

Research Paper

Modified Coring Tool Designs Reduce Iceberg Lettuce Cross-Contamination

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ABSTRACT

Contaminated coring tools may transfer bacteria to iceberg lettuce. The efficiency of coring tool design modifications in reducing bacterial transfer to lettuce heads was evaluated under simulated field operations. The standard coring tool consists of a stainless steel cylindrical tube welded to a tab that is inserted into a plastic handle. Design modifications included removal of the welded portion, incorporation of a shorter front straight bottom edge, or an angled bottom edge toward the front. In the first study, coring tools of four different designs were inoculated by dipping in a tryptic soy broth (TSB) suspension that contained 8.85 Log CFU/mL of *Escherichia coli* K-12 and then were used to core 100 lettuce heads, consecutively. Use of the standard tool resulted in 91% \pm 9% positive lettuce heads. Removing the welded surface from the standard tool resulted in the highest reduction of *E. coli* transfer (44% \pm 11.9% positive lettuce heads, $P < 0.05$), whereas incorporation of a short front straight edge with no welding resulted in 65.6% \pm 5.6% of the cored lettuce heads being positive for *E. coli*. Removal of the welded surface resulted in a 40% decrease in *E. coli* contamination among the last 20 cored lettuce heads (81 to 100), which indicates that coring tool design modifications resulted in reduced cross-contamination. In the second study, the transfer of *Salmonella* to coring tools after their immersion in rinsing solutions was evaluated using imaging. The tools were dip inoculated for 2 min in water, water with lettuce extract, or TSB containing 7 Log CFU/mL bioluminescent *Salmonella* Newport; they were then imaged to observe spatial distribution of bacteria. There was greater retention and spatial distribution of *Salmonella* on the surface of tools immersed in water containing lettuce extract than in TSB and water. The results of the second study indicate that rinsing solutions that contain lettuce particulate and organic load could facilitate cross-contamination of *Salmonella* Newport to tool surfaces.

Key words: Bioluminescent imaging; Coring tool; Cross-contamination; Food safety; Lettuce; *Salmonella* Newport

Escherichia coli O157:H7 and *Salmonella* have been implicated as disease-causing contaminants of leafy greens such as lettuce (1, 8). The sources of these clinical pathogens could include the environment, contact surfaces, harvest tools, and improper handling (8, 9). Postharvest contamination of lettuce could result in large-scale cross-contamination and redistribution of pathogens during cutting and washing operations before packaging (2, 3).

Salmonella and *E. coli* are able to survive on food contact surfaces and, consequently, can be transferred to produce surfaces upon contact, resulting in cross-contamination (13).

Precut iceberg lettuce is a popular leafy green that is a common ingredient of salads (15). Immediately after harvest, but while the crop is still in the field, heads of iceberg lettuce are often cored to reduce the weight of the product for transport and to obtain an even consistency of fresh-cut product (16). The lettuce heads are cored by placing a stainless steel cylindrical coring blade to the stem scar and applying pressure. The coring tool is connected to a handle with a blade on the other side that is used to cut the outer leaves and the head from the stem. The stainless steel coring ring is inserted into the lettuce head and, when pulled out, excises the core. A contaminated coring tool can transfer bacteria into the cored lettuce and, thereby, cause cross-contamination (5).

The portion of coring tools that comes in direct contact with heads of iceberg lettuce is made of stainless steel. *E. coli* and *Salmonella* can attach to stainless steel by means of

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appendages such as curli fimbriae and through biofilm development (7). The initial attachment of transferred cells could also result due to the hydrophobicity of the bacterial cell and the free energy of the organism, dispersal media, and surface material (14). Coring tools can be contaminated by foodborne pathogens through contact with hands, soil, contaminated rinse water, and contaminated tissue of the iceberg lettuce heads. These tools are often used to core heads of lettuce consecutively for several hours and are sanitized with chlorinated water, peroxyacetic acid, or hydrogen peroxide during breaks or gaps in activity (5). The presence of organic matter, pitting, and welded joints on the coring tool could result in bacterial attachment to the surface of the tool (4). Fallon et al. (5) indicated that as many as 75 heads of lettuce could be contaminated by a coring tool dipped in 8 Log CFU/mL *E. coli*. They observed that the level of bacterial contamination was reduced but not completely avoided when the cored produce tissue was sprayed with chlorine (100 ppm) or peroxyacetic acid (40 mL/50 L) (5). Because conventional coring tools could serve as a vehicle of contamination of lettuce heads, optimization of coring tool design and operation practices could aid in reducing pathogen transference during lettuce harvest.

In this study, the design of the coring tool was modified to evaluate whether changes in the coring blade, the angle of the ring, and welding, in comparison to the current conventional design, could help reduce lettuce head cross-contamination during coring. Elimination of welded regions, different lengths of the coring blade, and the presence of an angular edge or ring on the coring blade were tested to study their impact on reducing bacterial cross-contamination to lettuce heads. Bacterial transfer from inoculated dips to coring tools was also evaluated. The aim of the study was to provide growers with information about optimal coring tool design to reduce cross-contamination of foodborne pathogens. A spatial map was developed to show foodborne pathogen distribution on coring tools of different designs after immersion in different solutions, to demonstrate practices or conditions that can contribute to bacterial cross-contamination onto tool surface and to serve as an educational aid for extension education.

