Meat and Muscle Biology™

Biomapping and Enviromapping of Microbial Indicators and Pathogens in a Commercial Beef Processing Facility

Rodrigo M. Portillo-Alarcon*, Mindy M. Brashears, Marcos X. Sanchez-Plata, Ariana Roldan, and Markus F. Miller

International Center for Food Industry Excellence, Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA
*Corresponding author. Email: rodrport@ttu.edu (Rodrigo M. Portillo-Alarcon)

Abstract: The study aimed to perform biomapping and enviromapping of microbial indicators and pathogens to track microbial changes on carcasses and various surfaces throughout the facility, from harvest to fabrication. Additionally, the study sought to evaluate the effectiveness of interventions and food safety programs in 3 distinct areas of the facility. Samples were collected on 2 separate days to track the fabrication of carcasses from cattle harvested the previous day. On the first day, samples were taken from the lairage area and the harvest floor. On the second day, samples were collected from the fabrication floor. The surfaces tested included walls, equipment, handrails, drains, conveyor belts, cutting boards, and carcasses. MicroTally® cloths (FREMONTA), EZ Reach™ sponges (World Bioproducts), and boot covers (VWR® Basic Protection Anti-Skid Shoe cover, Avantor) were used to collect samples depending on the surface. Most samples, except those from the lairage area, were enumerated using the TEMPO® system (bioMérieux). All samples were tested for pathogens using the BAX® System (Hygiena). The microbial indicators for this study were aerobic counts, Enterobacteriaceae, and *Escherichia coli* Biotype I, and for pathogens were *Escherichia coli* O157:H7 (Shiga toxin-producing *Escherichia coli* [STEC] O157), non-O157 STEC (O26, O45, O103, O111, O121, and O145), and *Salmonella* spp. As anticipated, statistical reductions among different areas occurred for all 3 locations ($P < 0.05$). The results showed a trend of decreasing microbial loads, starting with high levels of indicators and gradually reducing in the later stages of the process. *Salmonella* and STEC were detected in the lairage and harvest areas, with some also found on surfaces in the fabrication area before the final interventions were applied. Biomapping and enviromapping are valuable tools for assessing the efficacy of interventions and the effectiveness of food safety programs, enabling manufacturers to make data-driven decisions.

Key words: antimicrobial intervention, indicator microorganisms, TEMPO®, BAX®
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Introduction

The United States is the largest producer of beef in the world, including grain-fed cattle for both domestic consumption and export purposes (USDA, 2023). Control of pathogens like Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* is a challenge for the beef industry. According to the U.S. Centers for Disease Control and Prevention (CDC), approximately 48 million people get ill from foodborne illnesses, 128,000 are hospitalized, and 3,000 die from foodborne illnesses every year (CDC, 2018). Non-typhoidal *Salmonella* and *E. coli* are responsible for 400 deaths, 21,000 hospitalizations, and 1,200,000 people getting ill annually (Scallan et al., 2011).

Between 2012 and 2019, 27 *Salmonella* outbreaks were linked to consumption of contaminated beef, mostly ground beef (44%) (Canning et al., 2023). The USDA Food Safety and Inspection Service

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(USDA-FSIS) declared *E. coli* O157:H7 an adulterant in non-intact beef in 1994 following an outbreak caused by this pathogen in 1993 (USDA-FSIS, 2002). Additionally, other serogroups of STEC, also known as the "Big Six" (O26, O45, O103, O111, O121, and O145), are associated with several cases of illness in humans in the U.S. and Europe (Smith et al., 2014).

Studies have proven that cattle hides are the primary source of carcass microbial contamination; however, loads tend to decline as different antimicrobial interventions target pathogenic bacteria before and after slaughter (Koohmaraie et al., 2007). The industry has implemented programs to evaluate all stages of the production process as a measure against contamination, including producers, processors, distributors, and other stakeholders involved in the value chain (Flach, 2023). Environmental conditions directly affect pathogenic contamination, and as a result, pre-harvest and post-harvest interventions are combined to reduce microbial loads in the food processing chain, including microbial indicators (Wheeler et al., 2014).

