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12-22-2021

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Journal of Food Protection, Vol. 85, No. 4, 2022, Pages 632–638 https://doi.org/10.4315/JFP-21-334 Published 2022 by the International Association for Food Protection Not subject to U.S. Copyright

Research Note

Evaluation of *Salmonella* Biofilm Cell Transfer from Common Food Contact Surfaces to Beef Products

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MS 21-334: Received 30 August 2021/Accepted 16 December 2021/Published Online 22 December 2021

ABSTRACT

Meat contamination by *Salmonella enterica* is a serious public health concern. Available data have suggested that biofilm formation at processing plants and contaminated contact surfaces might contribute to meat contamination. Because transfer from contact surfaces to food products via direct contact has been deemed the most common bacteria transmission route that can lead to contamination, we evaluated the effect of *Salmonella* biofilm forming ability, contact surface material, and beef surface tissue type on *Salmonella* biofilm transfer from hard surfaces to beef products. *Salmonella* biofilms developed on the common contact surfaces stainless steel (SS) and polyvinylchloride (PVC) were transferred consecutively via 30 s of direct contact to either lean muscle or adipose tissue surfaces of 15 pieces of beef trim. The *Salmonella* biofilm cells could be effectively transferred multiple times from the contact surfaces to the beef trim as indicated by quantifiable *Salmonella* cells on most meat samples. Biofilm forming ability had the most significant impact (P < 0.05) on transfer efficiency. More cells of *Salmonella* biofilms on SS transferred more efficiently than did those on PVC. In contrast, the two types of meat surface tissues were not significantly different (P > 0.05) in biofilm transfer efficiency. Beef trim samples that were in contact with biofilms but did not have quantifiable *Salmonella* cells were positive for *Salmonella* after enrichment culture. Our results indicate the high potential of *Salmonella* cells were positive for *Salmonella* after enrichment culture. Our results indicate the high potential of *Salmonella* cells were positive for *Salmonella* after enrichment culture. Our results indicate the high potential of *Salmonella* cells were positive for *Salmonella* after enrichment culture. Our results indicate the high potential of *Salmonella* cells were positive for *Salmonella* after enrichment culture.

HIGHLIGHTS

- Salmonella biofilms transferred multiple times from hard surfaces to beef products.
- Strong biofilm formers transferred more bacteria and contaminated more meat samples.
- · Biofilms on SS transferred more efficiently than did those on PVC.
- Beef samples in contact with biofilms did not always have quantifiable Salmonella cells.
- Salmonella-negative contact samples were sometimes positive after enrichment culture.

Key words: Bacterial transfer; Beef products; Food contact surface; Salmonella biofilms

The many serotypes of Salmonella enterica are estimated to cause 93.76 million illnesses worldwide annually (10). In the United States, exposure to Salmonella results in approximately 1.028 million illnesses, 19,300 hospitalizations, and 400 deaths each year (20). Most human salmonellosis cases are associated with the consumption of contaminated foods such as red meat and poultry (19). Cattle are one of the major known animal reservoirs of Salmonella, and pathogen transfer from hide to carcass during processing is an established route of product contamination in meat plants. However, contaminated food contact surfaces also may contribute to meat product contamination. Foodborne pathogen contamination was found more often on subprimals and steaks after 24 h of chilling than on the original beef carcasses (22), suggesting that additional contamination events might have occurred during fabrication, packaging, and distribution. In previous investigations (11, 23, 27–29, 31), contamination was attributed to other microbial sources such as bacterial biofilms in the processing and postprocessing environment.

Biofilm formation is an important strategy that helps bacteria survive under adverse conditions because cells in biofilms are much more resistant to sanitizers or other physical and chemical treatments than are planktonic cells. Biofilm cells that survive antimicrobial treatments can be a source of contamination when they detach from food contact surfaces. The most common transmission route leading to cross-contamination in the food processing environment is bacterial transfer from contact surfaces to food products via direct contact (14). A Salmonella Typhi infection outbreak that led to 469 cases of typhoid fever in

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TABLE 1. Salmonella enterica strains used to inoculate beef trim, their EPS (curli and cellulose) expression, and biofilm formation on S	S
and PVC surfaces	

Salmonella serotype	Strain	EPS expression ^a		Biofilm $(\log \text{CFU/cm}^2)^b$	
		Curli	Cellulose	SS	PVC
Anatum	MARC-MB-836	+	+	4.60 (0.09) A	4.46 (0.11) a
	FSIS1500556	_	_	3.58 (0.55) вс	3.17 (0.01) C
Dublin	FSIS1500571	+	+	4.73 (0.11) A	4.82 (0.10) A
	MARC-MB-463	_	_	3.71 (0.48) вс	4.24 (0.02) AB

^a EPS expression was determined based on colony morphology on Congo red indicator plates and LB agar plates containing calcofluor dye.