MATERIALS AND METHODS

Bacterial strain used for the cross-contamination study. *E. coli* K-12 (ATCC 25253) was used to study the transfer of bacteria during coring of 100 heads of iceberg lettuce. This strain is resistant to 300 µg/mL streptomycin. The frozen culture was revived by performing two consecutive subcultures of a loopful of frozen culture in tryptic soy broth (TSB; Difco, BD, Sparks, MD) every 24 h at an incubation temperature of 37°C. After the second subculture, a loopful of media was streaked onto eosin methylene blue (Hardy Diagnostics, Santa Maria, CA) agar with 300 µg/mL streptomycin sulfate (Amresco, Solon, OH). The plates were incubated for 24 to 48 h at 37°C, and typical colonies were used for the experiment.

Bacterial strain used for bioluminescent imaging. For bioluminescent imaging of the coring tool to visualize *Salmonella* cross-contamination to the tool surface from simulated dips, *Salmonella* Newport SN78 transformed with pAKlux1 plasmid

(25 µg/mL ampicillin resistant) was used. Electro-transformation of *Salmonella* Newport was performed using the protocol described previously by Kumar et al. (10). Briefly, a setting of 2.5 kV, 25 µF, and 400 Ω was used on the Gene Pulser II system (Bio-Rad, Hercules, CA). Transformants were screened on tryptic soy agar (TSA; Difco, BD) with 25 µg/mL ampicillin. The growth rates of this strain were similar to those of nonresistant or untransformed strains of *Salmonella* Newport SN78. Frozen stock culture of the bioluminescent *Salmonella* Newport SN78 strain was revived by inoculating a loopful of frozen stock in TSB with 25 µg/mL ampicillin and incubating for 48 h at 37°C. A loopful of the turbid broth was streaked on TSA with 25 µg/mL ampicillin and incubated at 37°C for 48 h. Typical bioluminescent colonies were isolated and used for imaging experiments.

Coring tool designs. Four coring tool designs (Figs. 1 and 2) were compared in this study. The length of the yellow handle of the tool was 17.78 cm for all tool designs tested. The length of the front of the coring blade was 2.3 cm for all blades. The standard tool had a straight bottom edge core knife attached to the rest of the tool with a welded attachment (Fig. 1). The length of the standard coring tool blade from the handle was 7.5 cm. Coring tool design 1 had a short coring region with straight bottom edge and no welding. The length of the tool blade from the handle was 10.5 cm (Fig. 1). Coring tool design 2 had the same coring region as the standard tool with the coring tool blade length of 10.5 cm (Fig. 1). This tool did not have a welded attachment, unlike the standard tool. Coring tool design 3 had the regular coring region with the bottom edge ring angled (20°) toward the front (Fig. 1). The length of the coring tool blade was 7.5 cm, and there was no welding.

Inoculation of tools for cross-contamination study. Before inoculation, the coring tools were placed in a 2,000-ppm sodium hypochlorite solution (bleach, Clorox Company, Oakland, CA) overnight and then rinsed using sterile deionized water. After drying the tool with paper towels, the tools were sprayed with 70% ethanol and allowed to dry for 45 min. The coring tools were inoculated by immersing the tool's cylindrical edge in 250 mL of the overnight culture of *E. coli* K-12 (8.8 Log CFU/mL). Cells were grown for 18 to 24 h at 37°C. Contact with *E. coli* culture was facilitated for 2 min, during which the tool was rotated for uniform contact with the culture. The tool was removed and allowed to dry for 45 min at room temperature (25°C) in the biosafety cabinet. The control tool was immersed in sterile TSB for 2 min and allowed to dry as previously described for the test tools.

Lettuce coring. Iceberg lettuce heads were obtained from the Yuma Agricultural Center, Yuma, AZ. The outer leaves of the lettuce heads were removed, the coring blade was placed over the stem scar, and the lettuce was cored. The cored portion was removed from the tool and discarded. One hundred lettuce heads were cored consecutively to evaluate the transfer of contamination from each coring tool design to cored lettuce heads.

Microbiological analysis of lettuce heads. The portions of the cored head that had come in contact with the coring tool were excised using a scraping knife (pumpkin carving kit, Amscan Inc., Elmsford, NY), and then 10-g portions were scooped out and placed into a sterile stomach Whirl-Pak bag (Nasco, Fort Atkinson, WI). The 10-g portions of the cored lettuce were mixed with 90 mL of TSB (with 300 µg/mL streptomycin) for enrichment and were massaged to release the attached *E. coli*

Coring tool design	Design Parameter description	Image
Standard Tool	Straight bottom edge core knife attached with welding. Standard coring tool blade length from the handle is 7.5 cm.	
Design 1	Straight bottom edge core knife. No welding. Design 1 coring tool blade length from the handle is 10.5 cm.	
Design 2	Same coring region as the standard tool. No welding. Design 2 coring tool blade length from the handle is 10.5 cm.	
Design 3	Angled (20°) bottom edged core knife. No welding. Design 3 coring tool blade length from the handle is 7.5 cm.	

FIGURE 1. Coring tool designs and descriptions.

cells. The lettuce-TSB suspension was incubated for 18 h at 37°C. After incubation, a loopful of the enriched broth was streaked on eosin methylene blue agar supplemented with 300 µg/mL streptomycin sulfate. The plates were incubated for 48 h at 37°C and were examined for typical colonies.

