*-contaminated surfaces to cucumbers ranged from 50% to 100%. Cucumber slices have a high water activity, as well as a flat surface. The resulting transfer rates being higher than those of our study could be the result of several different variables. Both the contaminated stainless steel and the cucumber have uniform surfaces, as opposed to a human hand. This results in high

surface-to-surface contact area, which will result in a greater transfer. In our study, the participants' hands were uniformly inoculated, however the volunteers commonly picked up the produce with only the index finger, middle finger, and thumb. Additionally, the volunteers handled the produce for less than a few seconds. This resulted in a smaller surface-to-surface contact area and contact time than with cucumber to steel surface.

Montville and Schaffner (2003) demonstrated the different transfer rates due to varying inoculum size, and the surfaces involved (cutting boards, hands, raw chicken, lettuce, and spigots) (*54*). This research determined that as inoculum size increases, the percent transfer decreases, but the number of bacteria transferred remains constant regardless of inoculum size. Our study used only 6 log CFU/source level of contamination. However, we initially used 3 log CFU/source and then 4 log CFU/source, but, with both contamination levels, an accurate transfer rates were not calculable, as many of the results were below the detection limit (2 log CFU/source). Our results suggest that, during the transfer of bacteria from inoculated produce (6 log CFU/25g) to gloved hands, the highest percent transfer (for any type of produce and bacteria) was 1%. This 1% transfer from produce to hands correlates to 4 log CFU being transferred to the gloved hands. The percent transfer from inoculated hands (6 log CFU/hand) to produce was 30%, correlating to a 5.5 log CFU transfer to produce. As indicated in Montville and Schaffner (2003) if the amount of bacteria transferred (during the 3 log CFU/source contamination levels) is constant, the transfer rates from our study should be close to 100% (*54*). However, because much of the data from our study deals with transfer rates below

our detection limit, drawing a correlation is difficult. It should be noted that the contamination level is log CFU/source, and not per log CFU/gram. Thus, while the inoculated hands or produce may have a certain amount log CFU/source, this amount is spread evenly over the entire source. If only a small part of the inoculated source is exposed to the previously uncontaminated source, a smaller portion of the bacteria will be transferred. It is possible that 100% of the bacteria were transferred per contact area, but because the contact area was small (tips of fingers to produce), the amount is below our detection limit when compared to the total surface area of the produce or hand.

Fischler et al. (2007) performed a similar study that aimed to determine transfer rates between inoculated hands (post wash) and 5 g melon balls (cantaloupe)(*26*). The hands were inoculated with either *Escheria coli* or *Shigella flexneri*, and hands were washed with plain or antimicrobial soap. In our study hands, were evenly inoculated via spot inoculation, while in Fischler et al. (2007), the hands were inoculated via touching saturated paper towels. In Fischler et al. (2007), both inoculated hands were sampled to get a baseline, and then washed thoroughly, re-inoculated and then handled the cantaloupe without allowing the hands to dry. With the dominant hand, subjects in the Fischler study rolled melon ball for 15 seconds, with palm, and thumb and fingers. This was a much longer contact time than our study and used more of the hands than in our studies. For *E .coli* O157:H7 contaminated gloved hands, after a plain soap wash, transfer about 3 log CFU/cantaloupe, when baseline was 6 log CFU/ hand. A single wash with plain soap resulted in a 2 log CFU / hand reduction. Therefore, if 4 log CFU/ hand was present

on the hand after the wash, and 3 log CFU/melon ball was measured on the melon ball, the resulting transfer rate is a 10% transfer from hands to cantaloupe piece. Antimicrobial soap has smaller load levels (2.5 log post wash, and 2 log on the melon balls), will have roughly 30% transfer(*26*). Our study demonstrated that when hands are inoculated with *E. coli* O157:H7 (6 log CFU/hand), 3% of the bacteria would transfer from hands to 25g of cantaloupe without a prior wash (~18% transfer rate for *Enterobacter aerogenes*). In the Fischler et al. study, the cantaloupe pieces were handled for more time, and with more of the hand than as in our study. These two factors may explain the higher transfer rates in the Fischler et al. study. An additional factor that may explain the difference is inoculum size. In 2003, Montville and Schaffner determined that inoculum size determines transfer rates. A higher inoculum size, such as in our study (when compared to Fischler et al) will result in a smaller transfer rates (*54*).

The Chen et al. (2001) study looked at cross contamination rates during common food service tasks(*16*). Hands were not inoculated directly, and instead were contaminated via handling inoculated chicken (1mL of 10^8 CFU/mL of *Enterobacter aerogenes*). Volunteers cut chicken into small pieces and then went to wash their hands. After handling contaminated chicken, participants washed their hands for 20 seconds or until participants felt their hands were clean. Two scenarios were tested: participants either touched two contaminated spigots, or had the water turned on for them. Generally, little difference in the microbial reductions was seen between the different handwashing regimes. The cross contamination rates from chicken to hands was 10%, but the transfer rates during preparation

varied between 0.5% to ~100%. Bacteria were still present after washing, at percentages between 0.001% to 12% of the original bacteria. Transfer of bacteria to lettuce from post-wash hands was 0.0003% to 100%, but most frequently was 1%. Hands had a variable prcentage left on them after washing, between 2 log CFU/hand to 6 log CFU/hand. Pre-washed hands generally had a mean of 7.43 log CFU/hands, while post-wash hands had a mean of 4.68 log CFU/hands. The post-wash hands in this study had similar contamination levels as our study, 6 log CFU/hand. Our study had higher transfer rates from hand to produce than in their study regardless of the higher inoculum size, which may be in part due to the washing regime used in the Chen et al. study. The use of soap and paper towels may have removed a majority of the "loose" bacteria, and what remained was difficult to transfer. Another possibility is that, in this study lettuce was used, which may have different transfer rates than celery, cantaloupe, and carrots. This study also had a higher transfer rates from chicken to hands (10%) than any of the handling scenarios in which the produce was the source of contamination (our highest was 1%). This may be due to a different type of material, raw chicken versus raw vegetation, higher surface moisture content on chicken, and longer handling time as the participants chopped and manipulated the chicken(*16*).

Verhoeff-Bakkenes et al. (2008) performed a study in which volunteers prepared a chicken and fruit salad (*96*). The chicken was contaminated at 8-9 log CFU/fillet levels. The study involved several scenarios in which the hands, knife, and cutting board were not washed. The second scenario in which hands were not washed after cutting the chicken fillet, but the knife and cutting board were replaced, is of

particular interest to our study. This scenario involved a contaminated hand preparing a fresh produce salad. The percent transfer was measured by (CFU per prepared salad/ CFU per chicken fillet) x100. Verhoeff-Bakkenes et al. (2008) had a percent transfer ranging from17-38% (*96*). The hands' bacterial levels were never quantified, but the percent transfers show a high percent transfer to the produce, with most replicates above 10%. It should be noted that the bacterial cocktail was also transferred to the knife and cutting board, which could lead a lower overall percent transfer. Our results indicate that inoculated hands will transfer 3-30% with *E. coli* O157:H7 or 18-30% with *E. aerogenes* of the bacteria to the produce. Verhoeff-Bakkenes et al. (2008) demonstrated higher transfer rates, which may be due to the volunteer preparing the fruit salad, rather than just handling it as we did in our study. In other words, a longer hand to food contact time occurred in their study. Nevertheless, a high transfer rates to produce is observed in both their study and our study (*96*). Verhoeff-Bakkenes et al. (2008) also had a worst-case scenario, which involved unwashed hands and no replacement of cutting board or knife. This scenario had a percent transfer between 10- 44%. The scenarios in which hands were washed generally had low percent transfers. This leads the authors to believe that hands play a large role in cross contamination.

Except under extreme conditions, hands and produce will generally not have bacterial level used in this study. The number of microorganisms on non-damaged portions of skin in the same person can vary from 2 log- 4log/cm^2 , and rarely are the bacteria of the same strain or even species (*43, 51*, *52*, *57*). However, our study demonstrates that if a pathogen contamination scenario exists on either the food,

such as in temperature abuse conditions, or on hands, such as in poor hygiene conditions, there will be transfer. A 1% transfer of 10,000 (4 log CFU) *E. coli* O157:H7 or *Salmonella spp.* cells, which is lower than the inoculum levels in our scenario, can still transfer enough cells (100 cells or 2 log CFU) to cause illness. However, for appropriate risk analysis, a dose-response curve, which looks at the average risk of illness from an ingested dose, should be consulted to appropriately assess the actual risk. For example, Cassin et al. (1998) developed a dose-response model for the risk of illness from *E. coli* O157:H7 in ground beef (*14*). They determined that 100 *E. coli* O157:H7 cells would result in the average person risking a 10% chance of developing an illness. Due to high nutrient and water content of fresh produce, bacterial growth is entirely possible, and bacterial populations equal to or greater than the prewash conditions could occur under temperature abuse given enough time (*22*, *50*, *73*, *91*). Even if produce could be supplied to the consumer fully decontaminated and risk-free, improper handling and temperature abuse in the home kitchen could lead to re-contaminated food and, subsequently, illness While not every person may develop an infection, those few who do develop an infection risk severe health complications. Therefore, it is imperative to fully understand the risk associated with cross contamination of bacteria to or from fresh produce, in order to complete the bigger picture of microbial contamination and risk from produce outbreaks.

Chapter III- Quantifying Transfer Rates of *Salmonella* and *E. coli* O157:H7 Between Fresh Cut Produce and Common Kitchen Surfaces

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III.1 Abstract

Cross contamination between foods and surfaces in industrial food processing environments and the home-kitchen may play a significant role in foodborne disease outbreaks. Quantitative understanding of the factors that influence cross contamination rates is still in its infancy, but prove very useful in the development of quantitative microbial risk assessment models. This study quantifies the cross contamination rates between a variety of fresh cut produce and common kitchen surface types using scenarios that differ by cross contamination direction, surface type, produce type, and moisture levels. A five strain cocktail of *Salmonella spp.* was used as the model organism in transfer scenarios involving celery, carrot, and watermelon, and a five strain cocktail of *E. coli* O157:H7 was used as the model organism in transfer scenarios involving lettuce. The food contact surfaces used were coupons of ceramic, stainless steel, glass, and plastic. Produce or contact surfaces were placed in a sterile filter bags, and homogenized, or, in the case of surfaces, mixed. The resulting solutions were serially diluted in 0.1 % peptone and surface plated (0.1 ml) onto tryptic soy agar with 80 mg/g rifampin (TSAR) and bismuth sulfite agar (BSA) with 80 mg/g rifampin (BSAR) for *Salmonella*, or Sorbitol MacConkey agar (SMAC) with 80 mg/g rifampin (SMACR) for *E. coli* O157:H7. If the surface was moist, all inoculated surfaces transferred essentially all (~100%) of the bacteria to produce. If the surfaces were allowed dry for one hour, carrots and watermelon were still received almost 100% of the bacteria from the inoculated surface. Transfer from dry surfaces to celery or lettuce were much more variable (0.5 to 100%). Freshly inoculated celery or lettuce transfer more bacteria (~5%-50%) compared to freshly inoculated carrots or watermelon (~1-10%). After one hour of

drying, inoculated celery, carrot, and lettuce have relatively low transfer rates to surfaces (0.1-1%), while watermelon has a higher transfer rates (1-5%). Moisture and direction of transfer appear to have the strongest effect on microbial transfer rates.