^b Each strain was allowed to form biofilms on SS or PVC surfaces for 5 days at 7°C. Values are means (n = 3) and standard deviations. Means followed by different letters are significantly different (P < 0.05).

Aberdeen, Scotland was traced back to one contaminated container of delicatessen-sliced corned beef; the pathogen was transferred from the delicatessen slicer to the deli meats (5). In our previous study (29), we characterized a wide collection of *S. enterica* strains isolated from contaminated beef trim and found that the vast majority of these strains were able to develop strong biofilms and had a high tolerance to common sanitizers. These results suggest that *Salmonella* biofilm cell transfer from contact surfaces to meat products could pose a serious risk to meat safety.

Many factors could affect the transfer efficiency of biofilm cells to food products. The specific properties of the biofilms, such as cell density, three-dimensional structure, cell surface expression of extracellular polymeric substances (EPS), attachment forces between colonized bacteria and contact surfaces, and the coexistence of other bacterial species in the mixed biofilm community, would most likely affect biofilm cell transfer (8, 12, 13, 16, 17). The composition of the food products also could play a role in the dissemination of biofilm cells. Some aspects of food composition, such as high fat and high moisture, could increase the transfer of Listeria monocytogenes biofilm cells to salmon products (4). The contact surface material and the surface hydration also could influence biofilm attachment, which would in turn affect biofilm detachment and dissemination of the biofilm cells. In one study, the attachment of L. monocytogenes biofilms to polyvinyl chloride (PVC) or polyurethane surfaces was significantly stronger than attachment to a stainless steel (SS) surface, and the type of surface substantially affected the detachment process and the transfer efficiency of the biofilm cells (12). However, the impact of bacterial biofilm forming ability, contact surface materials, and red meat surface tissue types on Salmonella biofilm transfer to beef product has not been investigated. The objective of the present study was to evaluate the impact of these factors by quantifying the Salmonella cells transferred from biofilms on common contact surfaces (SS or PVC) to beef trim. The effect of meat surface composition (lean muscle versus adipose tissue) on biofilm cell transfer efficiency also was evaluated. We also determined the prevalence of Salmonella in beef trim samples after brief direct contact with common surfaces colonized by various levels of Salmonella in biofilms.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and EPS expression. Four S. enterica strains of serotypes Anatum and Dublin were used in this study (Table 1). Two strains (FSIS1500556 and FSIS1500571) were isolated from contaminated beef trim by the U.S. Department of Agriculture, Food Safety Inspection Services (FSIS), and the other two (MARC-MB-836 and MARC-MB-463) were isolated from enriched samples of postintervention carcasses at the U.S. Meat Animal Research Center (MARC). All strains had been screened for their biofilm forming ability on 96-well polystyrene plates with crystal violet staining (29) and were identified as strong or weak biofilm formers (Table 1). The expression by these strains of curli and cellulose fimbriae, the two major bacterial extracellular polymeric structures associated with biofilm forming ability and sanitizer tolerance, were tested as previously described (26) using Congo red indicator plates and Lennox formula Luria-Bertani (LB) broth agar plates containing calcofluor dye, respectively.

Biofilm formation on materials commonly used in the meat industry. For the biofilm formation experiments, bacterial broth cultures at stationary phase were prepared in LB broth (Acumedia, Baltimore, MD) without salt (LB-NS) as described previously (26) then further diluted in fresh sterile LB-NS medium for each experiment. Biofilm formation by each strain on SS and PVC contact surfaces commonly used in the meat industry were quantified with a colony enumeration method on agar plates as previously described (28). Sterile SS (18 by 18 by 2 mm) and PVC (14 by 12 by 3 mm) chips were prepared as platforms on which biofilms were allowed to develop by incubation for 5 days at 7°C in 1:100 diluted overnight (18 to 20 h) bacterial cultures in LB-NS broth containing approximately 5×10^6 cells per mL. At the end of the incubation period, each chip was rinsed with 10 mL (5 mL per side) of sterile phosphate-buffered saline (PBS; pH 7.2; Sigma-Aldrich, St. Louis, MO). After rinsing, SS chips were transferred to 50-mL centrifuge tubes each containing 1.0 g of glass beads (425 to 600 µm; Sigma-Aldrich) in 10 mL of sterile LB-NS broth, and PVC chips were transferred to 15-mL centrifuge tubes each containing 0.5 g of glass beads in 5 mL of sterile LB-NS broth. All tubes were sonicated for 1 min then vortexed at maximum speed for 2 min to remove the attached biofilm cells. The vortexed suspensions were 10-fold serially diluted in sterile LB-NS broth, and the appropriate dilutions were plated onto xylose lysine deoxycholate (XLD) agar plates (Oxoid, Remel, Lenexa, KS) without antibiotics for colony enumeration after overnight incubation (18 to 20 h) at 37°C. Because biofilms were formed on both sides of the chips, biofilm formation by each strain was