Microbiological analysis of coring tool. An unused, inoculated coring tool and a coring tool that was used to core 100 lettuce heads were swabbed using a sterile cotton swab, using a template with a 9-cm² surface area. Five portions were evaluated for bacterial population by swabbing: back, side, front, bottom edge circumference, and inside (Fig. 3). After swabbing, the tip of the swab was broken and placed in 9 mL of TSB. The broth containing the tip was vortexed at a speed of 7 using a vortex mixer (VWR International, Radnor, PA) and was spread plated on eosin methylene blue agar supplemented with 300 µg/mL

streptomycin sulfate. The populations of *E. coli* were enumerated after appropriate serial dilutions were performed.

Evaluation of coring tool cross-contamination from wash water. The immersion of a coring tool in water to rinse the tool was simulated. The fluids for immersion included 400 mL of tap water with 50 mL of extract of pummeled iceberg lettuce to simulate reused wash water, 450 mL of TSB, and 450 mL of tap water as controls. Iceberg lettuce extract was prepared by pummeling 100 g of iceberg lettuce leaves in 100 mL of tap water for 2 min using a stomacher (Lab Blender 400, Seward, London, UK). All fluids were inoculated with 50 mL of suspension of the bacterial culture to obtain a final concentration of 7 Log CFU/mL *Salmonella* Newport SN78. To prepare the suspension, bacterial cells were centrifuged at 4,500 × g for 10 min (5810R, Eppendorf, Hamburg, Germany); then, the supernatant was decanted and vortexed to resuspend the pelleted cells in

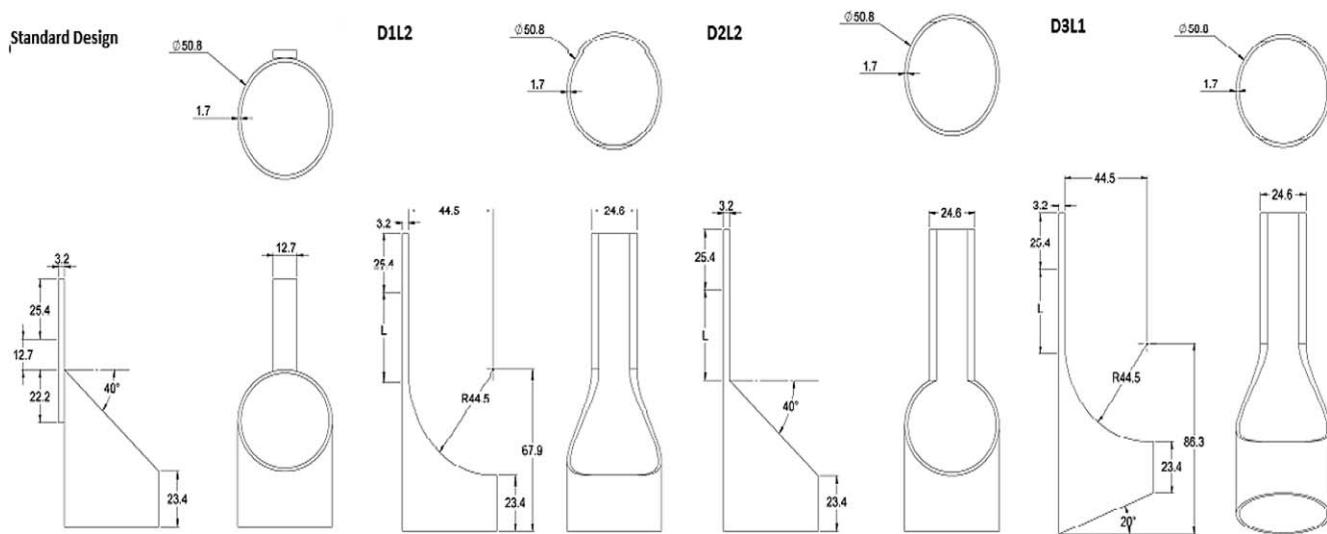


FIGURE 2. Coring tool design blueprints for (from left to right) standard design, design 1, design 2, and design 3.

sterile deionized water. This step was repeated to remove any residuals from the growth media. The dipping medium containing water, iceberg lettuce extract, and *Salmonella* Newport cells was used after 1 h to allow *Salmonella* cells to acclimatize to the suspension. The tools were dipped into the fluids for 2 min. Excess water was dabbed from the tool with a Kimwipe (Kimberly-Clark, Roswell, GA) to blot the fluid. The tool was then imaged using the AMI 1000 imaging system (Spectral Imaging, Tucson, AZ).

Bioluminescent imaging of lettuce core. To determine whether the cored portion of the lettuce also retained *Salmonella* Newport, bioluminescent imaging was done. The standard coring tool that was dip inoculated with bioluminescent *Salmonella* Newport, as described in the previous section, was used to core a head of lettuce. The core was imaged to visualize retention of the pathogen using the imaging system, as previously described.

Statistical analysis. The experiment was based on a randomized block design, with each experiment performed three times per treatment. The populations of *E. coli* on the front, back, sides, bottom edge circumference, and inside of the coring tool were analyzed and compared using the honest significant difference test. After enrichment of the tissues obtained from each lettuce head, the presence of colonies was considered positive for *E. coli* cross-contamination and the absence of colonies was considered negative. The percent positives were calculated for every 20 consecutive lettuce heads cored, and significant differences in the number of lettuce heads contaminat-

ed with *E. coli* from coring tool were determined. Significant differences between the percent transfer from coring tools to lettuce heads were determined using one-way analysis of variance (Minitab version 13.31 software, Minitab Inc., State College, PA). A level of significance lower than 5% was considered significant. Significant differences in bioluminescence from *Salmonella* Newport were determined by the software (Aura, Spectral Imaging) and expressed by different colors: the highest luminescence (photons per pixel) was denoted as red, lowest by blue, and average as green. Areas where no luminescence was detected were gray.