III.2 Intro

The American food supply is considered safe, but outbreaks and illness occur regardless of the safety policies implemented. Fresh cut produce is an increasing popular snack and meal substitute for many Americans, but has been linked to numerous outbreaks (*77*). In America, consumption of fresh produce is increasing due to increased availability and changes in eating lifestyles, and importation of fresh produce has increased to meet off-season demand (*50*). These imports, while convenient, can come with significant public safety issues, and the outbreaks can quickly become multistate and possible multinational (*50*, *72*). Annually, an estimated 2 to 4 million cases of salmonellosis are occur in the United States, and because many milder cases are not diagnosed or reported, the actual number of reported infections is lower (*15*, *24*). An estimated 400 persons die each year with acute salmonellosis, and an estimated 95% of cases of *Salmonella* infection are foodborne in origin (*15*, *42*).

In the Rayner et al. (2004), samples of common kitchen objects used in preparing of food, produce, and cleaning fabrics were examined under a microscope (*68*). The authors illustrated that multiple samples of carrots show bacterial colonies, some in biofilm formation, and many individual cells, especially in bagged carrots. Used dishrags and used sponges, especially if damp, displayed many types of bacteria and advanced biofilm formation. According to Prigent-Combaret et al. (2000), "bacterial colonization of both biological and non-biological surfaces is regarded as a primary aspect of bacterial pathogenesis and ecology"(*66*). These factors (poor sanitation, high presence, at risk populations) leave immunocompromised individuals, a

significant part of the population, continuously at risk from both a personal and environmental perspective (*49*). It is imperative to have a strong understanding of the transfer rates between various kitchen surfaces, hands, and foods in order fully understand the risk at home and in the industry. Studies have been done to estimate transfer rates between kitchens utensils, food, cutting boards, and hands (*16*, *50*, *55*, *93*). This study aims to quantify the cross contamination rates between four types of fresh cut produce and four types of common kitchen surfaces, under moist and dry conditions. The produce in question is commonly eaten raw and also comes into contact with kitchen surfaces. The four surfaces used are all found in kitchens from an industrial to at-home scale.

Bacterial attachment is complicated and multifaceted process. Surface charge and Van der Waals forces plays a role in initial attachment of microbes. Plants and bacteria both have negative electrostatic surfaces charges that act as repulsitory forces between the two (*90*). The extracellular matrix of *Salmonella spp.* and *Escherichia coli* O157:H7 is composed of proteinaceous and starchy molecules polymers, mainly cellulose and fimbriae, which assist with initial and irreversible attachment (*4*, *82*). Most natural surfaces and bacteria are negatively charged, and thus adherence is strongly dependent on hydrocarbons on either surface, either abiotic or biotic, due to the repulsive electrostatic force (*94*).

Moisture can affect cross contamination rates between surfaces. For this reason, and that *Salmonella* and *E. coli* O157:H7 are capable of surviving under desiccation

conditions, this study will examine the difference between immediate (zero hour) and one hour of drying time of the produce and surfaces (*101*).

Microbial preference to attach to hydrophobic, non-polar surfaces has been linked to their preference for plastics over glass and metals (*18*, *28*, *67*). *E. coli* will preferentially attach first to cuts, bruises, and cracks in produce surface, and then begin to occupy less advantageous sites, however *Salmonella* does not show surface attachment preference (*88*). Preference for cut portions increases the risk of contamination of fresh cut produce. Takeuchi and Frank (2000) determined that higher inoculum levels have shown increased attachment, when compared to lower inoculum levels (*87*). An inoculum concentration of *E. coli* O157:H7 of 9 log CFU/mL had a 1 log CFU/cm^2 greater attachment than 8 log CFU/mL concentration, and 2 log CFU/cm^2 greater attachment than 7 log CFU/mL.

Gram-negative bacteria produce lipopolysaccharides that will also assist with attachment (*84*). The O-antigen also has hydrophilic properties that affect the attachment of gram-negative bacteria, and lack of an O-antigen can improve adherence to hydrophobic materials (*84*). Cell surface polymers with nonpolar sites, such as fimbriae, primarily initiate attachment to hydrophobic substrata, while exopolysaccharides and lipopolysaccharides initiate attachment to hydrophilic materials (*18*).

Initial attachment can also be linked to molecules on the surface of bacteria. Exopolysaccharides, which also make up a large part of biofilms, can adhere colonized bacteria to a surface and reduce the transfer to offensive surfaces (*11*).

Similarly, planktonic bacteria can produce exopolysaccharides to better adhere to a surface by overcoming the electrostatic gap (*11*).

III.3 Materials and Methods

III.3.a Preparation of Domestic Food Contact Surfaces

Four different food contact surfaces typical of materials found in domestic kitchens were tested; glass (3/32"thick); ceramic tile (glazed); and plastic (styrene),; and stainless steel (type 304, 18 gauge; onlinemetals.com, Seattle, WA) were purchased from a local home improvement store (Winter Haven, FL). Surface materials were cut into 5 x 5 cm squares for cross contamination studies. Before use, the surfaces were disinfected with mixture of 30% sodium hypochlorite (Clorox, Oakland, California) and 70% distilled sterilized water, overnight. The surfaces were then scrubbed in hot water using an anionic active detergent and rinsed with hot water. Prior to artificial contamination, the surfaces were soaked in 70% ethanol for one hour then removed and air-dried.

III.3.b Produce

Fresh cut produce, including mini, peeled carrots, celery, watermelon, and romaine lettuce, in re-sealable bags or store containers, were purchased from a local supermarket (Winter Haven, FL). Produce was stored at 4°C and brought to ambient temperature prior to starting the experiment. Mini, peeled carrots, celery, and cubed watermelon (flesh only) were cut into 10 g pieces. Lettuce was cut into approximately 3 x 3 cm pieces.

III.3.c Selection of strains

A cocktail of five strains of bacterial isolates from produce or produce-related commodities were used. For transfer studies with watermelon, carrots, and celery *Salmonella* serovars were used (Tbl. III.3.c.1). Their sources and designations are as follows: Enteritidis (ATCC BAA-1045, raw almonds), Agona (LJH 517, Alfalfa

sprouts), Gaminara (F2712, Orange juice), Michigan (LJH 521, Clinical isolate cantaloupe outbreak), and Montevideo (G4639, Clinical isolate tomato outbreak).

For the lettuce transfer studies, a cocktail of five strains of *E. coli* O157:H7 was used (Table III.3.c.2), their designation and sources included; Odwalla outbreak (223), human isolate from a cantaloupe outbreak (F658), human isolate from a lettuce outbreak (H1730), human isolate from an Alfalfa Sprouts outbreak (F4546), and human isolate from a spinach outbreak (EC4042). All strains were adapted to grow in the presence of 80 mg/g rifampin (Thermo FisherScientific, Waltham, MA), through stepwise exposure (*62*).

Table III.3.c.1

***Salmonella* Serotype**	**Origin (Source)**	**Designation**
Enteritidis	2000-2001 Almond Outbreak	ATCC BAA-1045
Agona	Alfalfa sprouts	LJH 517
Gaminara	Orange Juice 1995	F2712
Michigan	Cantaloupe	LJH 521
Montevideo	Human-Tomato Outbreak	G4639

Table III.3.c.2

***Escherichia coli* Serotype**	**Origin (Source)**	**Designation**
O157:H7	Lettuce (human-feces)	H1730
O157:H7	Alfalfa sprout (human-feces)	F4546
O157:H7	1996 Odwalla (unpasteurized apple juice)	Odwalla strain # 223
O157:H7	Cantaloupe	CDC 658
O157:H7	Spinach	EC4042

III.3.d Inoculum preparation

Prior to each experiment, frozen cultures of each strain were streaked onto tryptic soy agar (TSA; Difco, BD,Sparks, MD) with 80 mg/g rifampin (TSAR), and incubated at 37°C for 24 h . One isolated colony from each plate was transferred to 10 ml of tryptic soy broth (TSB; Difco, BD) with 80 mg/g rifampin (TSBR), and incubated at 37°C for 24 h. Cultures were subsequently subcultured twice by transferring 0.1 ml of culture to 10 ml of fresh TSBR and incubated at 37°C for 24 h. Each strain was subjected to centrifugation at 0.6 x *g* for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). Cells were then washed twice by removing the supernatant and suspending the cell pellet in 10 ml of 0.1% peptone (Difco, BD). Washed cells were suspended in 0.1% peptone at half the original culture volume. Strains were diluted and combined in equal volumes to a concentration of 10^8 CFU/ml. Final concentrations were verified for each strain by enumeration on TSAR.

III.3.e Transfer between fresh cut produce and surfaces

Sixteen different produce transfer scenarios were evaluated for each produce type, taking into account the contact surface type, direction of transfer, and inoculation conditions, for a total for 64 individual scenarios. For each produce type, a 10 µl volume of inoculum was deposited in 5-8 droplets on the surface to obtain a final population of ca. 6 log CFU per cm^2. The produce was either touched (approximately 1-2 seconds) immediately to one of the four food contact surfaces (wet) or allowed to dry for 1 hour in a biosafety cabinet with the fan on (to dry) and then touched (approximately 1-2 seconds) to the contact surface. The transfer direction was then reversed inoculating the food contact surface and subsequently touching the produce surface.