calculated from the total CFU recovered from the chip surface divided by 2 and then divided by the surface area of the chip (3.24 cm^2 for SS chips and 1.68 cm^2 for PVC chips).

Biofilm cell transfer from contact surfaces to beef trim. Beef flanks were cut into uniform size and shape (ca. 40 by 50 mm). Each meat sample was placed in a sterile petri dish with either lean muscle surface or adipose tissue surface facing up and then treated by direct exposure to UV light for 30 min to reduce background microflora. Each Salmonella strain was allowed to develop biofilms on the SS or PVC chips as described above. After the PBS rinse and 5 min to air dry, each chip was placed on either the lean muscle or the adipose tissue surface of the meat sample in each petri dish, ensuring that the entire chip surface was in full contact with the meat surface. After 30 s of direct contact, the meat sample was aseptically transferred to a filtered stomacher bag containing 50 mL of sterile LB-NS broth, and the same meatcontact side of the chip was placed on the surface of the second meat sample in a new petri dish and processed following the same procedure as used for the previous meat sample. This experimental step was repeated using the meat-contact side of the same chip for a total of 15 meat samples. The chip-contact meat samples were then thoroughly homogenized in individual stomacher bags with a paddle blender stomacher (BagMixer 400 CC, Interscience, Woburn, MA) by vigorous agitation for 1 min. A 1.0-mL aliquot was removed from each bag, plated onto XLD agar plates at 100 µL per plate, and incubated at 37°C overnight (18 to 20 h) for enumeration and calculation of the transferred Salmonella cells after each contact. The transferred Salmonella cells could be distinguished from the meat surface background microorganisms by formation of unique black colonies. The Salmonella transfer rate was determined as the log CFU per square centimeter per transfer based on the counts on agar plates and the respective chip surface area. Meat samples without biofilm transfer were included as negative controls to monitor background microorganisms and to ensure no Salmonella presence on the noncontact meat samples.

Determination of *Salmonella* **prevalence after biofilm contact.** After the 1.0-mL aliquot was removed from each bag for enumeration of the transferred *Salmonella* cells, the stomacher bags containing the meat samples were enriched at 37° C overnight (18 to 20 h) and then plated onto XLD agar plates to determine *Salmonella* prevalence after the enrichment. The negative control samples without biofilm transfer were also enriched by following the same procedure. The isolated presumptive *Salmonella* black colonies were further confirmed by PCR amplification of the *Salmonella*-specific *invA* target gene (*30*).

Statistical analysis. Results were analyzed as the mean of three experimental replicates and three negative controls, which all were negative for *Salmonella* both before and after the enrichment procedure. An analysis of variance (ANOVA) and comparisons of mean \pm standard deviation biofilm cell density on SS and PVC surfaces and 95% confidence intervals were performed using Prism software (GraphPad Software, La Jolla, CA). A one-way ANOVA was performed with Tukey's post hoc test.

The number of *Salmonella* cells transferred to the meat surfaces from biofilms on SS or PVC surfaces was log transformed and analyzed as a complete block design with a $2 \times 2 \times 2$ (biofilm forming ability × contact surface material × meat surface tissue type) treatment structure. Within each sequential transfer event, data were analyzed using the PROC GLIMMIX procedure of SAS (SAS Institute, Cary, NC), and LSMeans were generated for significant interactions and main effects. Those LSMeans not

involved in higher order interactions were separated with the diff option. Results were considered significant at P < 0.05.