RESULTS

***E. coli* populations on the various regions of the coring tools.** The *E. coli* population in TSB into which the coring tools were dipped for 2 min was 8.85 ± 0.24 Log CFU/mL. No significant differences ($P > 0.05$) among the populations of *E. coli* on various coring tool designs (standard, design 1, design 2, design 3; Figs. 1 and 2) were observed. The back, sides, and front (Fig. 3) of the tools had *E. coli* populations of 4.39 ± 0.31 , 4.31 ± 0.49 , and 4.63 ± 0.70 Log CFU/9 cm², respectively (Fig. 4). The bottom edge circumference (Fig. 3) of the coring tools had 5.07 ± 0.42 Log CFU/9 cm². The inside portion (Fig. 3) of the coring tool had 5.12 ± 0.37 Log CFU/9 cm². All other portions of the four designs tested had no significant

- #1 Back
- #2 Side
- #3 Front
- #4 Bottom Edge Circumference
- #5 Inside

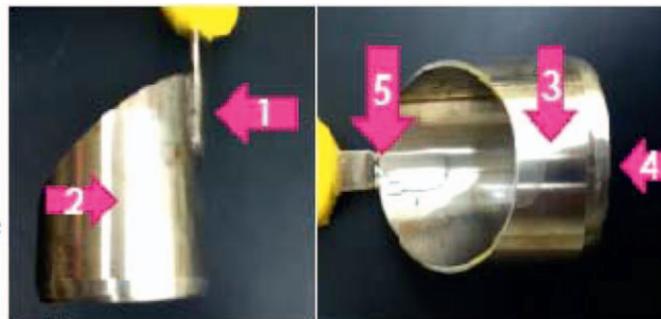
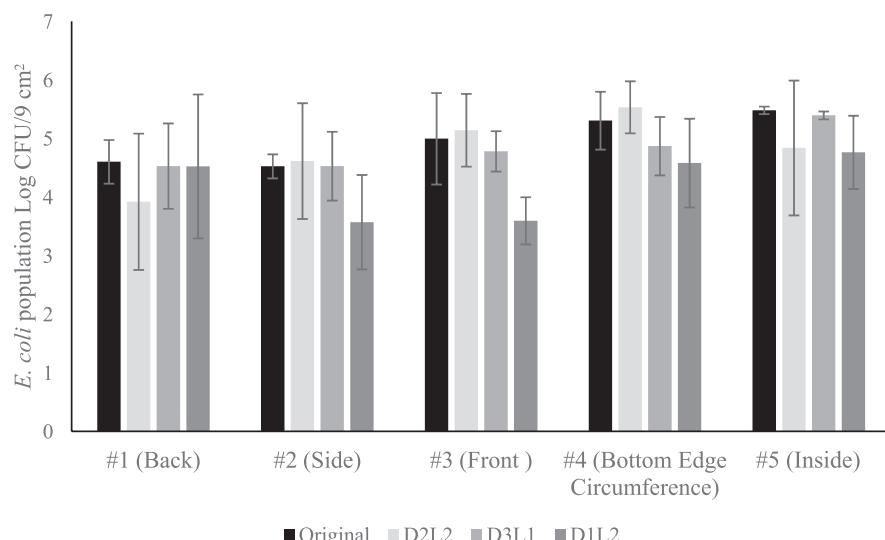


FIGURE 3. Areas of coring tools swabbed to determine *E. coli* populations before and after coring lettuce heads.

FIGURE 4. Population of *E. coli* on coring tool surface portions: 1, back; 2, side; 3, front; 4, bottom edge circumference; and 5, inside.



differences in the populations of *E. coli*. After coring 100 heads of lettuce, the only side on any of the tools that contained detectable levels of *E. coli* was the back portion of design 3 (0.37 ± 0.65), design 1 (0.37 ± 0.64), and design 2 (0.41 ± 0.57). *E. coli* was not recovered from the sides, front, bottom edge circumference, and interior portions of the coring tool after coring the 100 lettuce heads.

Standard design. The coring tool design affected the efficiency of *E. coli* transfer to cored lettuce heads. The standard coring tool (Figs. 1 and 2) resulted in the highest percentage ($91\% \pm 9\%$) of lettuce heads (Fig. 5) that were cross-contaminated with *E. coli* from the coring tool. The 26th lettuce head was the first lettuce head cored that was negative for the presence of *E. coli* when the standard coring tool was used. Two of three repeats with the standard tool resulted in the 100th lettuce head being positive for *E. coli*. When the standard tool inoculated with *E. coli* was used, 100% of the first 20 iceberg heads cored were positive (Fig. 6). Ninety percent of the cored iceberg lettuce heads from 21 to 40 and 41 to 60 were positive for *E. coli* (Fig. 6). Among lettuce heads 61 to 80 and 81 to 100, $88.33\% \pm 12.58\%$ and $86.66\% \pm 12.6\%$ of the cored iceberg lettuce heads were cross-contaminated with *E. coli*, respectively (Fig. 6).