III.3.f Enumeration of cells

Produce or contact surfaces were placed in a sterile 207 ml whirl pak filter bag (Nasco, Fort Atkinson, WI, USA) and 40 ml of DE (Dey/Engley, Thermo Fisher Scientific, Waltham, MA) was added to the samples and macerated in a smasher (AES Laboratories, Chemunex, France) for 1 minute, or, in the case of surfaces, mixed for 1 minute. Samples were serially diluted in 0.1 % peptone and surface plated (0.1 ml) onto TSAR and bismuth sulfite agar (BSA; Difco, BD, Sparks, MD) with 80 mg/g rifampin (BSAR) for *Salmonella*, or Sorbitol MacConkey agar (SMAC; Difco, BD, Sparks, MD) with 80 mg/g rifampin (SMACR) for *E. coli* O157:H7. Plates were incubated at 37°C for 24 h (TSAR) or 48 h (BSA). Following incubation, colonies were counted and *E. coli* O157:H7 or *Salmonella* population levels were expressed in log CFU/g for produce and CFU/cm^2 for food contact surfaces.

III.3.g Data Analysis

Data were compiled, log transformed, and used to create histograms, using Microsoft Excel (Microsoft, Redmond, Washington), and the data were then assembled into graphs using sigma plot (Systat Software Inc., Chicago, IL). The number of times a particular transfer rate occurred within a target data set (i.e. it's frequency) was plotted on the y-axis to visualize variability in log percent transfer rates during the different transfer events. The x-axis in these histograms is log percent transfer, as previous research in our lab has indicated that this transformation generally produces normally distributed data (*74*). The x-axis bin width used to create these histograms was either 0.25 or 0.5 log percent transfer as past experience in our lab indicates that this is generally satisfactory. Optimal bin size is determined by multiple factors, but generally the fewer observations

available, the larger the bin needs to be to visualize meaningful trends. The bin size used in this chapter is 0.25 log percent.

The inoculated source is defined as the surface sample or produce sample, depending on direction of transfer, that is the initial source of bacteria in each transfer scenario.

The Total CFU on the inoculated source= [Total CFU $_{(CFU/10\ g\ produce)}$ + Total CFU $_{(CFU/coupon)}$]

The Transfer Rate (%) when the source of contamination is a surface= [Total CFU $_{(CFU/10\ g\ produce)}$ / Total CFU $_{(on\ the\ inoculated\ source)}$] x 100

The Transfer Rate (%) when the source of contamination is produce=[Total CFU $_{(CFU/coupon)}$ / Total CFU $_{(on\ the\ inoculated\ source)}$] x 100

III.4 Results

III.4.a Statistical Analysis of Transfer Rates (%)

As can be seen from Table III.4.a and Table III.4.b (located in the appendix of the chapter), there are 64 different scenarios, which were evaluated using 5 different parameters: mean ($\bar{x}$), median (M), standard deviation (σ), min, max, and range. The resulting tables encompass 320 different values. The data in Table III.4.a describes the data found in fig 3.1-3.4 in greater detail, and the data in Table III.4.b describes the data found in fig 3.5-3.8 in greater detail. While each different scenario could be discussed in turn, and since many scenario results were indistinguishable, not every observation will be specifically noted. What will be

discussed are those results that seem particularly striking or different from the typical results. The tables will be referenced as needed to augment the discussion of the figures although each observation in the tables may not be specifically noted.

III.4.b Produce coming into contact with inoculated kitchen surfaces.

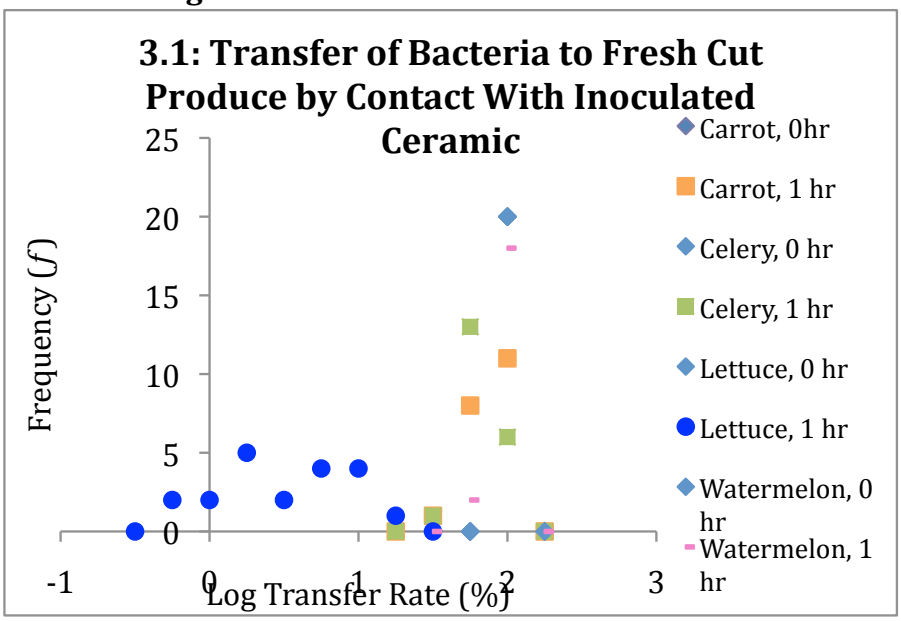

Figure 3.1 shows the transfer of *Salmonella* to carrots, celery, or watermelon, or *E. coli* O157:H7 to lettuce during contact with inoculated ceramic (both freshly inoculated and after drying for one hour). When freshly inoculated ceramic came into contact with produce there is a ~100% transfer of bacteria from inoculated ceramic to produce. After the ceramic had dried for one hour, transfer to watermelon samples displayed a ~100% transfer. After the ceramic had dried for one hour, transfer to carrot samples displayed transfer rates between 56-100% (or exactly $\bar{x}$=69.583%, Table III.4.a). After the ceramic had dried for one hour, transfer to celery samples displayed 56%-100% (or exactly $\bar{x}$ = 48.812% Table III.4.a). After

the ceramic had dried for one hour, transfer to lettuce displayed transfer rates between 0.3% -18% (or exactly $\bar{x}$ = 4.128%, Table III.4.a).

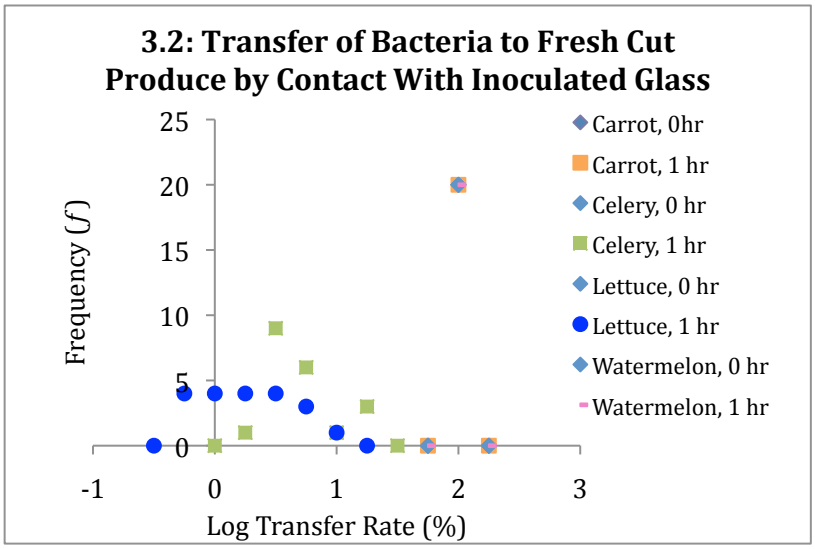

Figure 3.2 shows the transfer of *Salmonella* to carrots, celery, or watermelon, or *E. coli* O157:H7 to lettuce during contact with inoculated glass (both freshly inoculated and after drying for one hour). When freshly inoculated glass came into contact with produce there is a ~100% transfer of bacteria from inoculated glass to produce. After the glass had dried for one hour, transfer to carrot and watermelon samples displayed ~100% transfer rate. After the glass had dried for one hour, transfer to celery samples displayed a transfer between 3%-18% (or exactly $\bar{x}$=4.930%, Table III.4.a). After the glass had dried for one hour, transfer to lettuce samples displayed a transfer rate between 0.5%- 6% ($\bar{x}$=1.972%).

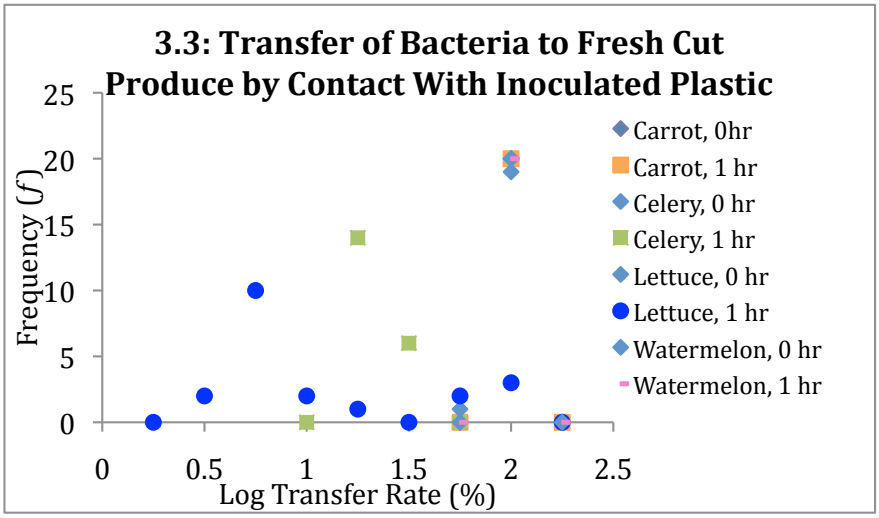

Figure 3.3 shows the transfer of *Salmonella* to carrots, celery, or watermelon, or *E. coli* O157:H7 to lettuce during contact with inoculated plastic (both freshly inoculated and after drying for one hour). When freshly inoculated plastic came into contact with produce, there is a ~100% transfer of bacteria from inoculated plastic to produce. After the plastic had dried for one hour, transfer to carrot and watermelon displayed ~100% transfer. After the plastic had dried for one hour, transfer to celery displayed a transfer rate between 18%- 32%(or exactly $\bar{x}$=17.435%, Table III.4.a). After the plastic had dried for one hour, transfer to lettuce displayed variability between 3% -100%, with the largest peek at 6%(or exactly $\bar{x}$=18.643%, Table III.4.a).