RESULTS AND DISCUSSION

In the present study, Salmonella serotypes Anatum (strains FSIS1500556 and MARC-MB-836) and Dublin (strains MARC-MB-463 and FSIS1500571) were used to evaluate the effect of biofilm forming ability, contact surface materials, and meat surface tissue types on the transferability of Salmonella biofilm cells from colonized surfaces to beef trim products. Strain selection was based on the type of biofilm forming ability (strong or weak) and the expression of EPS (positive or negative). The strong biofilm formers of each serotype had significantly higher levels (P < 0.05) of colonized biofilm cells than did the weak biofilm formers so the impact of biofilm forming ability on bacterial transfer efficiency could be determined. The biofilm density (log CFU per square centimeter) of each selected strain also was not significantly different (P > 0.05) on SS and PVC surfaces, so the effect of the contact surface materials on Salmonella biofilm transferability could be determined (Table 1).

Salmonella biofilm cells were transferred effectively from either SS or PVC surfaces to beef trim multiple times via brief direct contact of 30 s (Fig. 1). The 30-s contact time was based on the observation of mean contact time at commercial meat plants where beef cuts briefly rested on contact surfaces during processing. In the present study, the vast majority of the trim samples had quantifiable Salmonella cells on either lean muscle or adipose tissue surfaces after each direct contact with the biofilm-colonized chips. However, the trim samples consistently received the highest number of Salmonella cells from the first transfer, then the number of transferred cells decreased with each successive transfer event. This pattern differed from that observed in a previous study in which a dynamic slicing model was used to investigate transfer of surface-dried L. monocytogenes biofilms to turkey or salami (8). In that study, the level of bacteria transferred during the first slice was consistently lower than the level transferred to the second slice and beyond. However, in that study L. monocytogenes biofilms were transferred after prolonged desiccation (6 or 24 h), and higher levels might have been transferred after exposure to the contact surface (SS slicer blades) with moisture from the food product (turkey or salami) during the first slice and due to the friction from slicing. In contrast, the Salmonella biofilms in the present study were transferred without a desiccation period. The hydration level of the contact surfaces is important for biofilm transfer, which requires a capillary effect or liquid bridge between the biofilm cells and the moisture on the food product surface. In a previous study, the transfer of Staphylococcus aureus biofilms was more efficient when the contact surfaces were wet or moist (18). In our study, the meat samples received the highest number of Salmonella cells from the first transfer probably because freshly rinsed Salmonella biofilms were used for the transfer, so that the hydration level of the chip surface was sufficient and affected the initial transfer of the biofilm cells. The differences between the pathogens (Salmonella versus

S.S

P.V.C

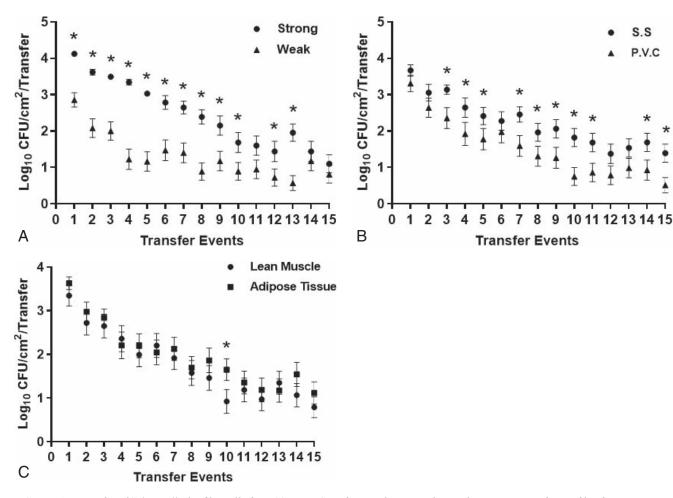


FIGURE 1. Transfer of Salmonella biofilm cells from SS or PVC surfaces to lean muscle or adipose tissue surfaces of beef trim. Data are the quantifiable transferred Salmonella biofilm cells at each transfer event separated by biofilm forming ability (A), contact surface material (B), and meat surface tissue (C). Differences within each sequential transfer event were evaluated with a complete $2 \times 2 \times 2$ block design (biofilm ability \times contact material \times meat tissue) as described in the "Materials and Methods." Data are means \pm standard deviations of three replicates. Asterisks indicate significant differences (P < 0.05) between the two comparators of each transfer event.

Listeria) and the research models (dynamic versus static contact) used in the two studies might also explain the differences in the initial transfer patterns, but these differences require further investigation.