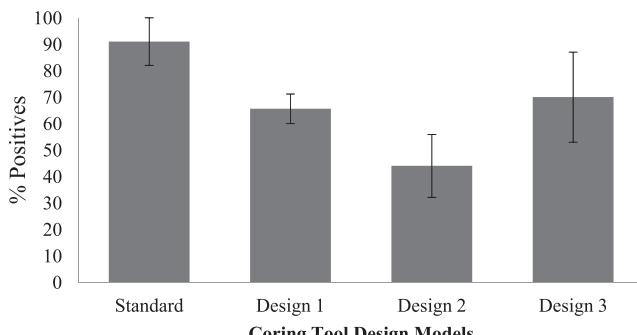


FIGURE 5. Percentage of the 100 lettuce heads that were positive for the presence of *E. coli* after coring, using the standard, design 1, design 2, and design 3 tools.

Coring tool design 1. The coring of iceberg lettuce heads with coring tool design 1 (Figs. 1 and 2) resulted in $65.6\% \pm 5.6\%$ (Fig. 5) of samples being positive for *E. coli*. The 21st lettuce head cored was the first found negative for the presence of *E. coli*. All three repeats of coring 100 heads resulted in the 100th head being negative for the presence of *E. coli*. The last lettuce head that was positive for the presence of *E. coli* was lettuce head number 98. Of the first 20 lettuce heads that were cored with the inoculated tool design 1, 100% were positive for the presence of *E. coli* (Fig. 6). The modified coring tool design 1 resulted in $73.34\% \pm 12.58\%$ of lettuces 21 to 40 being positive for *E. coli* (Fig. 6). Lettuces 41 to 60, cored in consecutive order, had an increase in cross-contaminated heads, with $81.66\% \pm 11.54\%$ positive for *E. coli* (Fig. 6). Lettuces 61 to 80 had $48.67\% \pm 11.67\%$ positive heads after coring. Of the final 20 lettuce heads cored with design 1 tool, $31.67\% \pm 10.4\%$ were positive for *E. coli* (Fig. 6).

Coring tool design 2. Coring tool design 2 (Figs. 1 and 2) was the most efficient in reducing cross-contamination of *E. coli* to iceberg lettuce heads. The number of positive heads that resulted from the use of this design were significantly different from that of the standard design ($P < 0.05$). Use of this tool resulted in $44\% \pm 11.9\%$ (Fig. 5) of iceberg lettuce heads being positive for the presence of *E. coli*. The first lettuce head that was negative for *E. coli* was the 16th consecutively cored head. Two of three repeats of the experiment resulted in the 100th cored lettuce head being positive for *E. coli*. Although coring the first 20 lettuce heads with a contaminated tool resulted in $96.66\% \pm 5.37\%$ positive samples, a significant decrease in lettuce heads contaminated with *E. coli* was observed in the next 20 lettuce heads cored (21 to 40), with only $56.66\% \pm 25.16\%$ lettuce heads being positive for *E. coli* ($P < 0.05$) (Fig. 6). Consecutive coring of lettuce heads 41 to 60 and 81 to 100 resulted in $26.67\% \pm 23.62\%$ and $26.67\% \pm 20.2\%$ of heads, respectively, being positive for *E. coli* (Fig. 6). The lowest percentage of *E. coli*-free lettuce heads occurred during consecutive coring of lettuce heads 61 to 80, because

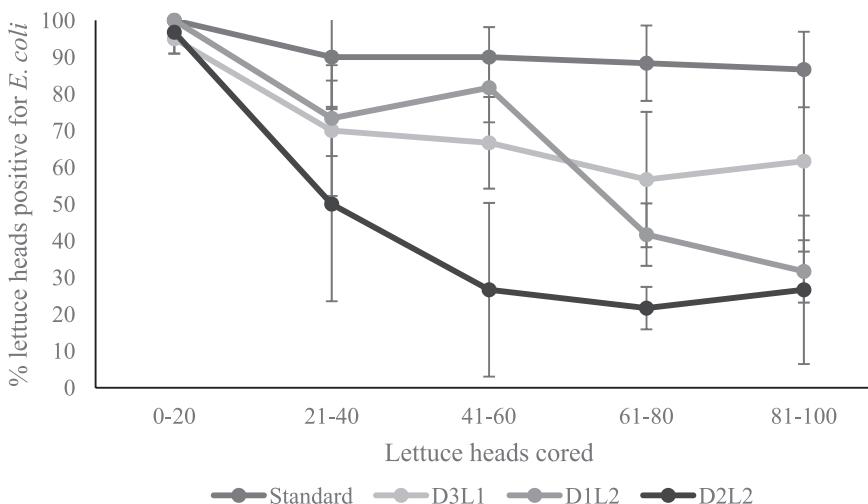


FIGURE 6. Percentage of progressively cored iceberg lettuce heads positive for the presence of *E. coli* using standard, design 1, design 2, and design 3 coring tools.

only $21.66\% \pm 1.15\%$ of the sampled heads were positive for *E. coli* (Fig. 6).

Coring tool design 3. Coring of iceberg lettuce heads with tool design 3 (Figs. 1 and 2) resulted in $70\% \pm 17.05\%$ (Fig. 5) of the cored heads being positive for the presence of *E. coli*. The 15th lettuce head cored was the first that was negative for *E. coli*. Two of three repeats that cored 100 iceberg lettuce heads using coring tool design 3 resulted in the 100th head being positive for *E. coli*. In the first 20 lettuce heads cored with tool design 3, $95\% \pm 5\%$ were positive for *E. coli* (Fig. 6). Of lettuce heads 21 to 40, $70\% \pm 21.8\%$ were positive for *E. coli*, and of 41 to 60, $66.66\% \pm 15.27\%$ were positive for *E. coli* (Fig. 6). Of lettuce heads 61 to 80 and 81 to 100, $56.67\% \pm 30.1\%$ and $61.67\% \pm 22.54\%$ were positive for *E. coli*, respectively (Fig. 6).