Additionally, environmental monitoring programs (EMP) employed in the meat industry enable the assessment of the effectiveness of overall microbial and process control (Mota et al., 2021). The objective of EMP is to monitor the environment through different steps to identify unsanitary conditions that could potentially increase the risk of direct or indirect product contamination (3M and Cornell, 2019). Before implementing an EMP in a meat processing facility, comprehensive studies must be conducted to identify sampling points that are critical to ensure process control (Sanchez, 2023). Another tool for monitoring the effectiveness of interventions is biomapping, a novel approach for processors that consists of visualizing microbial indicator loads numerically throughout different steps in food processing facilities (Vargas et. al, 2022). The objectives of the current study were to evaluate the effectiveness of antimicrobial interventions in reducing microbial loads at various stages of beef processing, to track the prevalence of pathogens such as *Salmonella* and STEC throughout the processing stages, and to utilize biomapping and enviromapping techniques to visualize and analyze microbial load data in a commercial beef processing facility.

Material and Methods

Beef processing facility description

Samples were collected in a beef processing facility inspected by the USDA-FSIS and located in the Midwestern U.S. This facility processes approximately 1,800 head of beef cattle per day. The process begins in the lairage area by receiving the cattle, and animals are moved from the outside to the inside of the plant in preparation for slaughter.

During the warmest months of the year, typically from April to November, a pre-harvest intervention of bacteriophages is applied to the hide of cattle in one of the holding pens. This process utilizes a multi-hurdle approach, incorporating various interventions to reduce the prevalence of pathogens and indicator loads. After de-hiding, a steam vacuum treatment is applied to the inside of the round, hock, and shank using steam at 82 ± 5°C, followed by a pre-evisceration carcass water wash at room temperature. Subsequently, a lactic acid spray (2–5% concentration) at 49 ± 5°C is uniformly applied over the carcass at a pressure exceeding 15 psi.

Post-evisceration, the carcass undergoes another water wash at room temperature, followed by a lactic acid spray treatment (2–5% concentration) at 49 ± 5°C and 15 psi. Visual inspections are conducted to remove impurities and any fecal residues by trimming the affected area. The carcass is then subjected to another hot water bath at 85 ± 5°C, followed by a final lactic acid spray treatment (2–5% concentration) at 49 ± 5°C and over 15 psi. The combined effect of hot water and lactic acid has been proven more effective than the individual use of either treatment in reducing indicator loads (Miah et al., 2014).

After the interventions on the harvest floor, the carcasses are moved to a cooler, where they are chilled for 18 to 24 h until their surface temperature drops below 7°C. Before the carcasses leave the cooler and enter the fabrication floor, they undergo another lactic acid spray treatment (2–5% concentration) at approximately 49 ± 5°C. On the conveyor belt, each sub-primal cut is also sprayed with lactic acid at the same concentration and temperature. Additionally, the conveyor belt on the chuck line (conveyor belt 2) is treated with ozonated water to reduce bacterial concentrations.

Sample collection

Samples were collected on 2 different days to track the same load of cattle from harvest to fabrication, aiming to understand the dynamics of carcasses as they progress through the plant. Each sampling was completed in 2 d. Samples from the lairage area and harvest floor were collected during the first day, and samples from the fabrication floor were collected during the second day. There were 3 separate collection areas for environmental samples: lairage, harvest, and fabrication. The lairage area

was divided into 3 sections (outside pens, intervention pen, and inside pens). The harvest area had 6 sections: 3 hide-on areas and 3 hide-off areas that tested the same surfaces before and after hide removal (surface, drain, and wall). The fabrication floor samples had 4 sections (drain, cutting board, conveyor belt 2, and conveyor belt 7). Biomapping samples included hide-on carcasses, hide-off carcasses (prior to the application of antimicrobial intervions), and cold carcasses.

Lairage area samples were collected to evaluate the differences in prevalence as cattle moved through the process and after they were treated with a bacteriophage intervention utilizing Finalyse™ from Arm & Hammer, a product that contains 3 phage strains that specifically target *E. coli* O157:H7. A 50 parts per million (ppm)™ solution was applied to cattle before it entered the harvesting facility through shower application. Finally, Finalyse™ intervention from Arm & Hammer was applied for one minute to 30 head of cattle, and approximately 8.8 mL of the solution was applied to the hides of each animal (Flach, 2023).

In the study, samples were collected from every holding pen ($n = 5$) per sampling day on 4 sampling days ($n = 20$ in the study). The harvest floor was divided into 2 sections (hide-on section and hide-off section) before hide removal and after hide removal. For both sections, samples were collected from similar surfaces including the foreshank of the carcass ($n = 15$ in the study), walls ($n = 20$ in the study), surfaces in contact with the carcasses ($n = 20$ in the study), and drains ($n = 20$ in the study). The next day, following production, samples from the fabrication floor were collected, including samples from conveyor belts (chuck and trim), cutting boards ($n = 20$ in the study), drains ($n = 20$ in the study), carcasses ($n = 15$ in the study), and intervention cabinet ($n = 15$ in the study).