Within each series of 15 consecutive transfer events, overall higher numbers of trim samples received quantifiable Salmonella cells from the strong biofilm formers than from the weak biofilm formers. Transfer of Salmonella cells below the level of quantification ($<2.7 \log CFU$) mostly occurred on trim samples that had been in contact with the PVC chips colonized by the two weak biofilm formers. Salmonella Anatum MARC-MB-836 (strong biofilm former) transferred quantifiable biofilm cells from the PVC surface to adipose tissue surface of all 15 trim samples, whereas only 6 adipose tissue samples received quantifiable Salmonella cells from biofilms of Salmonella Anatum FSIS1500556 (weak biofilm former) on the PVC surface. Salmonella Dublin FSIS1500571 (strong biofilm former) and MARC-MB-463 (weak biofilm former) transferred quantifiable biofilm cells from PVC surfaces to 13 and 3 trim samples, respectively, on the lean muscle surface. This observation is consistent with those of a previous study (24) of transfer of L. monocytogenes to turkey breast and salami sliced with inoculated SS knives, in which L. monocytogenes transfer was quantifiable on up to 30 slices with an initial inoculum of 10⁸ CFU per blade, whereas blades inoculated with 10^5 and 10^3 CFU yielded quantifiable L. monocytogenes only up to 20 and 5 slices, respectively. The strong biofilm formers contaminated higher numbers of food samples because of the general decrease in the biofilm cell population after each transfer. Keskinen et al. (9) investigated the effect of *Listeria* biofilm forming ability on bacterial transfer rate during slicing of delicatessen turkey meat and observed that Listeria biofilm populations decreased by 3 to 5 log CFU per slice after 16 slices. Thus, the cell density of the surface colonized bacteria had a significant impact on the number of food samples that could be contaminated by contact with surface biofilms.

Of more significance than the number of trim samples with quantifiable transferred bacteria was the actual mean log Salmonella cells detected on the meat surface after each transfer, which revealed that the biofilm transfer efficiency was highly strain dependent. Overall, bacterial transfer rate was proportional to the Salmonella biofilm cell density of each strain on the chip surface. When the data on the level of bacteria transferred from each square centimeter of the

contact surface were pooled and compared between the strong and weak biofilm formers, on the PVC surface the biofilm cell density of the weak biofilm former MARC-MB-463 was not significantly different from that of the two strong biofilm formers; however, the strong biofilm formers transferred significantly higher levels of bacteria (P < 0.05) to the trim samples for the first 10 consecutive transfers and at transfer events 12 and 13. This finding was consistent regardless of contact surface material and meat surface tissue type (Fig. 1A).

Numerous studies of L. monocytogenes biofilm transfer to various food types have revealed a similar pattern, indicating that biofilm cell population had the greatest effect on the level of quantifiable bacteria transferred to beef products (12), roast turkey breast (9), salmon products (4), and Genoa hard salami (8). Our observation of no significant difference (P > 0.05) in level of transferred biofilm cells between the strong and weak biofilm formers at transfer events 14 and 15 was likely due to the general decrease in the biofilm cell population after each transfer. This general decrease is referred to as the self-cleaning process (8, 9) and will finally result in low to nonquantifiable levels of transferred bacteria and will limit the number of food samples that could be contaminated by the biofilm-colonized contact surface. Involvement of such a biofilm self-cleaning process in the potentially biofilm-associated high event period contaminations (27, 28), which are usually resolved before any operational correction or specific intervention can be performed, is being investigated.

Statistical analysis further indicated that the contact surface material could affect Salmonella biofilm transfer efficiency. Of the two types of materials that we tested, SS chips appeared to allow more efficient transfer of Salmonella biofilms to trim samples than did PVC chips. When the data for transfer of the four Salmonella strains were pooled and compared between the SS and PVC surfaces, 10 of the 15 direct contacts transferred significantly higher levels of biofilm cells (P < 0.05) to the trim samples from each square centimeter of SS surface than was transferred from the PVC surface (Fig. 1B). No difference (P > 0.05)between the two types of materials was observed for transfer events 1 and 2, suggesting that bacterial transfer from biofilms with sufficiently high cell density would be much less affected by the contact surface material. During the initial transfer events, the transferred bacteria were mostly from the biofilm exterior surface; therefore, the tightness of adhesion between the biofilms and contact surface had less of an effect on the transfer efficiency. When the bacterial cell density decreased after a few transfer events, the adhesion between the biofilm cells at the bottom layers of the matrix and the contact surface would have a greater effect on bacterial detachment.

