



Research article

Quantification of pathogen cross-contamination during fresh and fresh-cut produce handling in a simulated foodservice environment

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Abstract: Fresh and fresh-cut produce are associated with a significant proportion of foodborne disease outbreaks, driving the need for proper food handling that mitigates the risk of pathogen spread in foodservice environments. The objective of this study was to investigate cross-contamination patterns resulting from preparing these foods. For the first part of the study, forty-five participants simulated preparing fresh and fresh-cut produce in a mock deli kitchen in three scenarios. Produce or participant hands were coated with an innocuous fluorescent compound (Glo Germ™), then, following the simulations, high touch areas were swabbed to quantify cross-contamination. For scenarios 1–3, the cutting board, leafy greens, and participant gloves had the highest log₁₀ percent of fluorescent compound (1.81, 1.31, 1.48, respectively). These results reinforce the need to properly sanitize kitchen equipment and to properly wash hands to reduce the likelihood of spreading harmful microorganisms. For the second part of the study, microbial experiments were conducted in a BSL-2 laboratory with two scenarios to determine how and to what extent *Listeria monocytogenes*, *E.coli* O157:H7, and *Salmonella* spp. spread from handling dip inoculated fresh and fresh-cut produce. Findings showed the propensity for pathogen harborage in utensils and wash water in scenario one. *E.coli* O157:H7 counts increased 33% from the inoculated lettuce sample to a second sample soaked in the same ice bath. By identifying and quantifying cross-contamination outcomes from food preparation, researchers can design task-specific educational materials that improve work flows which may reduce the risk of foodborne disease outbreaks.

Keywords: cross-contamination; foodborne disease; food safety; simulated food retail environment; fresh produce outbreaks; fresh-cut produce outbreaks; food safety training

1. Introduction

The National Restaurant Association (NRA) reports that 12.8 million employees in the U.S. serve over 70 billion meals at 960,000 commercial operations each year [1]. Consumption of food outside the home has increased since the 1970's [2], and evidence suggests this will continue over the next decade [3]. Dollar sales of fresh produce rose 22% from 2010–2014 in the U.S., driven in part by consumer buying habits that reflect desires for more healthy, convenience-based products that include ready-to-eat bagged or pre-made salads [4,5]. However, this shift in purchasing patterns of fresh and fresh-cut produce has been accompanied by an increase in foodborne disease outbreaks associated with produce consumption. These trends present a potential increase in the risk of foodborne disease outbreaks due to poor food handling practices [6] and necessitate more work to elucidate how pathogenic microorganisms are spread in a foodservice environment.

Although fresh produce was once considered to be one of the safest foods [7], it accounted for more than 46% of the foodborne disease outbreaks in the United States (U.S.) from 1998 to 2008 [8]. Foodborne disease outbreaks associated with human pathogens on plants caused 8,556 infections, 114 deaths, and 1 miscarriage from November 2010 to December 2015 [9]. According to the CDC, from 1998 to 2013 a total of 972 raw produce outbreaks were reported and resulted in 34,674 illnesses, 2315 hospitalizations, and 72 deaths [10]. Between 1973–2012, 606 leafy vegetable-associated outbreaks, with 20,003 associated illnesses, 1,030 hospitalizations, and 19 deaths were reported [11]. Eighty-five percent of leafy vegetable-associated outbreaks were prepared in a restaurant, often by an ill food handler. Among fresh-cut leafy greens alone there were approximately 100 U.S. outbreaks due to *Salmonella* spp., *Escherichia coli* O157:H7, *Shigella* spp., and *Campylobacter jejuni* reported between 2000 and 2007 [12]. As of July 2018, *Salmonella* Adelaide was the contaminant associated with a cut melon outbreak that caused 77 illnesses and 36 hospitalizations in nine states [13].

Fresh-cut produce undergoes minimal physical processing and can be especially conducive for pathogen growth. Contamination of such produce can occur on the farm, during processing, or during food handling [14]. Processing, (cutting, slicing, peeling, shredding) can destroy cell surfaces and expose the cytoplasm to provide a better source of nutrients for microorganisms as compared to intact produce [15]. Furthermore, much of the fresh-cut produce is consumed raw, which eliminates cooking as a kill step to decrease pathogen loads. Additional care by employees is required during handling, washing, and storage to prevent foodborne disease outbreaks [14,16].

Choi et al. [17] conducted observations in 31 foodservice operations to determine prevalence of high-risk practices of handling fresh and fresh-cut leafy greens. The results demonstrated low compliance rates of food safety behaviors related to improper use of thermometers, lack of documentation, and poor employee hygiene. Training workers on proper food handling practices and enforcing these behaviors through management oversight is instrumental for decreasing the risk of foodborne diseases transmission [18]. Choi et al.'s [17] research highlights the need to better understand how work flow may impact bacterial transfer. This information could then be used to design foodservice educational materials that minimize touch points with food contact surfaces. With

increases in foodborne disease outbreaks associated with fresh and fresh-cut produce, more produce-specific training must also be developed to ensure food handlers are properly trained on the potential risks associated with preparing fresh and fresh-cut produce. Simulating foodservice work environment conditions can help shed insight to supplement trainings without interfering with foodservice operations.