EZ Reach™ sponges were used for carcasses (foreshank), intervention cabinet, walls, and product contact surfaces in a 100-cm^2 area. MicroTally™ cloths were used for conveyor belts, drains, and cutting boards; for conveyor belts, MicroTally™ cloths were placed over the running conveyor belt holding it by the 2 upper corners for 15 s per side, and then were placed into their original bag. Cutting board samples were collected using a MicroTally™ Swab by rubbing the cloth over the cutting board for 15 s. Then, the other side of the MicroTally™ was used to sample the other side of the cutting board following the same procedure. Drain samples were collected on each sampling day using a MicroTally™ Swab over the drain to absorb all the liquid from the drain and surrounding areas until completely wet around 10 s, and subsequently folded into a square and placed inside the bag. Holding pen samples were collected using a boot cover (VWR® Basic Protection Anti-Skid Shoe cover, Avantor) on each foot and walking in a "Z" pattern throughout the holding pens for about 15 s; subsequently, boot covers were placed into a filtered sterile bag (Whirl-Pak™ Filter Bags for Homogenizer Blenders, 55 oz, Sterile).

Sample processing

Microbial indicators. Microtally™ samples were kept refrigerated and shipped overnight to the International Center for Food Industry Excellence (ICFIE) at Texas Tech University, and organized according to the area and surface. Microtally™ samples were hydrated with 200 mL of Buffered Peptone Water (BPW) and homogenized using a paddle blender at 230 revolutions per minute (RPM) for 1 min. All samples were tested for aerobic count (AC), Enterobacteriaceae (EB), and *Escherichia coli (E. coli)* on the bioMérieux TEMPO® System. TEMPO® vials for these indicators were filled with 3 mL of water and mixed to dissolve the dehydrated media in the vial. Then, 1 mL of pre-enriched sample was transferred to a vial. Samples were transferred to the TEMPO® Filler to hermetically seal the enriched medium with the sample in an individual TEMPO® Card. The cards were incubated for 22 to 27 h at 35°C; cards were read at 22 h for all indicators.

EZ Reach™ Swab. Upon arrival, samples were checked for temperature and sorted according to the area and surface. EZ Reach™ sponge samples were homogenized using a paddle blender at 230 revolutions per minute (RPM) for 1 min. All samples were tested for AC, EB, and EC on the TEMPO® system. TEMPO® vials for these indicators were filled with 3 mL of water and mixed to dissolve the dehydrated media in the vial. Then, 1 mL of pre-enriched sample was transferred to a vial. Samples were transferred to the TEMPO® Filler to hermetically seal the enriched medium with the sample in an individual TEMPO® Card. The cards were incubated for 22 h at 35°C and read as per manufacturer recommendations.

Pathogen prevalence

Microtally™. Microtally™ samples were hydrated with 200 mL of BPW and homogenized using a paddle blender at 230 RPM for 1 min. A total of 30 mL of enriched sample was transferred into a filtered sterile bag (Whirl-Pak® Bag) and 30 mL of Hygiena BAX MP was added. Microtally™ was incubated for 24 h at 42°C to run the prevalence of both *Salmonella* spp. and pathogenic *E. coli* strains. The prevalence of both

pathogens was assessed through the BAX® System prevalence protocol, which was applied using Hygena's Real-Time Polymerase chain reaction (PCR) detection kits for *E. coli* O157:H7 Exact, STEC Panel 1 (O26, O111, and O121), and STEC Panel 2 (O45, O103, and O145).

EZ Reach™ Swab. EZ Reach™ sponge samples were homogenized using a paddle blender at 230 RPM for 1 min. Then, 50 mL of BAX MP was transferred into each bag and homogenized at 230 RPM. Subsequently, 30 mL of homogenized sample was transferred into a 7 oz sterile bag (Whirl-Pak® Bag) and incubated for 24 h at 42°C to run the prevalence of both *Salmonella* spp. and pathogenic *E. coli* strains. The BAX® System prevalence protocol was applied using Hygena's Real-Time PCR detection kits for *E. coli* O157:H7 Exact, STEC Panel 1 (O26, O111, and O121), and STEC Panel 2 (O45, O103, and O145).