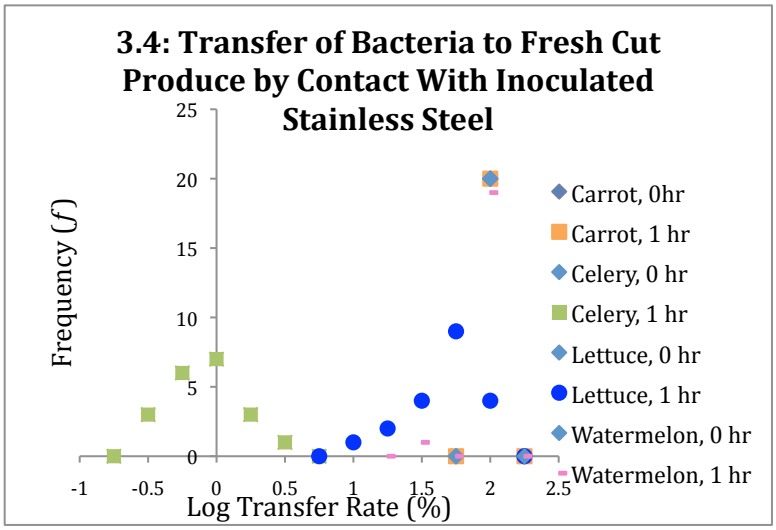

Figure 3.4 shows the transfer of *Salmonella* to carrots, celery, or watermelon, or *E. coli* O157:H7 to lettuce during contact with inoculated stainless steel (both freshly inoculated and after drying for one hour). When freshly inoculated stainless steel came into contact with produce, there is a ~100% transfer of bacteria from inoculated plastic to produce. After the stainless steel had dried for one hour, transfer to watermelon and carrot displayed a transfer rate a ~100% transfer rate. After the stainless steel had dried for one hour, transfer to celery between 0.3%-2%, but with a peek at 1% (or exactly $\bar{x}$= 0.742%, Table III.4.a). After the stainless steel had dried for one hour, transfer to lettuce displayed a transfer rate between 32%-100%, with a peek at 56% (or exactly $\bar{x}$=39.461%, Table III.4.a).

III.4.c Inoculated produce coming into contact with kitchen surfaces.

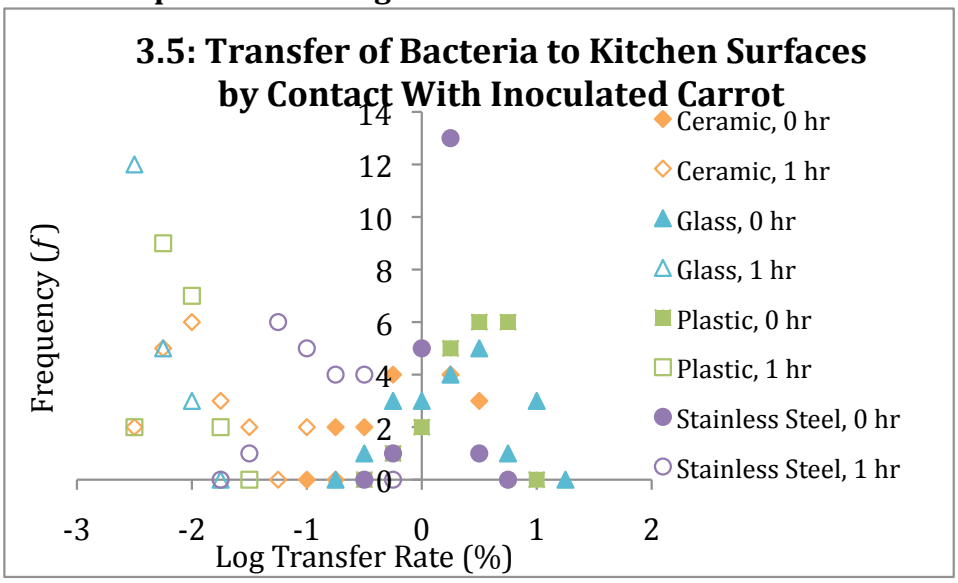

Figure 3.5 shows the transfer of *Salmonella* from inoculated carrots (both freshly inoculated and after drying for one hour) to four different contact surfaces. When freshly inoculated carrots contact a ceramic surface, the transfer rates are highly variable, with a transfer rate range of 0.3% to 3%, with its peak at ~1%, or more exactly as shown in Table III.4.b, where $\bar{x}$ = 0.898%. When carrots were freshly inoculated, the transfer rate to glass ranged from 0.5% to 10%, with it highest peak around ~3% (or exactly $\bar{x}$ = 2.322%, Table III.4.b). When carrots were freshly inoculated, transfer rates to plastic had a range between 2% to 6% (or exactly $\bar{x}$=2.469%). When carrots were freshly inoculated, transfer to stainless steel had a transfer rate of ~2% (or exactly $\bar{x}$= 1.238%, Table III.4.b). After the carrot had dried for one hour, transfer to ceramic was between 0.006% to 0.03% (or exactly $\bar{x}$=0.015%, Table III.4.b). After the carrot had dried for one hour, transfer to glass had transfer rates below the detection limit (12 out of 20 samples). The remaining transfer to glass samples vary from 0.006% to 0.01% (or exactly $\bar{x}$=0.004%, Table

III.4.b). After the carrot had dried for one hour, transfer to plastic had a small transfer rate of ~0.006% (or exactly $\bar{x}$=0.006%, Table III.4.b). After the carrot had dried for one hour, transfer to stainless steel had a transfer rate from 0.06% to 0.3% (or exactly $\bar{x}$=0.099%, Table III.4.b).

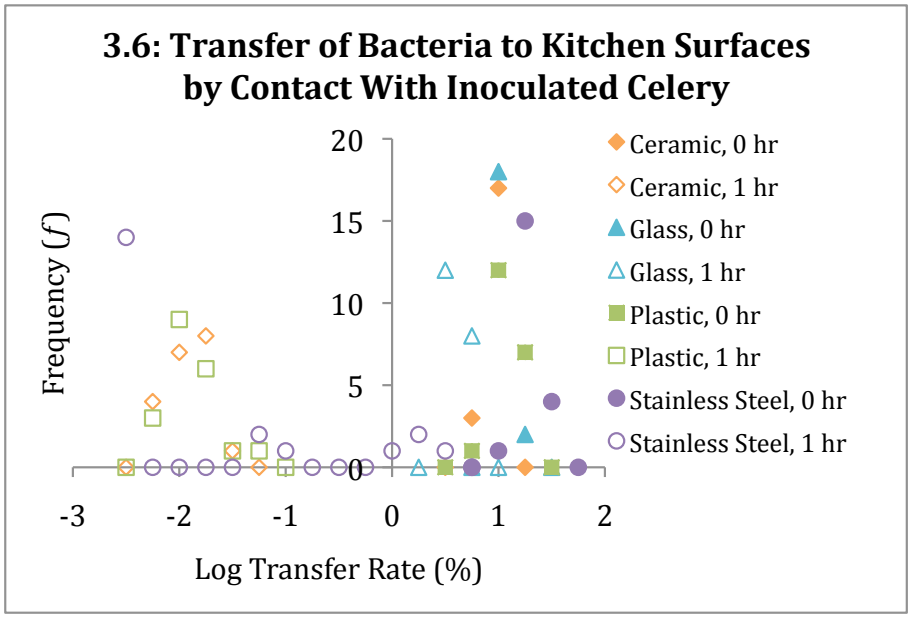

Figure 3.6 shows the transfer of *Salmonella spp.* from inoculated celery (both freshly inoculated and after drying for one hour) to four different contact surfaces. When celery were freshly inoculated, the transfer rate of *Salmonella* from celery to ceramic, to glass, and to plastic was 10%. When celery were freshly inoculated, the transfer rate of *Salmonella* from celery to stainless steel had higher transfer rate of 18% with slight variability (or exactly $\bar{x}$= 15.465%, σ= 4.110, Table III.4.b). After the celery had dried for one hour, the transfer to ceramic was ~0.02%. After the celery had dried for one hour, transfer to glass had a transfer rate of ~3%. After the celery had dried for one hour, transfer to plastic during contact was ~0.01%. After

the celery had dried for one hour, transfer to 14 out of 20 samples stainless steel samples were below the detection limit, and the remaining samples vary from 0.06%- 3% (or exactly $\bar{x}$=0.285% Table III.4.b).

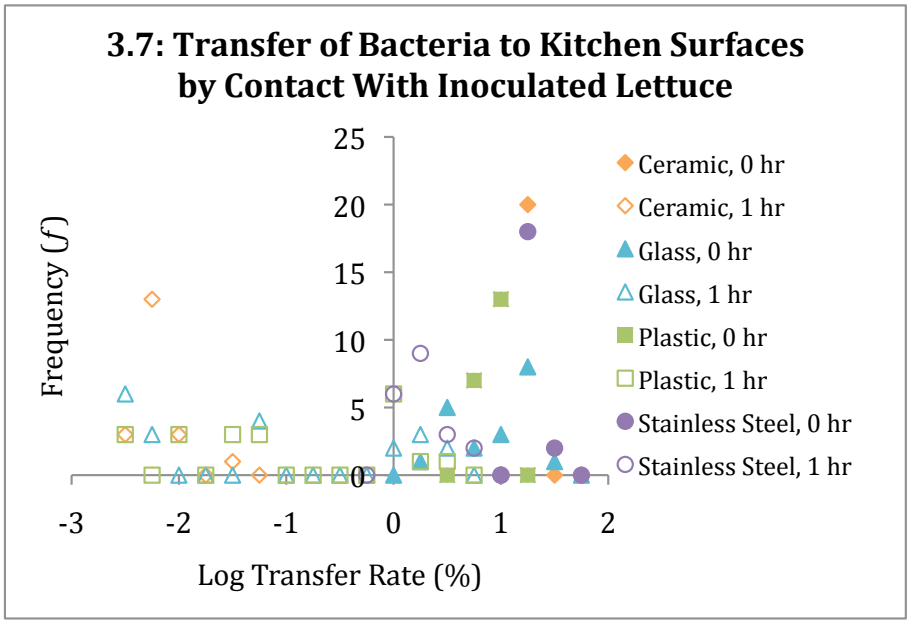

Figure 3.7 shows the transfer of *E. coli* O157:H7 from inoculated lettuce (both freshly inoculated and after drying for one hour) to four different contact surfaces. When lettuce were freshly inoculated, the transfer rate of *E. coli* O157:H7 to ceramic and stainless steel both had transfer rates of 18%, but ceramic displayed no variability. When lettuce were freshly inoculated, the transfer rate of *E. coli* O157:H7 to glass had a transfer rate range of 3%- 56%, with the highest peak at ~56% (or exactly $\bar{x}$=8.856%, Table III.4.b). When lettuce were freshly inoculated, the transfer rate of *E. coli* O157:H7 to plastic had a transfer rate of ~10%. After the lettuce had dried for one hour, transfer from inoculated lettuce to ceramic had low and variable transfer rates between 0.003%-0.01%, with 3 samples out of 20 below detection

limit (or exactly $\bar{x}$=0.006%, Table III.4.b). After the lettuce had dried for one hour, transfer from inoculated lettuce to stainless steel had a transfer rate of ~2%. After the lettuce had dried for one hour, transfer to glass had a transfer rate between 0.03%- 3% (or exactly $\bar{x}$=0.468%, Table III.4.b), and 6 out of 20 samples were below the detection limit. After the lettuce had dried for one hour, transfer from inoculated lettuce to plastic had transfer rates from 0.01%- 3% (or exactly $\bar{x}$=0.410%, Table III.4.b), with 3 out of 20 samples below the detection limit.