Dynamics of bacterial and viral transfer rates for fresh and fresh-cut produce are well-documented in the literature [19–26]. These studies have focused on commercial processing using automated slicing equipment, household kitchen practices, and preparation involving poultry. However, to date little is known about the dynamics of pathogen spread that may occur from preparing leafy greens and simulating the food handling behaviors previously observed in retail foodservice settings [17]. Information to fill these data gaps could be used for quantitative risk assessments tailored to be more commercial kitchen-specific and to improve training modules. The present study was concerned with filling some of these gaps for food safety practices of fresh and fresh-cut produce handling in the food industry.

The objectives of this study were: (1) to simulate the observed behaviors in a mock retail foodservice setting using a fluorescent compound and quantify cross-contamination in order to identify potential high-risk areas of contamination on food service equipment and food contact surfaces; (2) to conduct microbial experiments with high-risk behaviors in a contained environment with foodborne pathogens to quantify cross-contamination.

2. Materials and methods

2.1. Behavioral simulation

2.1.1. Participants

After receiving Internal Review Board approvals, participants were recruited from a large urban university. The participants included 45 faculty, staff and students (36% men and 64% women) with 82% being between the ages of 18–24.

2.1.2. Assessment

The behavioral simulation component was based on the methods described by Sirsat, Kim, Gibson et al. [27]. An innocuous fluorescent compound (Glo Germ™) (FC) was used to quantify how contamination can move through a retail foodservice environment when food handlers prepare fresh and fresh-cut produce. Forty-five participants were asked to complete a series of steps involving fresh and fresh-cut produce simulating the following behaviors:

1. Cross-contamination involving equipment, including salad spinners and cutting boards.
2. Preparing a salad with pre-washed leafy greens mix and fresh-cut produce.
3. Food handler glove changing.

For each scenario describe below, the researcher provided volunteers with detailed instructions and was present during the experiment. The following three scenarios were designed based on observational results and knowledge survey results obtained from Choi et al. [17]:

Scenario one. A head of romaine lettuce (purchased at retail) was coated with 5 g FC powder and labeled lettuce head “A”. The FC powder was measured and gloved hands were used to coat the 5 g onto romaine lettuce. Two additional heads were left uncoated and labeled “B” and “C”. The participant was read general instructions by the researchers, given gloves, an apron, hairnet and a series of tasks to complete without assistance:

1. Go to Refrigerator.
2. Remove lettuce head labeled “A”.
3. Place lettuce head “A” on cutting board.
4. Chop off end of the lettuce head A and throw it away.
5. Slice lettuce according to instructions.
6. Fill container A.
7. REPEAT steps 1–6 with lettuce “B” and “C” and place in appropriate containers marked B and C.
8. Take container A to sink and submerge salad in cold water for 30 seconds, stir water around.
9. Strain salad and place in Ziploc bag A.
10. REPEAT steps 8–10 with lettuce “B” and “C” and place in Ziploc bags B and C.

Scenario two. A bag of pre-washed leafy greens mix was coated with 5 g FC powder with gloved hands. The lettuce was combined with two additional bags of greens that were not coated with FC. Similar to scenario one, the participant was read instructions by the researcher, given gloves, an apron, hairnet and a series of different tasks to complete without assistance:

1. Go to Refrigerator.
2. Remove leafy greens tub.
3. Grab leafy green mix and place 1 handful in each of the 3 plastic containers.
4. Make 3 salads with: 2 cherry tomatoes, 5 shredded carrots, 2 cucumber slices, one sprig of cilantro.
5. Close containers.

Scenario three. The participant’s hands were covered with FC lotion (2 pumps). The participants were requested to rub their hands together so the lotions spread on both sides of their hands. This modeled an employee coming into work with unclean hands. Without washing their hands, they were given a series of tasks to complete:

1. Wear gloves.
2. Remove salad mix labeled “D.”
3. Fill 2 containers with salad mix.
4. Throw gloves in trash.
5. Put on new gloves.
6. Make 2 fruit salads with: 3 strawberry halves, 2 cantaloupe slices, 2 apple slices, and 3 grapes.
7. Place into fruit salad containers and seal.

2.2. Fluorescent compound quantification

Specific areas and fresh-cut produce items were swabbed using a 5 x 5 cm template and sterile calcium alginate tipped swabs in order to quantify the contamination. The swabs were placed in 15 mL sterile conical polypropylene tubes containing 7 mL ethanol and vortexed for 10 seconds. Next, 6 mL of the suspension was pipetted into a cuvette and inserted into a spectrophotometer (Milton Roy Spectronic 20) to calculate the absorbance at 370 nm. A standard curve was determined using a two-fold dilution of 1 g of the FC combined with 10 mL ethanol and measured by the

spectrophotometer at 370 nm. The concentration (y) for each swab in each scenario was determined by inserting the absorbency reading (x) for each swab, the slope (m) and intercept (b) into the equation:

$$y = mx + b$$

Following this, each participant's absorbency readings were normalized using the following formula:

$$\left(\frac{\text{Concentration of the swab } \left(\frac{\text{mg}}{\text{mL}}\right)}{\text{Sum of all swabs } \left(\frac{\text{mg}}{\text{mL}}\right)} \right) \times 100$$

Each participant's readings were totaled to determine the concentration of the swab divided by the sum of all swabs and multiplied by 100 to calculate the percentage of contaminant on each area or ingredient. The data was then logarithmically transformed to express concentration of FC as log₁₀ percent.