Bioluminescent imaging of coring tool. The TSB, water, and water with lettuce extract each had an average *Salmonella* Newport SN78 population of 7.73 ± 0.20 Log CFU/mL. Bioluminescent imaging of all the tools (standard, design 1, design 2, and design 3) after dipping into *Salmonella*-inoculated solutions to simulate contaminated wash water indicated that the highest cross-contamination of *Salmonella* Newport to the tool surface occurred from immersion in water containing the lettuce extract. Measurement of luminescence from the tool's surface (standard design) after immersion in water with lettuce extract resulted in the highest amount of luminescence from the inside of the tool, 3.1×10^7 photons per pixel (red coloration); this was significantly higher ($P < 0.05$) than the outside portions, 1.0×10^7 photons per pixel (blue coloration) (Fig. 7). *Salmonella* Newport was observed to be more dispersed on all tool surfaces that were immersed in water containing lettuce extract (C1, C2, C3, and C4; Fig. 7). Immersion of the tools in TSB containing *Salmonella* Newport also resulted in cross-contamination of the pathogen to the tool surface, and an even distribution of cells was observed for standard tools and design 3 tools (B1 and B4; Fig. 7). The highest luminescence measured was 1.35×10^7 photons per pixel (red) ($P < 0.05$) in the inside portion of the bottom edge. Other inoculated portions inside

the coring blade had a luminescence of 8.0×10^6 photons per pixel (yellow) (Fig. 7). Immersion of the tool in contaminated tap water resulted in lower or no cross-contamination of the pathogen to the tool surface in comparison to the other two solutions (D1, D2, D3, and D4; Fig. 7). The highest amount of luminescence detected was 1.36×10^7 photons per pixel (red) ($P < 0.05$); luminescence detected around the orange and yellow areas was 8.0×10^6 photons per pixel (Fig. 7). No luminescence was detected on any of the control tools (A1, A2, A3, and A4; Fig. 7). No significant difference was found among tools of different designs in bioluminescence after immersion in various solutions.

Bioluminescent imaging of lettuce core. Lettuce cores that came into contact with the coring tool contaminated by dipping in TSB-*Salmonella* Newport suspension were also positive for the presence of bioluminescent *Salmonella*. The luminescence measured from the cored portion was 2×10^8 photons per pixel (Fig. 8).

DISCUSSION

The results of this study indicate that coring tools are capable of transferring bacteria to the internal tissues of lettuce when consecutive lettuce heads are cored without a sanitation step. The design of the coring tool played an important role in bacterial cross-contamination to lettuce heads. Attachment of bacterial cells to a surface involves absorption, consolidation, and colonization over time (11); hence, the exposure of the stainless steel blade to the inoculum for 2 min, which was done for experimental purposes, might have aided in cross-contamination of bacteria due to physical forces such as surface charge of the cell, Van der Waals attraction, and ionic double layer interaction (11). The material of the coring tool, texture of the stainless steel (17), presence of a hydrophilic substrate, and negative electrostatic forces (6) could also have contributed to bacterial cross-contamination to lettuce tissue.

Different design models of coring tools were compared to the standard model that had the coring tool blade attached through welding. The changes in coring tool design