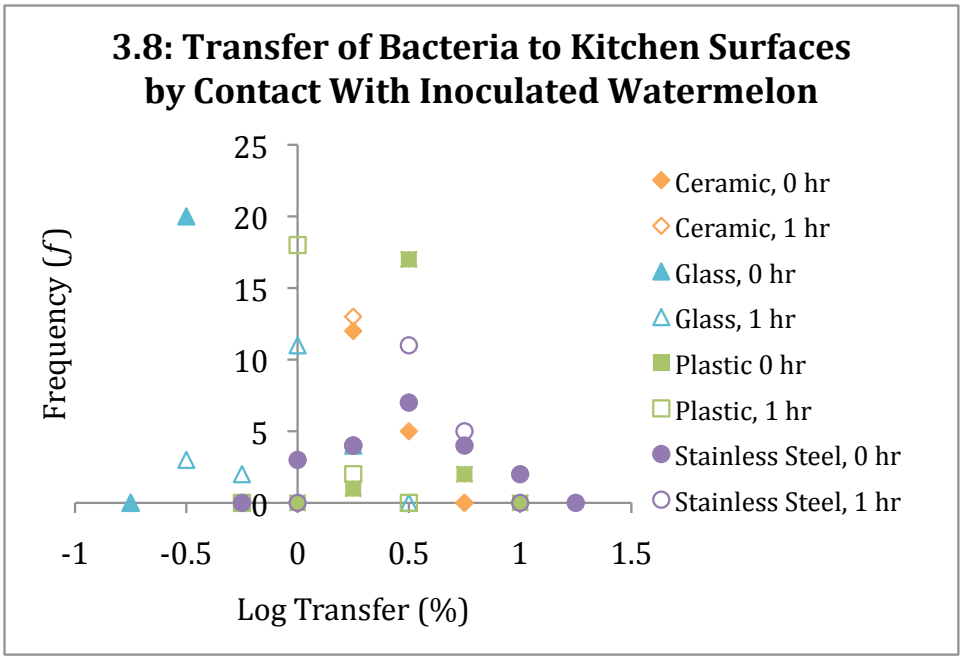

Figure 3.8 shows the transfer of *Salmonella spp.* from inoculated watermelon (both freshly inoculated and after drying for one hour) to four different contact surfaces. When freshly inoculated watermelon contact a ceramic surface, the transfer rates to ceramic was ~2%. When freshly inoculated watermelon contact a ceramic surface, the transfer rates to glass had a transfer rate of ~0.3%. When freshly inoculated watermelon contact a ceramic surface, the transfer rates to plastic had a transfer

rate of ~3%. When freshly inoculated watermelon contact a ceramic surface, the transfer rates to stainless steel range between 2%-10%, with a peak at ~3% (or exactly $\overline{x}$= 2.472). After the watermelon had dried for one hour, transfer from inoculated watermelon to ceramic had a transfer rate of ~2%. After the watermelon had dried for one hour, transfer from inoculated watermelon to glass had a transfer rate of ~1%. After the watermelon had dried for one hour, transfer from inoculated watermelon to plastic had a transfer rate of ~1%. After the watermelon had dried for one hour, transfer from inoculated watermelon to stainless steel had a transfer rate of ~3%.

III.5 Discussion

The first observation that stood out to the authors was the *Salmonella* and *E.coli* O157:H7 cocktails displayed greater transfer rates to produce than to surfaces. With or without drying, and regardless of produce or surface type, the transfer rates are greatest in the direction of inoculated surfaces to produce. The smallest of the transfer rates is displayed when the direction of transfer is from inoculated produce to surface, after an hour drying. The bacteria may have formed stronger attachment to the produce after an hour drying time due to available nutrients and starchy molecules on the produce, which significantly influence the attachment (*4*, *82*, *83*, *101*). However, a one-hour dryings time would not provide the *Salmonella* strains with enough time to form strong, irreversible adhesions to either surface. Therefore, the inoculated dried surfaces still transfer bacteria, albeit at a lesser rate, to produce. Similarly, the higher detachment from abiotic surfaces, regardless of drying time, could be due to these abiotic surfaces containing little, if any nutrients,

which can induce detachment (*11*). All produce samples had a ~100% transfer from inoculated surfaces that have not been dried, indicating that the strongest factor that affects the transfer rate after direction of transfer, is moisture. Moisture can play a large role in transfer. Solid-liquid interface between a surface and an aqueous medium provides the best surroundings for the attachment and growth of microorganisms (*18*).

Watermelon, after the surfaces has been dried for one hour, has the highest transfer rates (~100%) after touching inoculated stainless steel (Fig. 3.8). Without the surfaces being allowed to dry, watermelon also has a 100% transfer rate, but so do the three other produce samples. Watermelon has an extremely moist surface, which can facilitate transfer even on dried surfaces. Furthermore, the watermelon flat surfaces may provide more surface area for attachment as opposed to a lettuce leaf, carrot, or celery. Finally, the exposed watermelon surface (not the rind) can be considered an exposed surface, which bacteria will readily attach to (*87*).

Watermelon displayed a different pattern compared to other produce when the produce is the source of contamination. The transfer rates of watermelon from inoculated produce to surface remain about the same (3%) regardless of drying time. Inoculated carrot, celery, and lettuce show significantly reduced transfer rates before and after drying (reduced). Moisture may play a role in explaining this behavior. The watermelon's surface is still moist after an hour of drying, while carrots, celery, and lettuce have a dry surface. The only exception to this is when glass is involved, as watermelon transferred the least percentage to glass of all the

produce. However, it is important to note that a dry surface will not protect against cross contamination to or from watermelon, and possibly any moist fresh produce. Donlan (2002) suggested that when surfaces touch an aqueous medium, the surface will become coated with polymers, and that the change would affect both the rate and extent of microbial attachment (*18*).

Regardless of drying time, carrots also have high transfer rates from inoculated surfaces (Fig. 3.1, 3.2, 3.3, 3.4). The carrot samples had the highest surface-to-surface contact area after watermelon samples. However, independent of dry time, when the carrot is inoculated a (Fig. 3.5), the transfer rates are variable. The high rates at which the bacteria transfer to carrots from an inoculated source may be due to the carrots being peeled, which go through processing that exposes the inner surface. This exposes the inner surfaces of the carrot to the bacteria, rather than the out coating. The outer coating is generally discarded before consumption. As mentioned before, these inner surfaces are higher in nutrients and moisture, which can increase bacterial transfer rates.

Without drying, inoculated celery transfer the highest percentage of bacteria to a surface (Fig 3.6). After drying, inoculated celery has variable transfer rates. Celery has the lowest transfer rates from an inoculated surface that has been dried for one hour (Fig 3.1, 3.2, 3.3, 3.4). This may be due to the nature in which we prepare celery for eating. The outer protective layer is left relatively intact, with few bruises, peeling, or cuts when compared to watermelon or carrots. The surface of the celery with comes into contact with the kitchen surface is not the wounded or cut areas,

but the ridged outer surface. As stated before, cuts bruises, and cracks promote bacteria attachment, and an increase in nutritional concentration in a fluid can increase bacterial attachment (*17*, *18*).

Looking at figures 3.5, 3.6, 3.7, and 3.8, and specifically looking at the freshly inoculated produce transfer scenarios within these figures, a trend appears. Within each figure, when freshly inoculated produce comes into contact with a surface, generally the transfer rates were similar for ceramic, glass, plastic, or stainless steel. Freshly inoculated watermelon and freshly inoculated carrot displayed similar transfer rates to kitchen surfaces (~3%), while fresh inoculated celery and fresh inoculated lettuce displayed similar transfer rates to kitchen surfaces (~18%). This suggests that after direction of transfer and moisture, produce type is the next major factor, but not surface type.

Looking at figures 3.1, 3.2, 3.3, and 3.4, and specifically looking at the scenarios in which the inoculated surface were dried for one hour, another trend appears that indicates the role of produce type. Transfer from inoculated surfaces were allowed to dry for one hour, watermelon and carrots routinely had ~100% of the bacteria transferred to them, while celery and lettuce had low transfer rates. It is possible that the high transfer rates to watermelon and carrots were due to the moist surfaces of the produce. Another possibility is the way the watermelon and carrots were processed compared to celery and lettuce. Fresh cut watermelon and carrots have completely exposed inner surfaces, while lettuce and celery are only chopped, but most of the outer surface is left intact. Bacteria will preferentially attach to cuts

and bruises of produce, and therefore the inner surfaces could be considered a large "cut" that bacteria will attach too (*88*).

Previous studies indicate types of kitchen surface can affect microbial attachment and colonization (*16*, *18*, *28*, *50*, *55*, *93-95*). In our study, each kitchen surface coupon was pressed lightly for 1-2s against the produce. Increased pressure, contact time, type of contact (sliding vs. pressing) may all have an affect on transfer rates. Surface roughness, which can decrease shear forces from a liquid wash, can also protect against detachment (*18*). Additionally, organisms in *Enterobacteriaceae*, such as *Salmonella,* produce curli, (thin fimbriae like structures) which can enhance attachment to polystyrene, but not to stainless steel (*4*, *71*). Our study revealed that attachment is more dependent on produce type, moisture content, and drying time, but not kitchen surface type.

The major impact for food safety that this study reveals is that consumers should be sure to thoroughly sanitize and dry a surface before and after use. Furthermore, a consumer should use separate cutting boards, bowls, and utensils for raw meat and produce. The results of this study are that of a simple transfer, which includes limited contact time, and does not represent the complex transfer between a kitchen surface and a large amount of fresh produce that is normally performed in a kitchen. If either biotic or abiotic surface is contaminated, transfer of bacteria will occur. Whether the bacteria cause an infection, is dependent upon multiple factors, but eliminating a microbial hazard from a kitchen process is a step towards risk mitigation and a safer household.