2.3. Microbial experiments

Since the transfer dynamics of the FC may be different than actual pathogens, microbial experiments were conducted by the researcher. Participants were not recruited. Each scenario was replicated thrice and was conducted in a BSL-2 laboratory under a bio-safety cabinet.

Salmonella spp. [ATCC 14028 (poultry isolate), BAA-1604 (produce isolate from Roma tomato outbreak), BAA-1594 (produce isolate from Roma tomato outbreak)], *Escherichia coli* O157:H7 [ATCC 43895 (isolate from raw hamburger meat outbreak)], and *Listeria monocytogenes* [ATCC 51414 (isolate from raw milk outbreak), ATCC 43256 (isolate from Mexican-style cheese outbreak)] were obtained from the American Type Culture Collection (Manassas, VA) and stored at -80 °C in glycerol. These strains were chosen to represent key foodborne pathogens most commonly associated with leafy green outbreaks. While not all individual strains used were associated with produce-linked outbreaks, the species themselves have been. Each strain was streaked on Brain Heart Infusion (BHI) agar plates and incubated at 37 °C for 24 hours. A single colony from each strain was transferred to an individual tube with 5 mL Brain Heart Infusion (BHI) broth and incubated for 18 hours at 37 °C. After incubation, 0.1 mL of each strain was pipetted into 5 mL of BHI broth and incubated for 18 hours at 37 °C. On the day of the experiment, 0.5 mL of each strain was added to 47 mL of peptone water yielding a concentration of 10⁹ CFU/mL. This bacterial suspension was inoculated on romaine lettuce to obtain a final concentration of approximately 10⁸ CFU/g. The romaine lettuce was inoculated according to the dip method as prescribed by the National Advisory Committee on Microbiological Criteria for Foods' (NACMCF) Parameters for Determining Inoculated Pack/Challenge Study Protocols and Methods described by Buchholz et al. [19] and Zerio-Egli, Sirsat, and Neal [28]. Approximately 5.5 g of romaine lettuce was placed in a 50 mL sterile glass beaker and submerged in the cocktail for 10 min with slight agitation to ensure the entire sample was submerged during the inoculation period. Next, the sample was drained on a sanitized plastic strainer for 15 minutes to allow excess inoculum to drip off the sample. Following this, the sample was air dried under a ventilation hood for 10 minutes.

Three replications of each scenario described below were carried out.

Scenario one. After dip inoculation, 5.5 g of romaine lettuce (Lettuce A) was placed on a sanitized cutting board and was chopped using a sterile autoclaved knife. The chopped lettuce was then soaked in an ice bath for 30 seconds simulating soaking procedures observed in foodservice establishments [17]. Using sterile metal tongs, the lettuce was taken from the ice bath and put in a sanitized salad spinner (OXO® Steel Large Salad Spinner). The spinner pump was pushed 5–7 times until all excess water was spun from the lettuce sample in a bio-safety cabinet. The spun lettuce was placed in a stomacher bag with 50 mL peptone water and stomached using an easyMIX® Lab Blender (AES-Chemunex) for 120 seconds. Following this, to simulate cross-contamination, a separate 5.5 g sample of clean, un-inoculated romaine lettuce (Lettuce B) was weighed and chopped on the same cutting board using the same knife used in the initial cutting to simulate cross-contamination. The sample was placed in the same ice bath and salad spinner as mentioned in the steps above without washing or sanitizing. Finally, the sample was placed in a sterile stomacher bag with 50 mL peptone water and stomached for 120 seconds. These steps were repeated once more with 5.5 g of clean, un-inoculated romaine lettuce (Lettuce C).

In addition to the lettuce samples, 5 cm² swabs were taken from the cutting board, knife blade, and salad spinner after all the lettuce samples were run through. Serial dilutions were performed in 10-fold and spread plated onto Eosin methylene blue (EMB) agar to quantify *E.coli* O157:H7 and *Salmonella* colonies and PALCAM *Listeria* Agar Base with PALCAM *Listeria* Selective Supplement to quantify *L. monocytogenes* colonies. The EMB plates were incubated at 37 °C for 24 hours while the PALCAM plates were incubated at 37 °C for 48 hours. Following the incubation period the colonies were quantified.

Scenario two. A 5.5 g sample of pre-washed romaine lettuce was dip inoculated using the methods described above. Following the dip inoculation, 11 g of uncontaminated mix was combined with the 5.5 g of the inoculated leafy green mix and separated into 255 mL plastic cups to emulate a food handler preparing three small salads. Two cherry tomatoes, one sprig of cilantro, five shredded carrots, and one sliced cucumber were added to each of the salads. Each salad was given moderate agitation by shaking the salads briefly to simulate mixing the salad to coat all ingredients with dressing. Samples were taken from each ingredient to determine log CFU of the pathogens. Serial dilutions were performed and samples were spread plated onto EMB agar to quantify *E.coli* O157:H7 and *Salmonella* colonies and PALCAM *Listeria* Agar Base with PALCAM *Listeria* Selective Supplement to quantify *L. monocytogenes* colonies. The EMB plates were incubated at 37 °C for 24 hours while the PALCAM plates were incubated at 37 °C for 48 hours. Following the incubation period the colonies were quantified.