Boot swabs. Boot swab samples were collected by using boot covers (VWR® Basic Protection Anti-Skid Shoe cover, Avantor) on each foot and walking in a "Z" pattern throughout the holding pen. Subsequently, boot covers were placed into a filtered bag (Whirl-Pak™ Filter Bags for Homogenizer Blenders, 55 oz, Sterile). A single boot cover is considered as one individual sample (Flach, 2023). After collection, all samples were immediately chilled and shipped overnight to the ICFIE Food Microbiology Laboratory at Texas Tech University.

Statistical analysis

Data were analyzed using R Software (4.4.0) to evaluate the differences between sampled surfaces in the different areas starting from the lairage area, hide-on section, hide-off section, and fabrication floor. Data were analyzed using a Kruskal-Wallis followed by a pairwise Wilcoxon test with a significance level of 0.05 for indicators (AC, EB, and EC). A Chi-squared McNemar test-variable comparison and logistic regression followed by Tukey arrangement for a *P*-value comparison were used to evaluate differences between binary values of prevalence (*Salmonella* spp., *E. coli* O157:H7, O111, O121, O103, O45, O145, and O26) in different stages of the process. Essential R Software packages for the statistical analysis and plotting were agricolae, ggplot, and rstatix.

Results and Discussion

A summary of AC, EB, and EC Type I is presented in Tables 1, 2, and 3 for the Boot Swab – Inside, Boot Swab – Intervention Pen, Boot Swab – Outside, Hide-On – Carcass, Hide-On – Drain, Hide-On – Surface, Hide-On – Wall, Hide-Off – Carcass, Hide-Off – Drain, Hide-Off – Surface, Hide-Off – Wall, Cold Carcass, Cold Carcass – Cabinet, Cutting Board, Drain, Conveyor Belt 2, and Conveyor Belt 7.

Biomapping and enviromapping

Microbial indicators. The process of handling cattle as they enter the slaughter facility reveals significant insights into the reduction of microbial indicators like AC, EB, and EC Biotype I.

The biomapping demonstrated that the highest numbers of AC were found in the hide-on carcass, with an initial load of 7.04 log (Figure 1). A substantial reduction was observed as carcasses were sampled both hide-on and hide-off, showing a decrease to 0.20 logs on the hide-off samples. Cold carcass samples were collected, but the results were below the limit of quantification (LOQ < 1 log). This drastic reduction illustrates the effectiveness of hide removal in controlling microbial contamination, a trend consistent with other studies in similar environments. Similar reductions were observed across other surfaces in the facility, including walls, drains, and carcass contact surfaces; however, no differences were found between hide-on surfaces and walls, with average loads of 5.77 ± 0.35 and 6.49 ± 0.19, respectively. For instance, there was a significant reduction ($P < 0.05$) on the walls, from 6.49 logs to less than 1 log in hide-off areas, while drains and contact surfaces exhibited a decrease of 1–2 logs. In the chilled area, microbial counts on environmental surfaces were relatively low, with cutting boards averaging 2.80 logs and conveyor belts averaging 2.60–3.10 logs. The counts on drains in the chilled areas were significantly lower than those in the harvest area, likely due to more rigorous cleaning protocols in the chilled environment. These findings emphasize the importance of comprehensive sanitation practices across various environmental surfaces.

Another study in the same plant found AC averages of 3.52 ± 0.17 and 3.69 ± 0.25 logs, with no statistically significant differences between time points when testing conveyor belts for AC (Sanchez, 2023). In this study, Sanchez evaluated the differences between time points of data collection to determine if there was an accumulative effect. Although AC are not directly related to pathogenic bacteria, they serve as useful indicators for evaluating sanitary conditions, good manufacturing practices (GMP), and environmental hygiene (Ajcet-Reyes, 2023). In another study,