III.6 Future Work

This study was done with different strains of *Salmonella* and *E. coli* O157:H7. Future work would look at different pathogens that are common to fresh produce. Ukuku and Fett (2002) measured bacteria surface charge (electrostatic interactions) and hydrophobicity across *E. coli*, *Salmonella*, and *Listeria monocytogenes* strains. Hydrophobicity is variable across *Salmonella* strains, but not *Listeria monocytogenes* or *E. coli*. The study also indicated that a weaker charge is on the surface of the cells of *E. coli* than *Salmonella* and *Listeria monocytongenes*. This could lessen the gap between surfaces by reducing electrostatic repulsion. *Salmonella* displayed stronger attachment over time though (*90*).

III.7 Appendix

III.4.a Transfer rates (%) of bacteria from inoculated kitchen surfaces coming into contact with fresh cut produce						
	$\bar{x}$(%)	σ	Median	Max	Min	Range
Ceramic						
Carrot	79.571	4.268	80.333	86.649	71.575	15.074
Carrot (1 hr)	69.583	25.047	90.909	90.909	25.000	65.909
Celery	90.400	2.244	90.761	93.051	85.855	7.196
Celery (1hr)	48.812	11.185	52.632	65.789	22.222	43.567
Lettuce	91.375	2.513	91.441	94.845	86.379	8.467
Lettuce (1hr)	4.128	4.308	2.542	16.667	0.490	16.176
Watermelon	92.545	2.242	92.521	96.272	88.617	7.655
Watermelon(1hr)	86.914	14.857	91.076	95.975	40.670	55.305
Glass						
Carrot	96.835	2.017	97.634	98.974	92.486	6.488
Carrot (1 hr)	99.167	1.576	99.719	99.859	94.391	5.468
Celery	89.883	2.058	89.490	93.066	86.312	6.754
Celery (1hr)	4.930	4.305	3.229	16.393	1.429	14.965
Lettuce	84.515	4.102	85.544	89.431	76.389	13.042
Lettuce (1hr)	1.972	2.036	1.322	9.091	0.369	8.722
Watermelon	86.621	2.217	86.731	90.196	82.836	7.360
Watermelon(1hr)	92.517	2.329	92.131	96.970	88.692	8.278
Plastic						
Carrot	97.697	0.972	97.679	99.244	95.063	4.182
Carrot (1 hr)	99.914	0.051	99.927	99.980	99.776	0.204
Celery	87.725	2.062	87.778	90.842	84.004	6.839
Celery (1hr)	17.435	3.432	16.775	27.586	12.862	14.724
Lettuce	86.185	11.442	90.009	94.111	47.087	47.025
Lettuce (1hr)	18.643	25.827	4.773	73.770	2.273	71.498
Watermelon	97.411	0.544	97.421	98.299	95.659	2.640
Watermelon(1hr)	89.393	3.216	90.048	93.561	83.333	10.228
Stainless Steel						
Carrot	91.749	5.443	93.607	97.151	76.484	20.667
Carrot (1 hr)	84.750	6.707	86.298	95.406	70.081	25.325
Celery	83.356	6.717	85.613	89.590	62.130	27.460
Celery (1hr)	0.742	0.462	0.672	2.174	0.204	1.969
Lettuce	91.283	1.680	91.479	93.982	87.850	6.132
Lettuce (1hr)	39.461	19.455	40.833	71.964	7.092	64.872
Watermelon	94.636	2.138	95.181	97.686	89.003	8.683
Watermelon(1hr)	79.999	13.673	83.399	95.599	34.884	60.716

III.4.b Statistical analysis of transfer rates (%) of bacteria from inoculated fresh cut produce coming into contact with kitchen surfaces						
	$\bar{x}$(%)	σ	Median (M)	Max	Min	Range
Ceramic						
Carrot	0.898	0.641	0.724	1.968	0.113	1.855
Carrot (1 hr)	0.015	0.019	0.008	0.071	0.003	0.068
Celery	7.722	1.472	8.279	9.774	5.233	4.542
Celery (1hr)	0.009	0.004	0.009	0.019	0.005	0.014
Lettuce	13.768	2.037	13.968	16.978	10.241	6.737
Lettuce (1hr)	0.006	0.005	0.004	0.028	0.003	0.025
Watermelon	1.481	0.550	1.461	2.875	0.686	2.189
Watermelon(1hr)	1.653	0.467	1.611	2.708	1.040	1.668
Glass						
Carrot	2.322	2.332	1.475	8.252	0.205	8.047
Carrot (1 hr)	0.004	0.002	0.003	0.009	0.002	0.007
Celery	8.847	1.050	9.123	10.577	6.741	3.836
Celery (1hr)	2.958	0.444	3.023	3.540	1.943	1.597
Lettuce	8.856	5.926	8.461	19.192	1.587	17.605
Lettuce (1hr)	0.468	0.706	0.036	1.984	0.001	1.983
Watermelon	0.211	0.020	0.209	0.239	0.180	0.059
Watermelon(1hr)	0.739	0.347	0.682	1.443	0.249	1.194
Plastic						
Carrot	2.469	1.372	2.516	5.040	0.561	4.479
Carrot (1 hr)	0.006	0.003	0.005	0.012	0.003	0.009
Celery	8.768	2.375	8.118	13.556	4.996	8.560
Celery (1hr)	0.011	0.007	0.009	0.032	0.005	0.027
Lettuce	6.398	1.496	6.664	8.917	3.859	5.058
Lettuce (1hr)	0.410	0.576	0.039	1.988	0.001	1.987
Watermelon	2.635	0.559	2.628	4.306	1.647	2.659
Watermelon(1hr)	0.734	0.178	0.671	1.171	0.564	0.607
Stainless Steel						
Carrot	1.238	0.400	1.278	1.857	0.535	1.322
Carrot (1 hr)	0.099	0.073	0.076	0.308	0.026	0.282
Celery	15.465	4.110	15.502	23.729	6.452	17.277
Celery (1hr)	0.285	0.607	-	2.019	-	2.019
Lettuce	13.971	2.537	13.812	18.231	10.131	8.100
Lettuce (1hr)	1.544	0.977	1.174	4.470	0.641	3.829
Watermelon	2.855	2.140	2.296	8.314	0.629	7.685
Watermelon(1hr)	2.472	0.950	2.116	4.441	1.167	3.274

"-" indicates that the value is zero. A zero value in this analysis indicates a value below the detection limit.

Chapter IV - Cross Contamination of *Escherichia coli* O157:H7 Between Lettuce and Wash Water During Washing in a Stainless Steel Bowl

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[B] Data collection

[C] Supervised data collection, experimental design

[D] Supervised data analysis, editing

Key Words: Cross Contamination*, Escherichia coli*, Produce, Wash Water

IV.1 Abstract

Lettuce consumption has increased over the past few decades due to increase consumer demand and increase availability off-season. Fresh produce, including lettuce, has an inherent risk of foodborne disease due to the product frequently being consumed raw, often with minimal or ineffective washings beforehand, and no lethal inactivation steps during preparation. Another concern with lettuce is the risk of spreading contamination via wash water to other pieces of lettuce during washing by a consumer. Wash water quickly becomes contaminated with microbes from the lettuce surfaces, and will transfer those microbes to other leaves during the wash. This studies aims to quantify the transfer rates of five strains of rifampicin resistant *E. coli* O157:H7 between a single inoculated lettuce leaf to non-inoculated lettuce leaves under various washing regimes (30 s, 1 min, 2 min, 5 min). The study involved washing lettuce leaves with sterilized municipal water in a stainless steel bowl. Ten leaves of green romaine lettuce pieces were used as the non-inoculated lettuce pieces, and one leaf of red romaine lettuce was used for the inoculated piece. Homogenized lettuce samples and wash water were serially diluted in 0.1 % peptone and surface plated (0.1 ml) onto Tryptic soy agar with 80 mg/g rifampicin (TSAR) and Sorbitol MacConkey agar with 80 mg/g rifampicin (TSAR & SMACR). Our results demonstrate that wash water will become contaminated with 90-99% of bacteria originally present on the lettuce leaves, regardless of washing time. During the 30 s and 5 minute wash each non-inoculated lettuce had ~1% of the *E. coli* O157:H7 of the inoculated pieces' *E. coli* O157:H7 transferred to them. The 1 minute and 2 minute washes typically displayed less than ~1% of the *E. coli* O157:H7 of the inoculated pieces' *E. coli* O157:H7 transferred to them. This indicates that a 1-2min wash is optimal

to remove the greatest amount of bacteria from the inoculated lettuce piece, yet minimizes reattachment to non-inoculated lettuce pieces. Ultimately, a simple wash with tap water may not be sufficient to significantly reduce the microbial load on lettuce, and may result in contamination spread to previously uncontaminated leaves.

IV. 2 Intro

Lettuce and leafy greens have been implicated as a carrier of pathogens in many outbreaks (*20*, *30*, *35*, *49*, *59*, *78*). Pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella*, *Campylobacter*, *Yersinia enterliticus*, and *Salmonella spp* have all been linked to lettuce, salads, and leafy greens (*8*, *30*, *35*, *59*). Increased outbreaks with lettuce have been linked partially to increased consumption of leafy greens, because Americans are choosing healthier eating habits, and diets that include higher amounts of minimally processed fruits and vegetables {Doyle 2007; Froder Martins 2007). Lettuce presents a microbial risk, especially to immunocompromised individuals, due to people traditionally consuming it raw (*49*). From farm to fork, contamination of lettuce can occur from touch points at all steps of the processing route (*8*, *30*, *35*). This includes irrigation, soil, harvesting, packaging, storage, and in the home kitchen (*8*, *30*, *35*).

Lettuce harbor pathogens in part due to their large surface area and layering of the leaves, which can inhibit a thorough wash (*80*). Some structures of the lettuce surface, such as pores, will protect the pathogens from being washed(*78*). Smith et al (2003) found that 88% of unwashed lettuce samples had greater than 2 log CFU of aerobic, mesophilic microbes per gram of unwashed lettuce. Sixty five percent of lettuce samples had greater than >3 log CFU/g, which were similar to Vijayakumar et al. (2002) findings (*79*, *98*). All samples had a total coliform count <100CFU/g (*79*). Soriano et al (2000) found a range of 3-7.8 log CFU of aerobic, mesophilic microbes per gram of untreated lettuce in homes (*81*).

Consumer demand for more convenient, yet healthy meals has lead to the rise in popularity of precut, bagged lettuce. However many pathogens, especially *E. coli* O157:H7 display a preference, and at times better survival, to the cut portions of lettuce(*12*, *33*, *88*, *100*). In their 2000 paper, Takeuchi et al. showed that roughly 0.2 log CFU/g more would attach to cut portions of lettuce. This may be due to cut vegetables being more susceptible to microbial attachment and growth, due to the nutritious and starchy exudate that is released from the wound (*30*, *100*).