2.4. Data analysis

For the behavioral simulations component, the amount of FC on each sample was quantified to determine the concentration (mg/mL). First, the standard curve for FC was established, second, the concentrations were calculated using the standard curve or slope intercept equation, and third, the absorbency readings were normalized using the formula described above. The log₁₀ percent of FC on each area was determined for each scenario. Three replications of each scenario were carried out. Dependent t-tests were used to compare concentration of FC between the participants for the salads and equipment for the behavioral simulations.

Data from the microbial experiments was converted to log CFU/cm² for environmental swabs of equipment and log CFU/g for produce samples.

3. Results

3.1. Behavioral simulation

Scenario one. For scenario one, the amount of cross-contamination from leafy greens to processing equipment was analyzed to determine high touch areas on equipment. The results (Figure 1) indicate that areas with the highest log₁₀ percent of FC concentration were the cutting board (1.81 log₁₀ percent FC), salad A (0.80 log₁₀ percent FC) and salad B (0.64 log₁₀ percent FC). Salad A had a significantly higher log₁₀ percent of FC concentration compared to Salad B [t(14) = 4.38, P = 0.00061] and Salad C [t(14) = 4.58, P = 0.00041]. There was no significant difference in FC between Salad B and Salad C [t(14) = 2.04, P = 0.06]. The knife blade (0.26 log₁₀ percent FC) had lower FC concentration than the knife handle (0.44 log₁₀ percent FC), though this difference was not statistically significant across participants (t[14] = -0.99, P = 0.34).

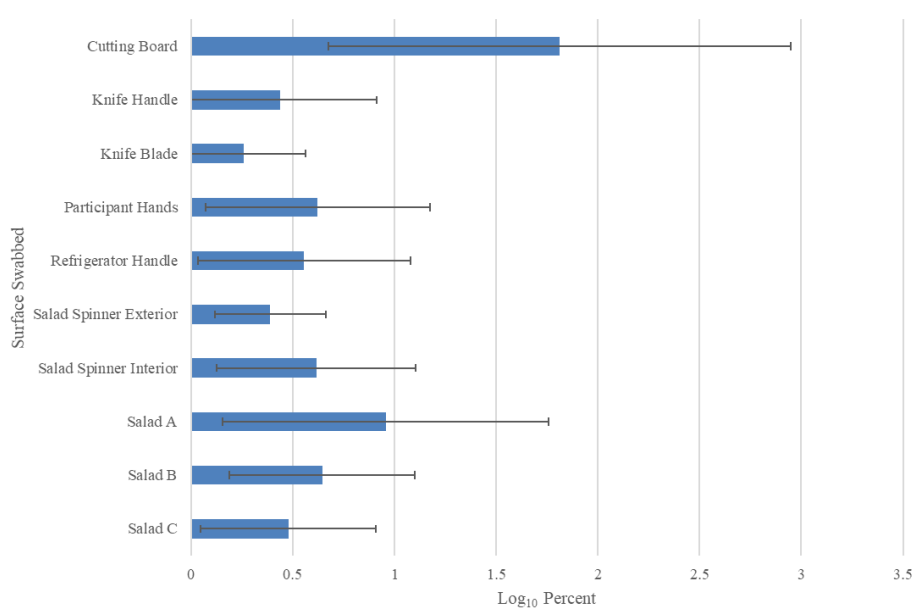


Figure 1. Log₁₀ percent of fluorescent compound concentration of each surface swabbed in scenario 1 for the behavioral simulation. Error bars indicate standard deviation.

Scenario two. For scenario two, the amount of FC from pre-washed leafy greens to other fresh-cut produce was examined to simulate cross-contamination rates between ingredients in packaged salads. The results (Figure 2) indicated that areas with the highest amount of FC concentration were the lettuce from the pre-washed mix (1.32 log₁₀ percent FC), cucumbers (1.17 log₁₀ percent FC), and salad 2 (1.17 log₁₀ percent FC). Other fresh produce ingredients including shredded carrots (0.65 log₁₀ percent FC), cherry tomato (0.91 log₁₀ percent FC), and cilantro (0.61 log₁₀ percent FC) had moderate amounts of concentration in relation to equipment like plastic container closures (0.49 log₁₀ percent FC).

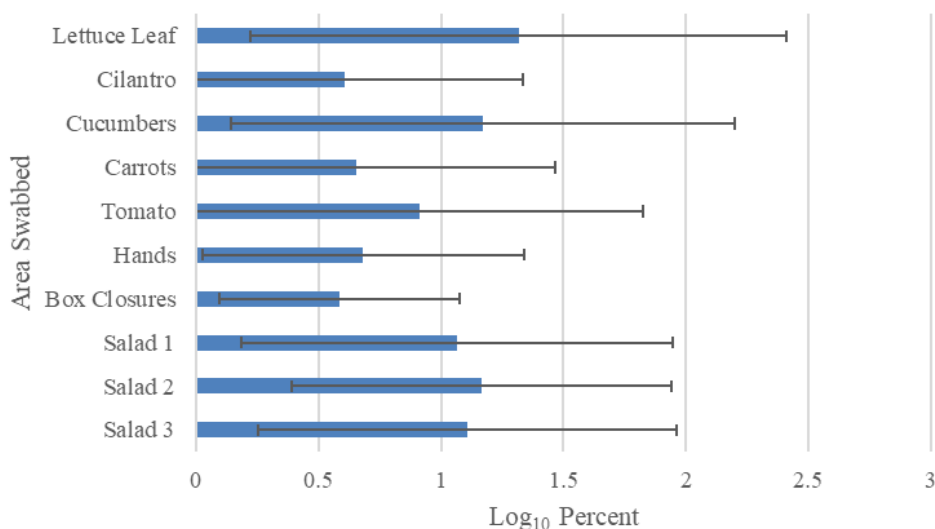


Figure 2. Log₁₀ percent of fluorescent compound concentration of each surface swabbed in scenario 2 for the behavioral simulation. Error bars indicate standard deviation.