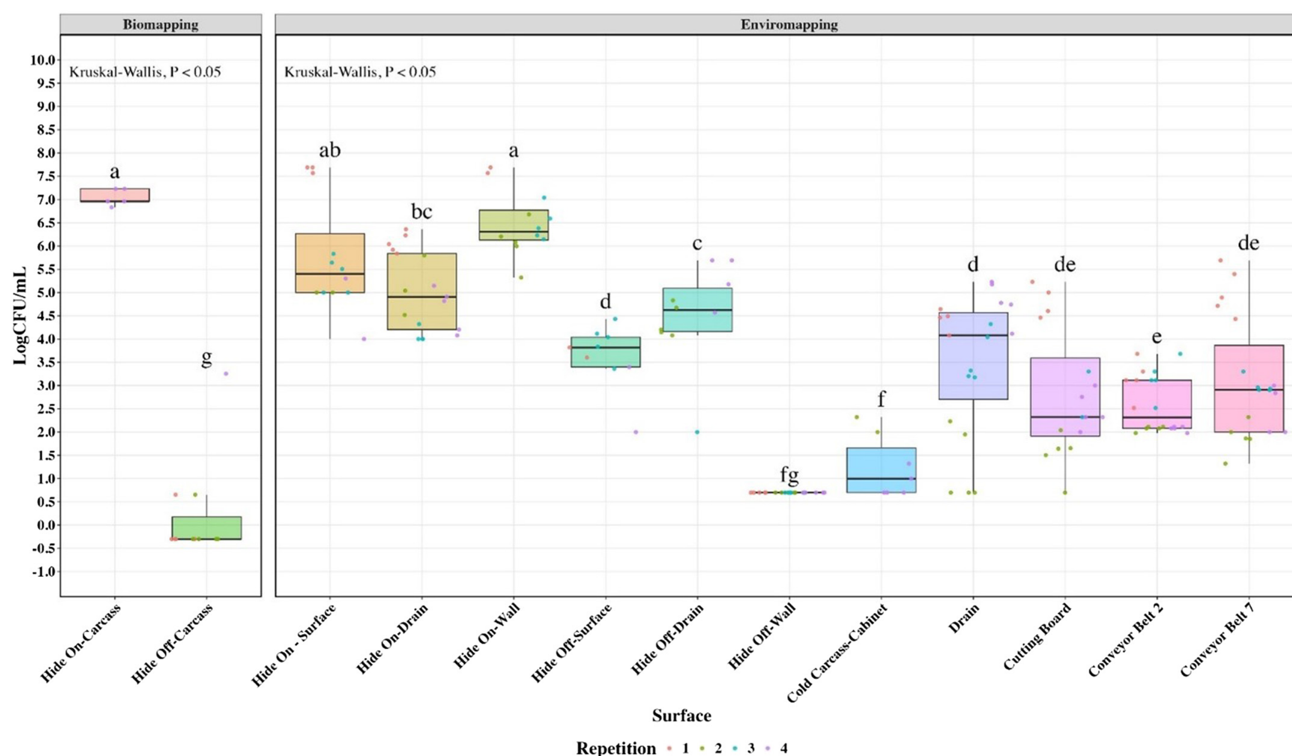

Figure 1. Aerobial plate counts (Log CFU/mL) at different surfaces and sampling locations throughout the processing environment. The figure is divided into biomapping and enviromapping sections. In each box plot, the horizontal line crossing the box represents the median, the bottom and top of the box are the lower and upper quartiles, and the vertical lines extend to show the data range. Individual points represent the actual data points, with colors indicating different repetitions (1–4). For each sampling location, boxes with different letters (a–g) are significantly different according to Kruskal-Wallis analysis ($P < 0.05$). The y-axis shows Log CFU/mL, while the x-axis displays various sampling locations including hide areas, processing surfaces, drains, and equipment.

after hide removal, AC on carcasses were significantly reduced to an average load of 0.87 ± 0.18 logs (Bosilevac et al., 2005), compared to the average value for carcasses with hide, which ranged from 8.20 to 12.50 logs (Log10 CFU/100 cm^2) (Bacon et al., 2000). The incoming load at this slaughter facility was lower than in other U.S. facilities, suggesting higher baseline hygiene levels.

Levels of EB were also notably reduced throughout the process. The highest loads were found on the hide-on wall, with an average of 5.00 ± 0.22, which dropped significantly after hide removal ($P < 0.05$). The lowest EB loads were found on hide-off carcasses at 0.28 ± 0.13, further supporting the critical role of hide removal in reducing contamination. The reduction in EB levels on walls, surfaces, and drains—by over 4 logs in some cases—highlights the effectiveness of interventions in controlling bacterial presence across different areas of the facility. The levels of EB counts were significantly reduced throughout the process, although an increase was observed starting in the cold carcass stage. As shown in Figure 2, hide-off drains were not significantly different from drains on the fabrication floor, nor were cold carcass samples significantly different from cutting boards and conveyor belts.