Another focus of this paper is the wash water's ability to serve as a cross contamination medium for pathogens. The point of a wash is to remove debris, bacteria, fertilizers, and pesticides, which may have latched on during cultivation or processing. However studies have demonstrated that washes, even multiple times, may not be enough to remove a significant amount of pathogens from the lettuce leaves (*25*, *30*, *32*, *36*, *58*, *59*, *100*, *102*). Reusing wash water is not advisable, as a microbial build up may occur due to ineffective or no sanitation of the wash water(*32*). Gil et al (2009) suggested that while wash water may remove pathogens from produce, sanitizing agents would not remove pathogens from produce. The sanitizing agents primarily reduce cross contamination rates between produce by maintaining the quality of the wash water. High organic loads, from dirt or small produce pieces, rapidly reduce the effectiveness of wash water and sanitizers by compromising its quality (*1*, *32*). Furthermore, long-term storage of produce after a wash could bring the original microbial load back to or above original levels (*32*, *47*). One of the goals of this study is to determine the cross contamination rates of bacteria to wash water from a single inoculated piece of lettuce. The wash will

mimic what is commonly performed in a consumer's kitchen, and using supplies that consumer would have access to, such as a stainless steel bowl and municipal tap water.

IV.3 Materials and Methods

IV.3.a Preparation of Stainless Steel Bowls.

The stainless steel bowls have a 15cm circumference and were 4cm deep at the center**.** Before use, the stainless steel bowl surfaces were disinfected with 30% sodium hypochlorite (Clorox, Oakland, California), overnight. The surfaces were then scrubbed with hot water with an anionic active detergent, and rinsed with hot water. Bowl surfaces were soaked in 70% ethanol for one hour, then removed, and air-dried prior to each washing regime.

IV.3.b Produce.

One bag of fresh cut romaine lettuce and one bag of fresh cut romaine spring mix, in re-sealable bags or storage containers (clam shells), were purchased from a local supermarket (Winter Haven, FL). Produce was stored at 4°C and brought to ambient temperature prior to starting the experiment. Lettuce was cut, with an autoclaved, non-serrated knife, into approximately 3 x 3 cm pieces.

IV.3.c Selection of strains.

A five strain cocktail of *E. coli* O157:H7 isolates from produce or produce-related commodities were used (Tbl. IV.3.c.1). Their designation and sources included; Odwalla outbreak (223), human isolate from a cantaloupe outbreak (F658), human isolate from a lettuce outbreak (H1730), human isolate from an Alfalfa Sprouts outbreak (F4546), and human isolate from a spinach outbreak (EC4042)**.** All strains were adapted to grow in the

presence of 80 mg/g rifampicin (Thermo FisherScientific, Waltham, MA), through stepwise exposure (*62*).

Table IV.3.c.1

Escherichia coli Serotype	Origin (Source)	Designation
O157:H7	Lettuce (human-feces)	H1730
O157:H7	Alfalfa sprout (human-feces)	F4546
O157:H7	1996 Odwalla (unpasteurized apple juice)	Odwalla strain # 223
O157:H7	Cantaloupe	CDC 658
O157:H7	Spinach	EC4042

IV.3.d Inoculum preparation.

Prior to each experiment, frozen cultures of each strain were streaked onto tryptic soy agar (TSA; Difco, BD,Sparks, MD) with 80 mg/g rifampin (TSAR), and incubated at 37°C for 24 h . One isolated colony from each strain was transferred to 10 ml of tryptic soy broth (TSB; Difco, BD) with 80 mg/g rifampin (TSBR), and incubated at 37°C for 24 h. Cultures were subsequently subcultured twice by transferring 0.1 ml of an overnight culture to 10 ml of fresh TSBR and incubated at 37°C for 24 h. Each strain was subjected to centrifugation at 0.6 x *g* for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). Cells were then washed twice by removing the supernatant and suspending the cell pellet in 10 ml of 0.1% peptone (Difco, BD). Washed cells were suspended in 0.1% peptone at half the original culture volume. Strains were diluted and combined in equal volumes to a concentration of 10^8 CFU/ml. Final concentrations were verified for each strain by enumeration on TSAR.

IV.3.e Transfer between fresh cut produce and surfaces.

Ten leaves of green romaine lettuce pieces were used as the non-inoculated lettuce pieces. One leaf of red romaine lettuce was used for the inoculated piece. Spot

deposit of 10ul (5-8drops) of 10^6CFU/ml *E. coli* O157:H7 cocktail were put onto the inoculated piece. One hundred milliliters of sterile potable city water was poured into a sterile stainless steel bowl. All the lettuce pieces (both inoculated and non-inoculated) were added to the bowl and mixed with constant agitation using a sterile spoon for 30s, 1, 2 or 5 minutes. Each piece of lettuce was pulled out of the mixing bowl using sterile forceps, and each leaf was placed in a stomacher bag with 10 ml of DE (Dey/Engley, Thermo Fisher Scientific, Waltham, MA) neutralizing buffer, taking care to note when the red (initially inoculated) piece was removed. Lettuce samples were stomached for 60s, serially diluted and plated onto SMACR and TSAR plates. A 1mL sample of the wash water was pulled for sampling and was also plated onto SMACR and TSAR.

IV.3.f Enumeration of cells.

Lettuce samples were placed in a sterile 207 ml whirl pak filter bag (Nasco, Fort Atkinson, WI, USA) and 40 ml of DE (Dey/Engley, Thermo Fisher Scientific, Waltham, MA) was added to the samples and macerated in a smasher (AES Laboratories, Chemunex, France) for 1 minute. Homogenized lettuce samples and wash water were serially diluted in 0.1 % peptone and surface plated (0.1 ml) onto TSAR and Sorbitol MacConkey agar, a more selective media, (SMAC; Difco, BD, Sparks, MD) with 80 mg/g rifampicin (SMACR). Plates were incubated at 37°C for 24 h (TSAR, SMACR). Following incubation, colonies were counted by hand and *E. coli* O157:H7 population levels were expressed in log CFU/g for produce and wash water.

IV.4 Data Analysis

Data were compiled, log transformed, and used to create histograms, using Microsoft Excel (Microsoft, Redmond, Washington), and the data were then assembled into graphs using sigma plot (Systat Software Inc., Chicago, IL). The number of times a particular transfer rate occurred within a target data set (i.e. it's frequency) was plotted on the y-axis to visualize variability in log percent transfer rates during the different transfer events. The x-axis in these histograms is log percent transfer, as previous research in our lab has indicated that this transformation generally produces normally distributed data (*74*). The x-axis bin width used to create these histograms was either 0.25 or 0.5 log percent transfer as past experience in our lab indicates that this is generally satisfactory. Optimal bin size is determined by multiple factors, but generally the fewer observations available, the larger the bin needs to be to visualize meaningful trends. The bin size used in this chapter is 0.50 log percent.

Transfer rates or reduction were determined by:

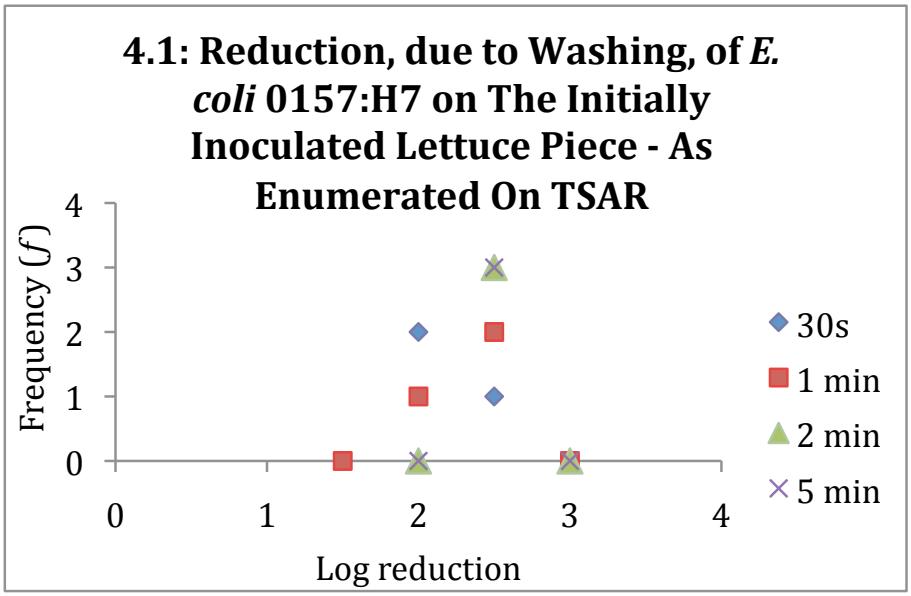
Log Reduction on initially inoculated piece: [Log (Total $CFU_{(\text{previously non-inoculated lettuce pieces})}$+Total $CFU_{(\text{wash water})}$)-Log(Total $CFU_{(\text{initially inoculated piece post-wash})}$)]

Previously non-inoculated pieces was calculated by: [Total $CFU_{(\text{on previously non-inoculated lettuce piece})}$/(Total $CFU_{(\text{previously non-inoculated lettuce pieces})}$+Total $CFU_{(\text{wash water})}$)]x100= Transfer Rate (%)

Transfer rates to the wash water was determined by: [Total $CFU_{(\text{wash water})}$/(Total $CFU_{(\text{previously non-inoculated lettuce pieces})}$+Total $CFU_{(\text{wash water})}$)]x100= Transfer Rate (%)

The wash water had a detection limit of 1 logCFU/mL, and the lettuce samples had a detection limit of 2 log CFU/mL.