Scenario three. The results (Figure 3) indicated that employee gloves (1.48 log₁₀ percent FC), cantaloupe (1.04 log₁₀ percent FC), and fruit salad (1.12 log₁₀ percent FC) had the highest levels of FC concentration. While the participants prepared salad 1 (0.80 log₁₀ percent FC) and salad 2 (0.86 log₁₀ percent FC) first, fruit salad 1 (1.12 log₁₀ percent FC) and fruit salad 2 (0.99 log₁₀ percent FC) had higher levels of FC concentration. The fresh-cut fruit including cantaloupe (1.04 log₁₀ percent FC), strawberry (0.96 log₁₀ percent FC) and apple (0.94 log₁₀ percent FC) had higher concentration than the grapes (0.63 log₁₀ percent FC).

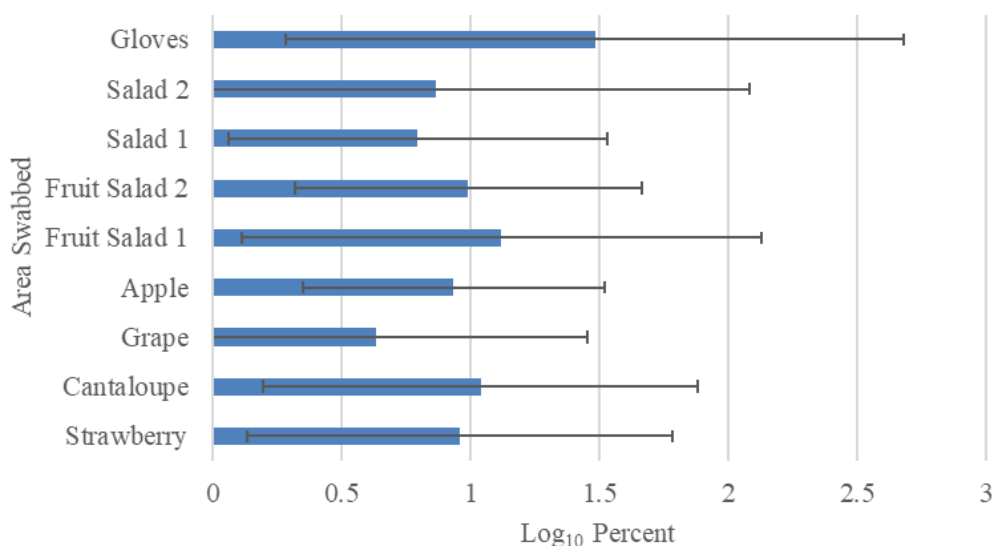


Figure 3. Log₁₀ percent of fluorescent compound concentration of each surface swabbed in scenario 3 for the behavioral simulation. Error bars indicate standard deviation.

3.2. Microbial experiments

Scenario one. Lettuce A, the inoculated sample, had the highest pathogen counts: 6.55 log CFU/g *Salmonella* spp., 5.68 log CFU/g *Escherichia coli* O157:H7, and 3.18 log CFU/g *Listeria monocytogenes* (Figure 4). Lettuce B had 5.15 log CFU/g *Salmonella* spp., 4.71 log CFU/g *E.coli* O157:H7, and 4.16 log CFU/g *Listeria monocytogenes*. Lettuce C had similar counts to Salad B with 5.30 log CFU/g *Salmonella* spp., 4.89 log CFU/g *E.coli* O157:H7, and 4.45 log CFU/g *Listeria monocytogenes*. Additionally, the salad spinner and cutting board had nearly identical counts of all three pathogenic bacteria above 4 log CFU/cm², while the knife blade had the lowest counts with 3.71 log CFU/cm² *Salmonella* spp., 3.53 log CFU/cm² *E.coli* O157:H7, and 3.63 log CFU/cm² *Listeria monocytogenes* (Figure 5).

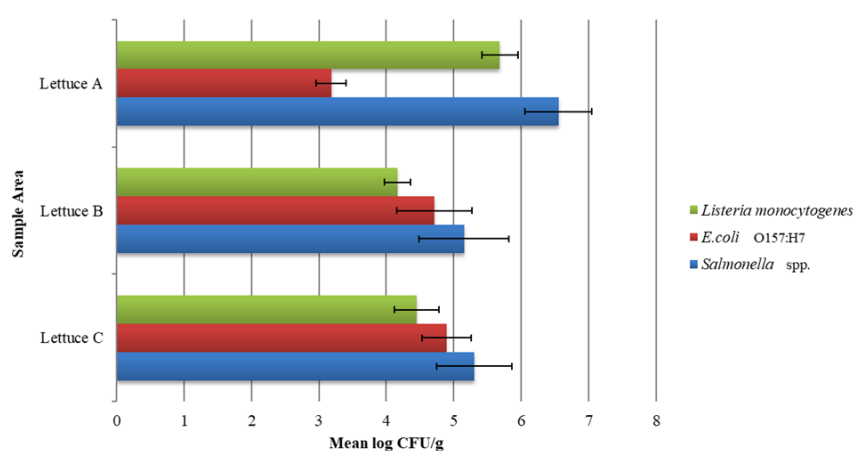


Figure 4. Mean log data of three replicates demonstrating the pathogen amounts on lettuce samples in the microbial experiment scenario 1. Error bars indicate standard deviations.