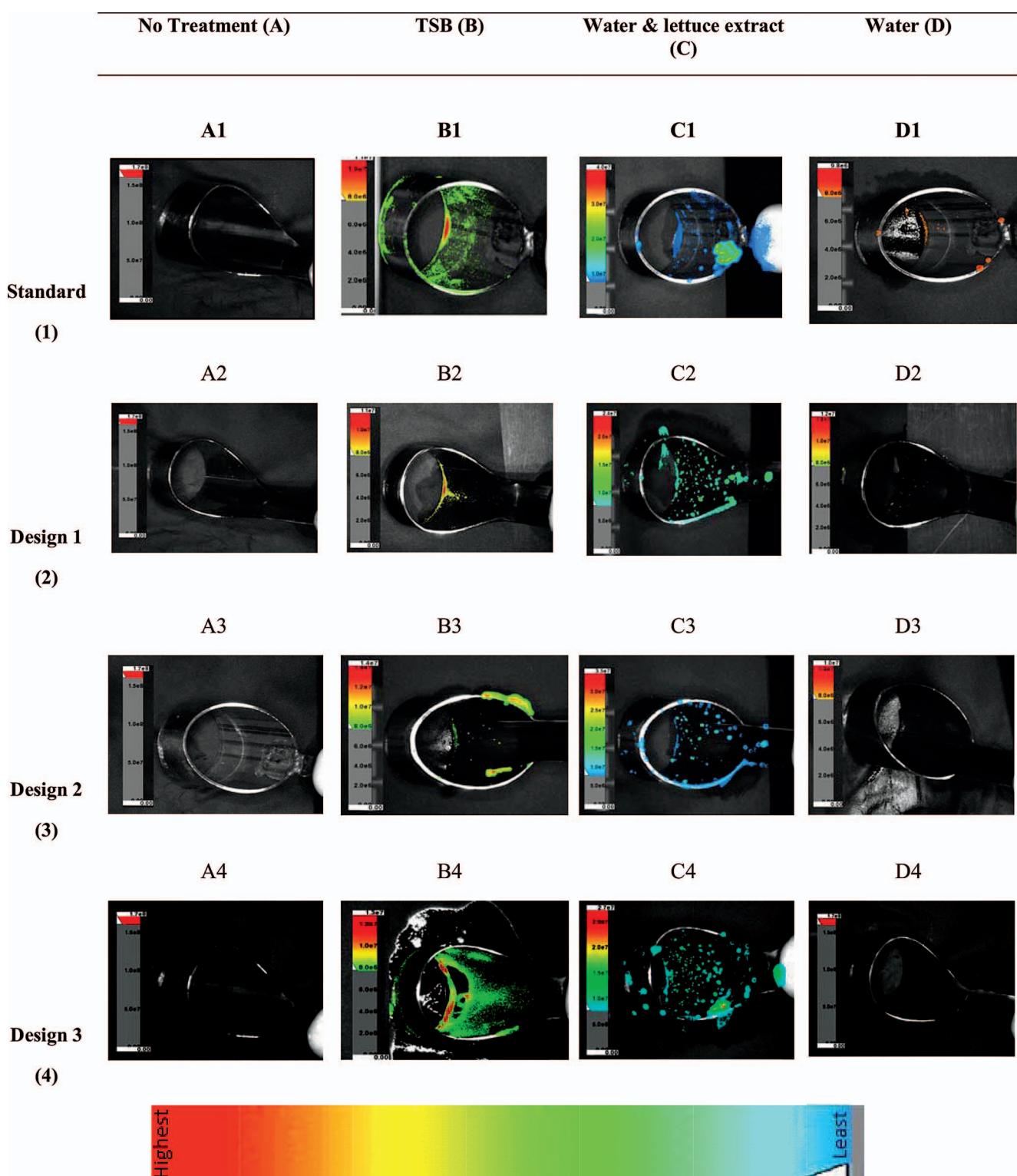


FIGURE 7. Spatial distribution of *Salmonella* Newport on coring tools with no treatment (A), after immersion in TSB (B), water with lettuce extract (C), and water (D). Rows 1 to 4 indicate standard, design 1, design 2, and design 3 coring tools, respectively. The color scale on the left of each panel indicates the intensity of luminescence.

included absence of a welded edge (designs 1, 2, and 3), a shorter coring region (design 1), and an angled edge (design 3) (Figs. 1 and 2). The results of the study indicate that changes in design, such as adding a shorter coring region or an angled edge and removing the welding, affected cross-contamination of *E. coli* to lettuce. The standard tool design

had a welded region at the junction of the handle and coring knife and resulted in over 90% of the cored lettuce heads being contaminated with *E. coli*. All other modified designs lacked a welded portion and were seamless (Fig. 1). Tools with seamless designs (Fig. 1) resulted in lower percentages of lettuce heads being contaminated after coring (Figs. 5

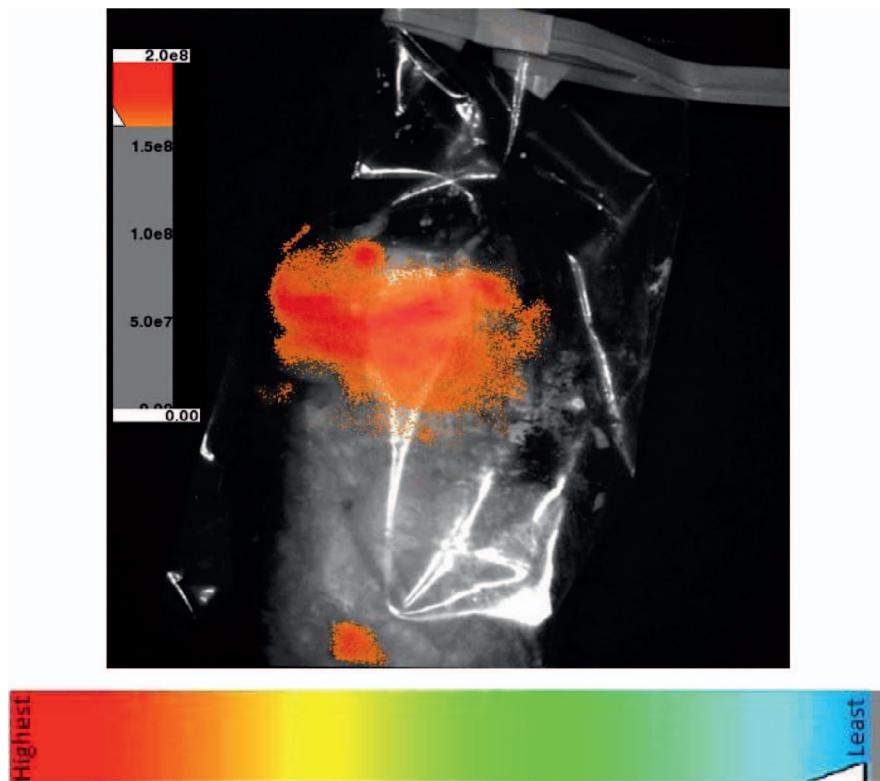


FIGURE 8. Lettuce core removed from a lettuce head using a coring tool that was immersed in a TSB suspension of bioluminescent *Salmonella* Newport. Colored portions of the core are indicative of contamination. The color scale on the left of the panel indicates the intensity of luminescence.

and 6). The recommended finish for stainless steel for food contact surfaces in the United States is the US No. 4 finish with a surface roughness of 1 μm or less (17) because rough surfaces could harbor bacteria and contribute to cross-contamination when abrasive contact occurs during coring (17). The retention of *E. coli* at the back of the coring tools even after coring 100 lettuce heads could have occurred because of increased roughness at the juncture of coring blade and handle.