IV.5 Results



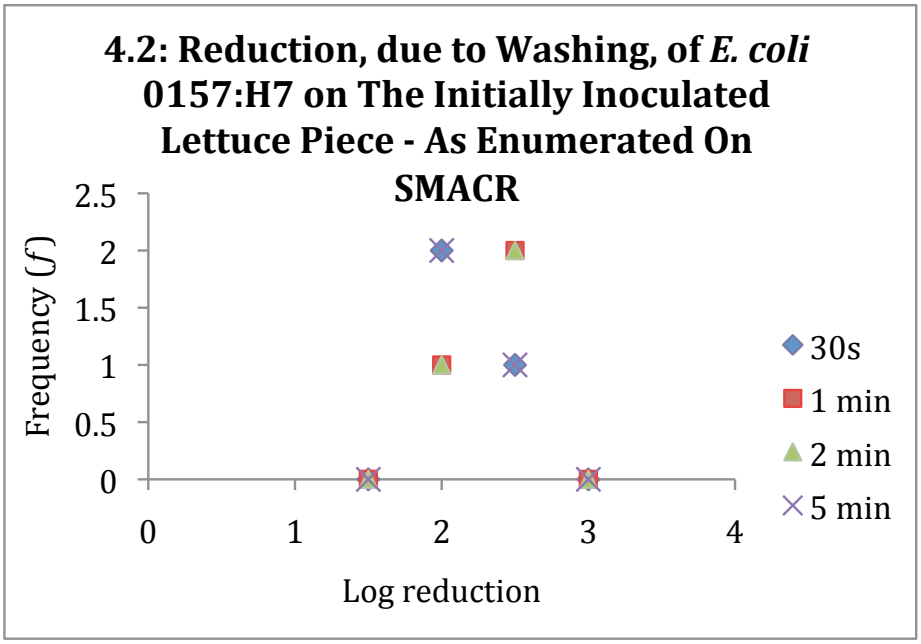
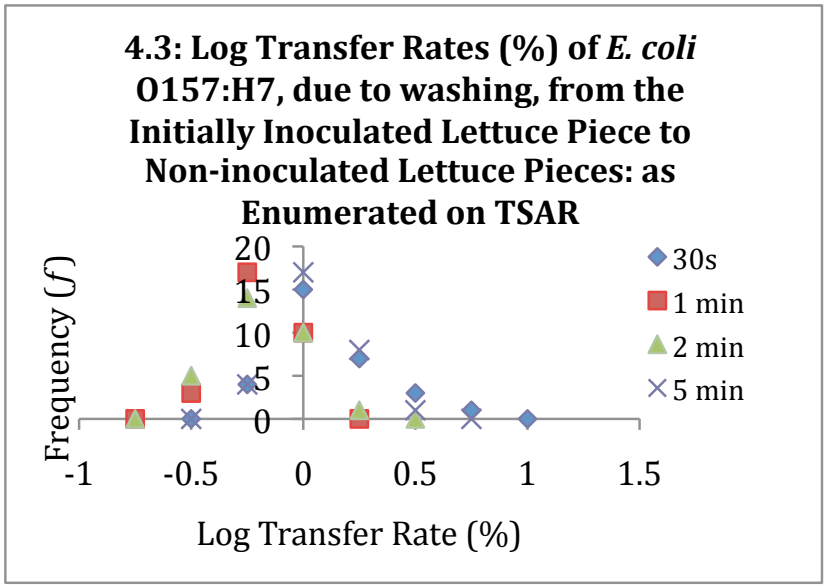


Figure 4.1 represents the reduction of *E. coli* O157:H7 on the initially inoculated piece after being washed with the non-inoculated pieces, when samples were plated

onto TSAR. The highest reduction, 2.5 log CFU/g, is observed at both 2 and 5 minutes. A thirty second and 1 minute wash had a reduction between 2-2..5 logCFU/g, with the 1-minute wash having a higher instance of 2.5 log CFU/g reduction. Figure 4.2 also represents the reduction of *E. coli* O157:H7 on the initially inoculated piece after being washed with the non-inoculated pieces, when the samples were plated onto SMACR. SMACR is a more selective media than TSAR, and as a result will show reduced growth when compared to TSAR. The 5min and 30s wash both frequently displayed a 2 log CFU/g reduction, while the 1 minute and 2 minute wash displayed a 2.5 log CFU/g reduction.



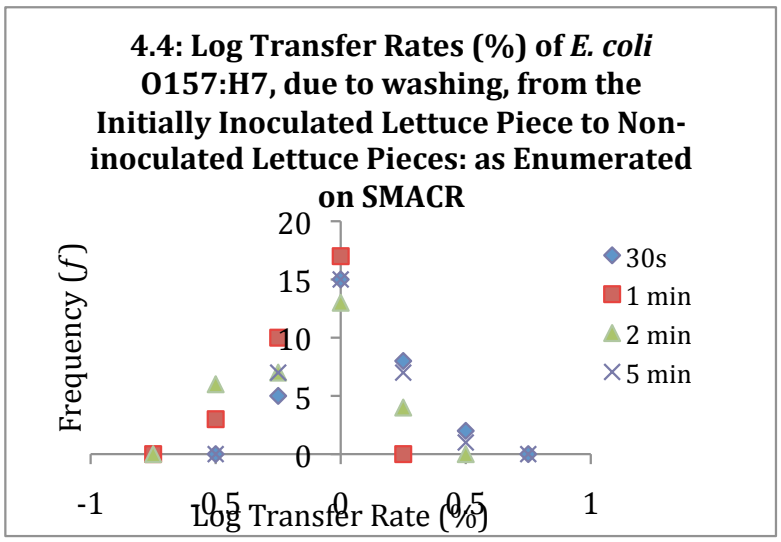


Figure 4.3 represents the frequency of the log % transfer of *E. coli* O157:H7 from the inoculated lettuce piece to each of the non-inoculated pieces of lettuce during washing, when samples were plated onto TSAR. During the 30 s and 5 minute wash each non-inoculated lettuce had 1% of the *E. coli* O157:H7 on inoculated pieces transferred to them. The 1 minute and 2 minute washes displayed 0.5% of the *E. coli* O157:H7 on inoculated pieces transferred to them. Figure 4.4 represents the log % transfer of *E. coli* O157:H7 from the inoculated lettuce piece to the non-inoculated pieces of lettuce during washing and the samples were plated onto SMACR. All wash times displayed a 1% of the *E. coli* O157:H7 on inoculated pieces transferred to them.

In all scenarios, between 90-99% of *E. coli* O157:H7 transferred from the inoculated lettuce piece to the 500mL of wash water (Not shown).

IV.6 Discussion

IV.6.a Cross contamination between inoculation lettuce piece and the non-inoculated pieces.

In 2002, Wachtel and Charkowski simulated a restaurant's lettuce preparation procedure to measure cross-contamination rates and growth during handling and storage (*98*). They mixed dry lettuce leaves with an inoculated lettuce leaf. Their results showed that at 4 log CFU/piece inoculation levels, 7% of lettuce leaves mixed displayed presence of *E. coli* O157:H7, and at 5 log CFU/piece inoculation levels, 96% of the non-inoculation pieces showed presence of *E. coli* O157:H7 (Detection limit was 10 CFU/piece) (*98*). In our study we mix the lettuce leaves in water for a maximum of 5 minutes, and our results quantified the amount present on the non-inoculated pieces after mixing. Wachtel and Charkowski put lettuce into water inoculated with *E. coli* O157:H7 (3L at 7 logCFU/mL inoculum), and after 24 hours the lettuce pieces displayed 6 log CFU/g. There was no agitation of the lettuce in the water as in our study, but our results indicate that even a short immersion time in the wash water (<5min) can results in at minimum a ~1% cross contamination rates between lettuce pieces. During the 30 s and 5 minute wash, each non-inoculated lettuce had ~1% of the *E. coli* O157:H7 on inoculated pieces transferred to them. The 1 minute and 2 minute washes typically displayed less than 1% of the *E. coli* O157:H7 on inoculated pieces transferred to them. This indicates that a 1-2min wash is optimal to remove the greatest amount of bacteria from the inoculated lettuce piece, yet minimizes reattachment to non-inoculated lettuce pieces.

IV.6.b Reduction of *E. coli* O157:H7 on initially inoculated piece and transfer of *E. coli* O157:H7 between inoculated lettuce piece and the wash water.

There was 90-99% transfer rates to the wash water from the initially inoculated lettuce leaf during washing, and there is a 2-2.5 logCFU/g reduction on the initially inoculated piece. This is slightly higher reduction than previous studies, which suggest that washing can achieve a 1-2 log CFU/g reduction on lettuce pieces (*30*, *36*, *78*, *100*). However, it should be noted that this is the reduction on one piece, and not the entire batch. Our study demonstrated that other pieces would become contaminated.

Zhang et al. (2009) performed a similar study to ours (*102*). In their study the iceberg lettuce was spot inoculated with an *E. coli* O157:H7 cocktail to achieve roughly 5.6 log CFU per piece. They placed the inoculated lettuce in a plastic container and held it at 4°C for 2h. We spot inoculated at room temperature and allowed the inoculated piece to dry for only a few minutes. Zhang et al. made a small cut on the inoculated lettuce leaves to differentiate between non-inoculated lettuce leaves. Zhang et al. had the wash done in 4°C room with 100mL sterile water for 1.5 min. Inoculated lettuce leaves had a 2 log CFU/piece reduction after a water wash, which was similar to the reduction we observed (2-2.5 log reduction per piece). Their processing water contained 1.83 log CFU/mL after wash, and non-inoculated lettuce pieces had 2.5 log CFU/piece after wash. A single inoculated leaf would transmit about 2 log CFU/mL, or two percent of the *E. coli* O157:H7, to wash water, with or without organic material present. Ours wash water had almost 90-99% of the *E. coli* O157:H7 transferred to it. A reason for the difference in transfer of bacteria to the wash water may be due to different inoculation methods, and

different washing temperatures (the lettuce leaves in our study were washed at room temperature) (*32*, *102*).

Smith et al (2003) performed a lettuce washing experiment in which they had Rutgers University dining services food handlers wash the lettuce under running water (not in a bowl as we did) (*78*). Smith et al showed that a rinse under tap water resulted in a 0.1-1.42 log CFU/g reduction in total aerobic microbes. Our result indicated a 2-2.5 reduction of *E. coli* O157:H7 on the initially inoculated piece, but we did have significant transfer to the other pieces. The difference may be due to difference in target organism and type of wash, but a wash under tap water may result in less cross contamination than a wash in a bowl (*32*). In the Smith et al (2003) study, wash water is quickly removed from the lettuce leaves, and the force of the water rushing over the lettuce is greater than the force lettuce leaves experience during a bowl wash. The downside to this technique is that it will use more water, which may cause fiscal issues on an industrial scale or problems in fresh water limited communities.

While our study demonstrated that there could be a 2-2.5 log CFU/g reduction on the initially inoculated piece, under heavy contamination conditions, such as temperature abuse and or poor hygiene practices, a wash may not necessarily ensure the safety of the lettuce being eaten. Furthermore, some pathogens may be removed from the inoculated lettuce leaves, but the non-inoculated lettuce leaves are sources of attachment sites for the bacteria, and they will become contaminated. Care must be taken to dispose of the wash water properly and it should never be

reused for other purposes. A safer alternative to washing in a bowl may be to wash the lettuce under running water, and before cutting, to reduce cross contamination between lettuce leaves. The sink should be thoroughly sanitized afterwards (*58*).

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