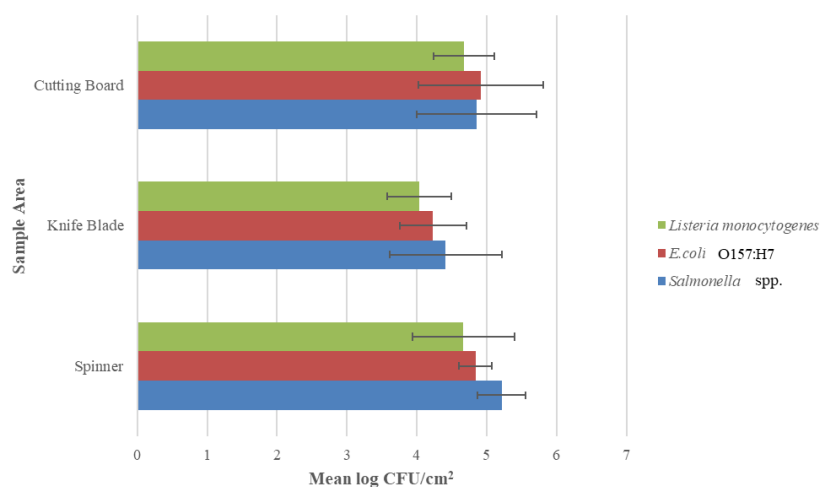


Figure 5. Mean log data of three replicates demonstrating pathogen amounts on surface samples in the microbial experiment, scenario 1. Error bars indicate standard deviations.

Scenario two. For scenario two, the pathogen count on varying fresh and fresh-cut produce ingredients in a packaged salad was analyzed (Figure 6). The inoculated lettuce had counts of 6.47 log CFU/g *Salmonella* spp., 5.80 log CFU/g *E.coli* O157:H7, and 5.73 log CFU/g *Listeria monocytogenes*. Cherry tomatoes had the lowest counts at 4.30 log CFU/g *Salmonella* spp., 3.82 log CFU/g *E.coli* O157:H7, and 4.03 log CFU/g *Listeria monocytogenes*. Shredded carrots had high counts of 6.13 log CFU/g *Salmonella* spp., 5.59 log CFU/g *E.coli* O157:H7, and 3.95 log CFU/g *Listeria monocytogenes*. Additionally, sliced cucumbers had counts of 5.06 log CFU/g *Salmonella* spp., 5.59 log CFU/g *E.coli* O157:H7 and 3.94 log CFU/g *Listeria monocytogenes*.

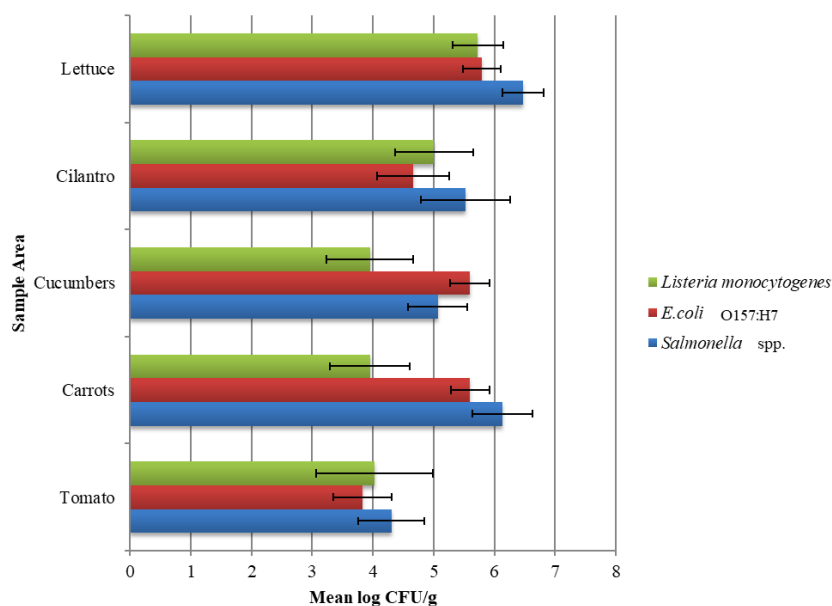


Figure 6. Mean log data of three replicates demonstrating pathogen amounts of “Lettuce” to other produce in the microbial experiment scenario 2. Error bars indicate standard deviations.