The influence of material types on the adhesion (attachment strength) between biofilms and contact surfaces, which would in turn affect biofilm detachment and bacterial transfer was reported previously. The attachment strength between *L. monocytogenes* biofilms with polyvinylchloride or polyurethane surfaces was found to be significantly greater than that with stainless steel surface, which substantially affected the detachment process and the

transfer efficiency of the biofilm cells (12). Similarly, Rodríguez et al. (17) observed that *L. monocytogenes* biofilms on stainless steel surface could transfer higher amounts of the bacteria to bologna and American cheese than those on high-density polyethylene surface. Conversely, a more recent study by Jeon et al. (6) reported that *L. innocua* biofilm cells were transferred to duck meat more efficiently from polypropylene surface than those from stainless steel surface. The different observations are likely due to the pathogen species, food types, the adhesion strengths of the microorganisms on the surfaces and the various experimental methods/conditions that were applied. Therefore, the impact of contact surface materials on transfer efficiency of biofilms by the various pathogens warrants further investigation.

In contrast, the two types of meat surface tissues (lean muscle and adipose tissue) had no significant effect (P >0.05) on Salmonella biofilm transfer efficiency. However, previous report suggest that the characteristics of the food surface receiving the biofilm cells could affect the dissemination and transfer of these cells. Higher fat and moisture contents have been related to the increased transfer of L. monocytogenes biofilm cells to some meat products (4, 24). Differences in fat globule size and variations in carbohydrate, protein, and moisture composition of the food types were associated with the higher transfer efficiency of L. monocytogenes biofilm cells to chicken and pork bologna compared with transfer to cheese and hard salami (16, 17). More relevant to our study, Wang et al. (25) found that the transfer of Salmonella biofilm cells to five types of meat products after 30 s of direct contact was highly dependent upon the product, with bacon and emulsified sausage receiving higher levels of bacteria than roast pork. Those authors attributed this finding to differences in the food surface texture, moisture content, and fat content. However, that study was conducted with Salmonella biofilms formed at 20°C, whereas we developed Salmonella biofilms at 7°C to simulate meat fabrication conditions. Hydration of the biofilms (40 min of air drying versus freshly rinsed in our study) and contact pressure (500 g over the contact area of 10 cm² versus no added weight in our study) could be the other factors affecting the differences in these findings.

Compared with the adipose tissue surface, the lean muscle surface has a higher moisture content, which favors bacterial transfer due to the capillary effect and presence of a liquid bridge between the bacteria and food surface (14). However, in the present study the meat samples were UV sterilized for 30 min to minimize surface background microorganisms, which might also reduce the difference in the moisture content between the two types of meat tissues. Thus, various factors and their interactions would add additional unpredictability and variations to these results.

All meat samples without quantifiable *Salmonella* (<2.7 log CFU) immediately after contact with the biofilm-colonized chips were positive for *Salmonella* in the overnight enrichment culture, regardless of the biofilm forming ability of the strain, contact surface material, or meat surface texture. This high pathogen prevalence after brief contact with surface-colonized biofilms has also been reported previously. Possas et al. (15) observed that slicer

blades inoculated with *Salmonella* Enteritidis at 10^8 CFU/mL could cross-contaminate turkey products for up to 20 slices. In another study (24) of *L. monocytogenes* transfer to turkey breast, bologna, and salami products, the pathogen was found in up to 27 and 15 slices of meat sliced by the blades inoculated at 10^5 and 10^3 CFU/cm², respectively. In our study, *Salmonella* biofilm cell density on SS and PVC surfaces was ca. 3.5 to 5.0 log CFU/cm², and the presence of *Salmonella* in all 15 transfer events indicates the efficiency with which *Salmonella* biofilms can cross-contaminate meat products from food contact surfaces at processing plants.

Although we conducted this study with materials and temperatures commonly applied in the meat industry to simulate actual processing conditions, other aspects of the industrial practice and environment can be more complicated due to many factors such as contact surface liquid; the presence of meat juices (instead of laboratory broth medium), soil, and various bacteria brought by animals; and other unpredictable operation disruptions that may impact biofilm formation and the pattern of bacterial transfer. Nevertheless, the meat processing environment and equipment, if not properly cleaned and sanitized, could become a major harborage for foodborne pathogens such as S. enterica. Product contamination during meat processing operations such as fabrication is a serious food safety concern to the meat industry and consumers; therefore, much research effort has been directed toward understanding and preventing cross-contamination by pathogens at commercial meat plants. The potential involvement of pathogenic biofilms in meat contamination at processing plants has been suggested previously (23, 27-29, 31). Numerous studies have been conducted (1, 3, 4, 8, 9, 12, 21) to investigate the transferability of Escherichia coli O157:H7 and L. monocytogenes biofilm cells in model systems simulating dynamic (slicing) or static contact between food products and biofilm-colonized contact surfaces.