Biophotonic imaging has previously been used to demonstrate the presence of foodborne pathogens on produce (10). Studying cross-contamination of foodborne pathogens in real time helps in a better risk assessment and can also serve as an educational tool. This study demonstrates the applicability of biophotonic imaging as a potential teaching tool for extension purposes. Using bioluminescent pathogens to educate stakeholders about cross-contamination of foodborne pathogens is novel. Determination of the spatial distribution of *Salmonella* using bioluminescent imaging indicated that the presence of lettuce extract in the water resulted in increased dispersion of *Salmonella* cells to the blade surface. Lettuce exudates in water could have resulted in increased adhesion of *Salmonella* to the coring tool in comparison to water without lettuce extract, by modifying the chemical and physical attributes of the water (12). Bioluminescent imaging of the cored portion of the lettuce also demonstrated retention of bacteria by the core (Fig. 8). This observation indicated that lettuce cores should not be discarded in the field and should be composted to prevent the reintroduction of pathogens into the field.

The results of our study indicate that contaminated coring tools can transfer bacteria to iceberg lettuce heads

during coring. The presence of foodborne pathogens in even a single head of lettuce can result in dissemination of foodborne pathogens when lettuce heads are shredded or washed. Coring tool design modifications, such as removal of welded regions, seamless edges, coring blade length, and angling of the blade, can significantly affect the cross-contamination of bacteria from the tool to the lettuce heads. Although it was observed that the presence of lettuce exudates and pieces in the wash water could result in increased dispersal of *Salmonella* on coring tool surfaces, additional research is required to understand the role of produce exudates in bacterial persistence on surfaces. The study also indicates that biophotonic imaging could serve as an effective tool to study the spatial distribution of bacteria after a cross-contamination event.

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REFERENCES

1. Ackers, M.-L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barrett, and L. Hutwagner. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.* 177:1588–1593.
2. Beuchat, L. R., B. B. Adler, and M. M. Lang. 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *J. Food Prot.* 67:1238–1242.
3. Buchholz, A. L., G. R. Davidson, B. P. Marks, E. C. Todd, and E. T. Ryser. 2014. Tracking an *Escherichia coli* O157:H7-contaminated

batch of leafy greens through a pilot-scale fresh-cut processing line. *J. Food Prot.* 77:1487–1494.

- 4. Casarin, L. S., A. Brandelli, F. de Oliveira Casarin, P. A. Soave, C. H. Wanke, and E. C. Tondo. 2014. Adhesion of *Salmonella* Enteritidis and *Listeria monocytogenes* on stainless steel welds. *Int. J. Food Microbiol.* 191:103–108.
- 5. Fallon, S. D., D. V. Rios, and J. M. Fonseca. 2011. Scrutinizing the sanitizing process involving aqueous chlorine from field to fresh-cut iceberg lettuce. *Acta Hortic.* 906:37–41.
- 6. Flint, S., J. Brooks, and P. Bremer. 2000. Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci. *J. Food Eng.* 43:235–242.
- 7. Hood, S. K., and E. A. Zottola. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *Int. J. Food Microbiol.* 37:145–153.
- 8. Horby, P., S. O'Brien, G. Adak, C. Graham, J. Hawker, P. Hunter, C. Lane, A. Lawson, R. Mitchell, and M. Reacher. 2003. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. *Epidemiol. Infect.* 130:169–178.
- 9. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog. Dis.* 1:27–35.
- 10. Kumar, G. D., R. C. Williams, H. M. Al Qublan, N. Sriranganathan, R. R. Boyer, and J. D. Eifert. 2017. Airborne soil particulates as vehicles for *Salmonella* contamination of tomatoes. *Int. J. Food Microbiol.* 243:90–95.
- 11. Notermans, S., J. Dormans, and G. Mead. 1991. Contribution of surface attachment to the establishment of microorganisms in food processing plants: a review. *Biofouling* 5:21–36.
- 12. Palmer, L., W. Merrill, M. Niederman, R. Ferranti, and H. Reynolds. 1986. Bacterial adherence to respiratory tract cells. Relationships between *in vivo* and *in vitro* pH and bacterial attachment. *Am. Rev. Respir. Dis.* 133:784–788.
- 13. Ravishankar, S., L. Zhu, and D. Jaroni. 2010. Assessing the cross contamination and transfer rates of *Salmonella enterica* from chicken to lettuce under different food-handling scenarios. *Food Microbiol.* 27:791–794.
- 14. Rivas, L., N. Fegan, and G. A. Dykes. 2007. Attachment of Shiga toxicogenic *Escherichia coli* to stainless steel. *Int. J. Food Microbiol.* 115:89–94.
- 15. Salgado, S. P., A. J. Pearlstein, Y. Luo, and H. Feng. 2014. Quality of iceberg (*Lactuca sativa* L.) and romaine (*L. sativa* L. var. longifolia) lettuce treated by combinations of sanitizer, surfactant, and ultrasound. *Lebensm-Wiss. Technol. Food Sci. Technol.* 56:261–268.
- 16. Saltveit, M. E., and L. Qin. 2008. Heating the ends of leaves cut during coring of whole heads of lettuce reduces subsequent phenolic accumulation and tissue browning. *Postharvest Biol. Technol.* 47:255–259.
- 17. Tide, C., S. R. Harkin, G. G. Geesey, P. J. Bremer, and W. Scholz. 1999. The influence of welding procedures on bacterial colonization of stainless steel weldments. *J. Food Eng.* 42:85–96.