4. Discussion

The behavioral simulations provided insight and helped identify equipment areas and fresh-cut ingredients that can become contaminated due to common handling procedures and behaviors. Previous research in a mock deli kitchen has shown using FC to simulate behaviors can help quantify cross-contamination potential and target high risk areas [27]. In scenario one, the cutting board was the predominant fomite, as it contained the highest log₁₀ percent of FC compared to the equipment, hands, and produce. These results are in line with prior work on cross-contamination, which has indicated longer contact times and non-porous surfaces are associated with greater transfer of microorganisms [29]; the cutting board was contacted frequently by the three salads and could be considered a non-porous surface conducive for pathogen transfer. This reinforces the need for training that emphasizes minimizing contact points with surfaces and sanitizing equipment since these can become the primary vehicles for transporting bacteria. The significant difference in FC between Salad A and Salads B & C shows how risk of cross-contamination may change gradually over time given FC compound loss to the wash water and salad spinner.

In scenario two, the risks associated with handling packaged salads were investigated. While the pre-washed leafy green mix was inoculated, other ingredients in the salad became contaminated, as would be expected. This confirms the propensity for cross-contamination given a myriad of exposure routes and contact points that occur in a commercial kitchen setting.

In scenario three, there were two significant findings in relation to glove changing. In this study, participants began with FC on their hands, simulating an employee that does not wash their hands prior to preparing food. Previous research has shown unwashed hands are more conducive for microorganism transfer than washed hands [29]. An observational study of fresh and fresh-cut produce practices of retail employees showed how poor employee hygiene is commonplace [17]. First, while gloves were put on before handling prewashed bagged lettuce, both salads became contaminated. This implies that only using gloves without properly washing hands can easily contaminate ready-to-eat food. Prior work has shown gloves are permeable to bacteria during food handling, serving as an inadequate substitution for proper hand-washing [30]. Glove usage among food handlers can also lead to increased risk taking with a false sense of security [31]. Second, fresh-cut produce, such as the cantaloupe, can become contaminated at a higher concentration than other fresh produce, like grapes. This could be partly due to the increased handling required to prepare the produce through peeling and slicing or the greater amount of free water on the cantaloupe surface, as moisture facilitates bacterial transfer. Additionally, food handlers must touch the ingredients of the fruit salad as there are many components compared to the green salad, which came straight from the ready-to-eat bag of lettuce.

The second part of the study addressed a knowledge gap by quantifying bacterial transfer through experiments that mimic foodservice operations. In scenario one, bacterial counts decreased 97% and 96% from Lettuce A to Lettuce B for *Listeria monocytogenes* and *Salmonella* spp., respectively, as might be expected due to loss of pathogens to the equipment from cutting, rinsing, and drying. Interestingly, there was an increase in bacterial counts of 94% and 42% for *Listeria monocytogenes* and *Salmonella* spp., respectively, from Lettuce B to C. An uptick in *E.coli* O157:H7 counts were observed from Lettuce A to B (33%) and Lettuce B to C (53%). The above findings point to the potential for pathogen harborage in utensils and wash water that can spread to other food through subsequent usage of kitchen equipment. A previous study found wash water contained approximately 90% of the *E.coli* O157:H7 inoculum used for lettuce processing [19]. Faour-Klingbeil et al. [22] showed how surfaces such as cutting boards can re-contaminate up to 6 batches of fresh-cut produce following an initial batch of inoculated produce. These results reinforce the need for cleaning regimens in foodservice establishments that sanitize food preparation materials and monitor water safety on a consistent basis. Robust cleaning and sanitation procedures function as strategies that mitigate risk of foodborne disease transmission. Foodservice establishments in the U.S. are required by law under the FDA Food Code [32] to clean food contact surfaces and equipment every four hours and whenever contamination may have occurred. Based on data obtained from scenario one from both behavioral simulation and microbial experiment, retail foodservice operations may consider more frequent cleaning and sanitizing regimes than what is currently mandated.

In scenario two, the tomatoes had the lowest CFU/g of the five types of produce used, taking the average of the three replicates done for each pathogen. This may be due to the shape of the tomato, the hydrophobic nature of the skin, or the surface having little free water. As was observed with the grapes in scenario three of the behavioral simulation, the low availability of moisture could have

affected the transfer of microorganisms. More replications that control for surface area across produce types would determine whether tomatoes are comparatively more conducive for pathogen harborage.

5. Conclusion

Greater consumption of fresh-cut produce coupled with an increase in foodborne disease outbreaks associated with produce places an impetus on proper food handling behaviors to decrease risk of foodborne disease transmission. This study consisted of a behavior simulation and microbial experiments designed to improve our understanding of contamination patterns. Results from the study can be used to educate workers with behavior-based training material designed to improve handling of fresh and fresh-cut produce. Food preparation instructions should be designed to create work flows conducive for risk mitigation that minimizing touch points of food, equipment, and food contact surfaces. The study reinforced the need for proper hand-washing and sanitizing of equipment to decrease microbial loads in foodservice environments.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. National Restaurant Association (2011) 2011 Restaurant industry forecast: Inside the mind of today's consumer: why restaurants are poised for success in 2011 and beyond. Washington, D.C., National Restaurant Association.
2. Michelle JS, Abigail MO, Tobenna DA (2018) America's eating habits: Food away from home. United States Department of Agriculture (USDA), Economic Research Service (ERS), Economic Information Bulletin Number 196.
3. Cushman & Wakefield (2017) The global food & beverage market. Available from: <http://www.cushmanwakefield.com/en/research-and-insight/2017/report-global-food-and-beverage-market-2017/>.
4. Nielsen Perishables Group (2015) Fresh Produce Industry Overview. Available from: <https://www.pma.com/~media/pma-files/research-and-development/fresh-produce-industry-overview-2015.pdf?la=en&la=en>.
5. Berger C, Sodha S, Shaw R, et al. (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* 12: 2385–2397.
6. Redmond E, Griffith C (2003) A comparison and evaluation of research methods used in consumer food safety studies. *Int J Consum Stud* 27: 17–33.
7. Tauxe R, Doyle M, Kuchenmüller T, et al. (2010) Evolving public health approaches to the global challenge of foodborne infections. *Int J Food Microbiol* 139: S16–S28.