Available data indicate that many common sanitizers cannot eradicate mature *Salmonella* biofilms on food contact surfaces (2, 7); however, relatively few studies have been conducted on the transferability of *S. enterica* cells from biofilms on contact surfaces to meat products. Our results revealed the high transferability of *Salmonella* biofilm cells from two surface materials to beef trim and the important impact of biofilm contact on *Salmonella* prevalence in meat products. Thus, *S. enterica* biofilms, if present, are highly likely to cause cross-contamination at commercial meat processing plants. Therefore, proper sanitization procedures and biofilm control are essential to prevent contamination and enhance meat safety.

ACKNOWLEDGMENTS

The authors thank Alberto O. Alvarado, Bruce Jasch, and Sydney Brodrick for technical support and Jody Gallagher for secretarial assistance. This study was partially supported by research funding from the Beef Checkoff through the North American Meat Institute (grant 58-3040-5-003). Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name in this article implies no approval of the product to the exclusion of others that may also be suitable.

REFERENCES

- Beauchamp, C. S., D. Dourou, I. Geornaras, Y. Yoon, J. A. Scanga, K. E. Belk, G. C. Smith, G.-J. E. Nychas, and J. N. Sofos. 2012. Transfer, attachment, and formation of biofilms by *Escherichia coli* 0157:H7 on meat-contact surface materials. *J. Food Sci.* 77:M343– M347.
- Corcoran, M., D. Morris, N. De Lappe, J. O'Connor, P. Lalor, P. Dockery, and M. Cormican. 2014. Commonly used disinfectants fail to eradicate *Salmonella enterica* biofilms from food contact surface materials. *Appl. Environ. Microbiol.* 80:1507–1514.
- Flores, R. A., M. L. Tamplin, B. S. Marmer, J. G. Phillips, and P. H. Cooke. 2006. Transfer coefficient models for *Escherichia coli* 0157:H7 on contacts between beef tissue and high-density polyethylene surfaces. *J. Food Prot.* 69:1248–1255.
- Hansen, L. T., and B. F. Vogel. 2011. Desiccation of adhering and biofilm *Listeria monocytogenes* on stainless steel: survival and transfer to salmon products. *Int. J. Food Microbiol.* 146:88–93.
- Howie, J. W. 1968. Typhoid in Aberdeen, 1964. J. Appl. Bacteriol. 31:171–178.
- Jeon, H. R., M. J. Kwon, and K. S. Yoon. 2018. Control of *Listeria* innocua biofilms on food contact surfaces with slightly acidic electrolyzed water and the risk of biofilm cells transfer to duck meat. *J. Food Prot.* 81:582–592.
- Joseph, B., S. K. Otta, I. Karunasagar, and I. Karunasagar. 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *Int. J. Food Microbiol.* 64:367–372.
- Keskinen, L. A., E. C. Todd, and E. T. Ryser. 2008. Impact of bacterial stress and biofilm-forming ability on transfer of surfacedried *Listeria monocytogenes* during slicing of delicatessen meats. *Int. J. Food Microbiol.* 127:298–304.
- Keskinen, L. A., E. C. Todd, and E. T. Ryser. 2008. Transfer of surface-dried *Listeria monocytogenes* from stainless steel knife blades to roast turkey breast. *J. Food Prot.* 71:176–181.
- Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50:882–889.
- 11. McSharry, S., L. Koolman, P. Whyte, and D. Bolton. 2021. The microbiology of beef from carcass chilling through primal storage to retail steaks. *Curr. Res. Food Sci.* 4:150–162.
- Midelet, G., and B. Carpentier. 2002. Transfer of microorganisms, including *Listeria monocytogenes*, from various materials to beef. *Appl. Environ. Microbiol.* 68:4015–4024.
- 13. Midelet, G., A. Kobilinsky, and B. Carpentier. 2006. Construction and analysis of fractional multifactorial designs to study attachment strength and transfer of *Listeria monocytogenes* from pure or mixed biofilms after contact with a solid model food. *Appl. Environ. Microbiol.* 72:2313–2321.
- Pérez-Rodríguez, F., A. Valero, E. Carrasco, R. M. García, and G. Zurera. 2008. Understanding and modelling the bacterial transfer to foods: a review. *Trends Food Sci. Technol.* 19:131–144.
- Possas, A. M. M., G. D. Posada-Izquierdo, F. Pérez-Rodríguez, and R. M. García-Gimeno. 2016. Modeling the transfer of *Salmonella* Enteritidis during slicing of ready-to-eat turkey products treated with thyme essential oil. *J. Food Sci.* 81:M2770–M2775.
- Rodríguez, A., W. R. Autio, and L. A. McLandsborough. 2007. Effect of biofilm dryness on the transfer of *Listeria monocytogenes* biofilms grown on stainless steel to bologna and hard salami. *J. Food Prot.* 70:2480–2484.
- Rodríguez, A., and L. A. McLandsborough. 2007. Evaluation of the transfer of *Listeria monocytogenes* from stainless steel and highdensity polyethylene to bologna and American cheese. *J. Food Prot.* 70:600–606.
- Sattar, S. A., S. Springthorpe, S. Mani, M. Gallant, R. C. Nair, E. Scott, and J. Kain. 2001. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model. *J. Appl. Bacteriol.* 90:962–970.
- Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011.

Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.

- Scallan, E., R. M. Hoekstra, B. E. Mahon, T. F. Jones, and P. M. Griffin. 2015. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiol. Infect.* 143:2795–2804.
- Silagyi, K., S. Kim, Y. M. Lo, and C. Wei. 2009. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. *Food Microbiol.* 26:514–519.
- Sofos, J. N., S. L. Kochevar, G. R. Bellinger, D. R. Buege, D. D. Hancock, S. C. Ingham, J. B. Morgan, J. O. Reagan, and G. C. Smith. 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J. Food Prot.* 62:140–145.
- Visvalingam, J., H. Wang, M. K. Youssef, J. Devos, C. O. Gill, and X. Yang. 2016. Spatial and temporal distribution of *Escherichia coli* on beef trimmings obtained from a beef packing plant. *J. Food Prot.* 79:1325–1331.
- Vorst, K. L., E. C. Todd, and E. T. Ryser. 2006. Transfer of *Listeria monocytogenes* during slicing of turkey breast, bologna, and salami with simulated kitchen knives. *J. Food Prot.* 69:2939–2946.
- Wang, H., X. Zhang, Q. Zhang, K. Ye, X. Xu, and G. Zhou. 2015. Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat. *Food Control* 50:574–580.

- Wang, R., J. L. Bono, N. Kalchayanand, S. Shackelford, and D. M. Brichta-Harhay. 2012. Biofilm formation by Shiga toxin–producing *Escherichia coli* O157:H7 and non-O157 strains and their tolerance to sanitizers commonly used in the food processing environment. *J. Food Prot.* 75:1418–1428.
- Wang, R., N. Kalchayanand, D. A. King, B. E. Luedtke, J. M. Bosilevac, and T. M. Arthur. 2014. Biofilm formation and sanitizer resistance of *Escherichia coli* O157:H7 strains isolated from "high event period" meat contamination. *J. Food Prot.* 77:1982–1987.
- Wang, R., B. E. Luedtke, J. M. Bosilevac, J. W. Schmidt, N. Kalchayanand, and T. M. Arthur. 2016. *Escherichia coli* O157:H7 strains isolated from "high-event period" beef contamination have strong biofilm-forming ability and low sanitizer susceptibility, which are associated with high pO157 plasmid copy number. *J. Food Prot.* 79:1875–1883.
- Wang, R., J. W. Schmidt, D. M. Harhay, J. M. Bosilevac, D. A. King, and T. M. Arthur. 2017. Biofilm formation, antimicrobial resistance, and sanitizer tolerance of *Salmonella enterica* strains isolated from beef trim. *Foodborne Pathog. Dis.* 14:687–695.
- Wang, R.-F., W.-W. Cao, and C. E. Cerniglia. 1997. A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. J. Appl. Microbiol. 83:727–736.
- Yang, X., F. Tran, M. K. Youssef, and C. O. Gill. 2015. Determination of sources of *Escherichia coli* on beef by multiple locus variable-number tandem repeat analysis. *J. Food Prot.* 78:1296–1302.