8. Painter J, Hoekstra R, Ayers T, et al. (2013) Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *J Emerg Infect Dis* 19: 407–415.
9. Hami A, Filiz Y, Yesim S, et al. (2017) Recent outbreaks of human pathogens on plants (HPOPs) on fresh produce-lessons learned from the practice. In: *Practical Tools for Plant and Food Biosecurity*, Springer, Cham, 77–96.
10. Bennett S, Sodha S, Ayers T, et al. (2018) Produce-associated foodborne disease outbreaks, USA, 1998–2013. *Epidemiol Infection* 146: 1397–1406.
11. Herman K, Hall A, Gould L (2015) Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. *Epidemiol Infect* 143: 3011–3021.
12. Anderson M, Jaykus L, Beaulieu S, et al. (2011) Pathogen-produce pair attribution risk ranking tool to prioritize fresh produce commodity and pathogen combinations for further evaluation. *Food Control* 22: 1865–1872.
13. Center for Disease Control (2018). Multistate outbreak of *Salmonella* Adelaide infections linked to pre-cut melon (final update). Available from: <https://www.cdc.gov/salmonella/adelaide-06-18/index.html>.
14. Lynch M, Tauxe R, Hedberg C (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol Infect* 137: 307–315.
15. Francis G, Gallone A, Nychas G, et al. (2012) Factors affecting quality and safety of fresh-cut produce. *Crit Rev Food Sci Nutr* 52: 595–610.
16. Little C, Gillespie I (2008) Prepared salads and public health. *J Appl Microbiol* 105: 1729–1743.
17. Choi J, Norwood H, Seo S, et al. (2016) Evaluation of food safety related behaviors of retail and food service employees while handling fresh and fresh-cut leafy greens. *Food Control* 67: 199–208.
18. Food and Drug Administration (2010) FDA trend analysis report on the occurrence of foodborne illness risk factors in selected institutional foodservice, restaurant, and retail food store facility types (1998–2008). Available from: <https://wayback.archive-it.org/7993/20170113095247/http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodborneIllnessRiskFactorReduction/UCM369245.pdf>.
19. Buchholz A, Davidson G, Marks B, et al. (2012) Quantitative transfer of *Escherichia coli* O157:H7 to equipment during small scale production of fresh-cut leafy greens. *J Food Prot* 75: 1184–1197.
20. Chai L, Lee H, Ghazali M, et al. (2008) Simulation of cross-contamination and decontamination of *Campylobacter jejuni* during handling of contaminated raw vegetables in a domestic kitchen. *J Food Prot* 71: 2448–2452.
21. Chen Y, Jackson K, Chea F, et al. (2001) Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J Food Prot* 64: 72–80.
22. Faour-Klingbeil D, Kuri V, Todd E (2016) The transfer rate of *Salmonella typhimurium* from contaminated parsley to other consecutively chopped batches via cutting boards under different food handling scenarios. *Food Res Int* 89: 495–503.
23. Grove F, Suriyanarayanan A, Puli B, et al. (2015) Norovirus cross-contamination during preparation of fresh produce. *Int J Food Microbiol* 198: 43–49.
24. Jensen D, Danyluk D, Harris J, et al. (2017) Quantifying bacterial cross-contamination rates between fresh-cut produce and hands. *J Food Prot* 80: 213–219.

25. Jensen D, Friedrich L, Harris L, et al. (2013) Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157: H7 between fresh-cut produce and common kitchen surfaces. *J Food Prot* 76: 1530–1538.
26. Zilelidou E, Tsourou V, Poimenidou S, et al. (2015) Modeling transfer of *Escherichia coli* O157: H7 and *Listeria monocytogenes* during preparation of fresh-cut salads: impact of cutting and shredding practices. *Food microbiol* 45: 254–265.
27. Sirsat S, Kim K, Gibson K, et al. (2014) Tracking Microbial Contamination in Retail Environments Using Fluorescent Powder-A Retail Delicatessen Environment Example. *J Vis Exp* 85: e51402.
28. Zerio-Egli C, Sirsat S, Neal J (2014) Development of a novel economical device to improve post-harvest processing practices on small farms. *Food Control* 44: 152–158.
29. Lopez G, Gerba C, Tamimi A, et al. (2013) Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity. *Appl Environ Microbiol* 79: 5728–5734.
30. Montville R, Chen Y, Schaffner D (2001) Glove barriers to bacterial cross-contamination between hands to food. *J Food Prot* 64: 845–849.
31. Todd EC, Michaels BS, Greig JD, et al. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 8. Gloves as barriers to prevent contamination of food by workers. *J Food Prot* 73: 1762–1773.
32. Food and Drug Administration (2017) FDA Food Code 2017. Washington, D.C. Available from: <https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm595139.htm>.



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