Some studies have tried to correlate the presence of EB to pathogen prevalence. A study published in 1996 found that the EB family is not correlated with pathogens in raw meat products (Johnson, 1996). Another study suggested that EB absence can indicate *Salmonella* absence on beef carcasses (Ruby & Ingham, 2009). After hide removal, EB loads were significantly reduced ($P < 0.05$) to an average of 0.28 ± 0.13; however, total EB counts for cold carcass samples slightly increased compared to hide-off carcass samples, with an average of 0.70 ± 0.00. The hide-off wall had the lowest counts in all processes, with an average of 0.70 ± 0.00, as it is not in constant contact with carcasses like other surfaces. A recent study found averages of 2.93 ± 0.48 and 2.92 ± 0.41 on conveyor belts 2 and 7, respectively (Sanchez, 2023). The study performed by Sanchez focused on the enviromapping baseline of microbial indicators in a beef fabrication floor as a tool for sanitary design, sanitizing interventions, and pathogen monitoring.

The highest EC loads were observed on the kill floor, particularly in the hide-on section (Figure 3).

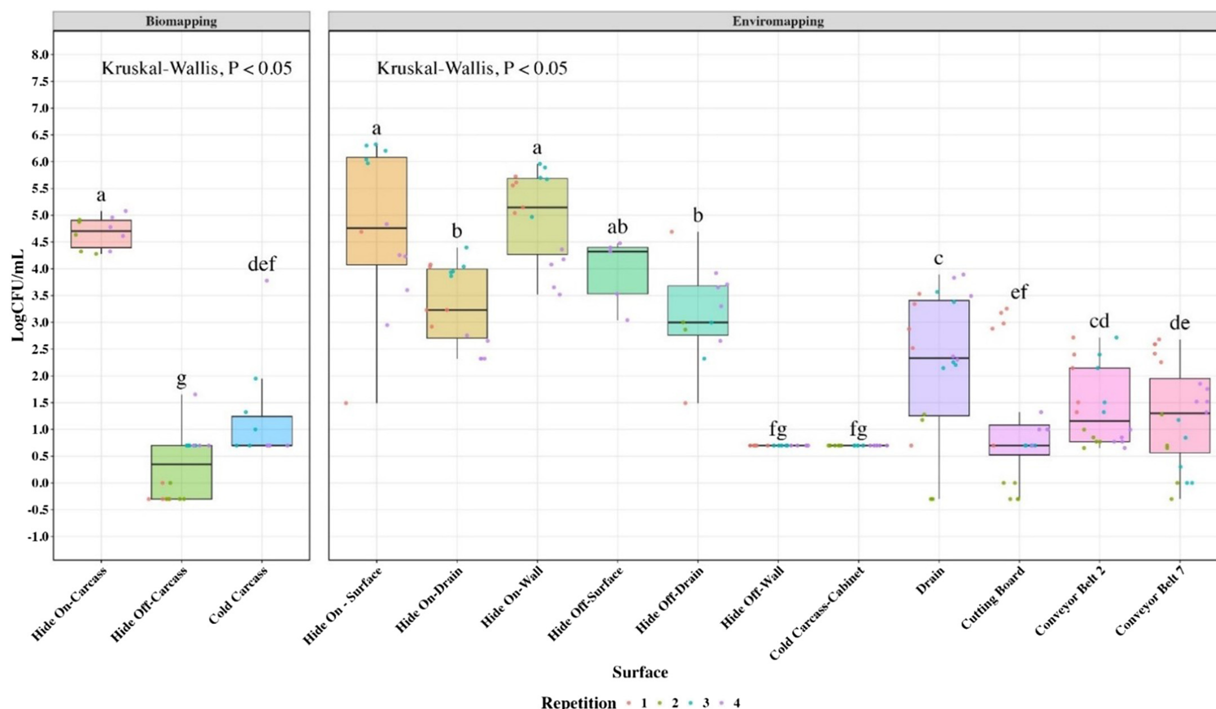

Figure 2. Enterobacteriaceae counts (Log CFU/mL) at different surfaces and sampling locations throughout the processing environment. The figure is divided into biomapping and enviromapping sections. In each box plot, the horizontal line crossing the box represents the median, the bottom and top of the box are the lower and upper quartiles, and the vertical lines extend to show the data range. Individual points represent the actual data points, with colors indicating different repetitions (1–4). For each sampling location, boxes with different letters (a–g) are significantly different according to Kruskal-Wallis analysis ($P < 0.05$). The y-axis shows Log CFU/mL, while the x-axis displays various sampling locations including hide areas, processing surfaces, drains, and equipment.

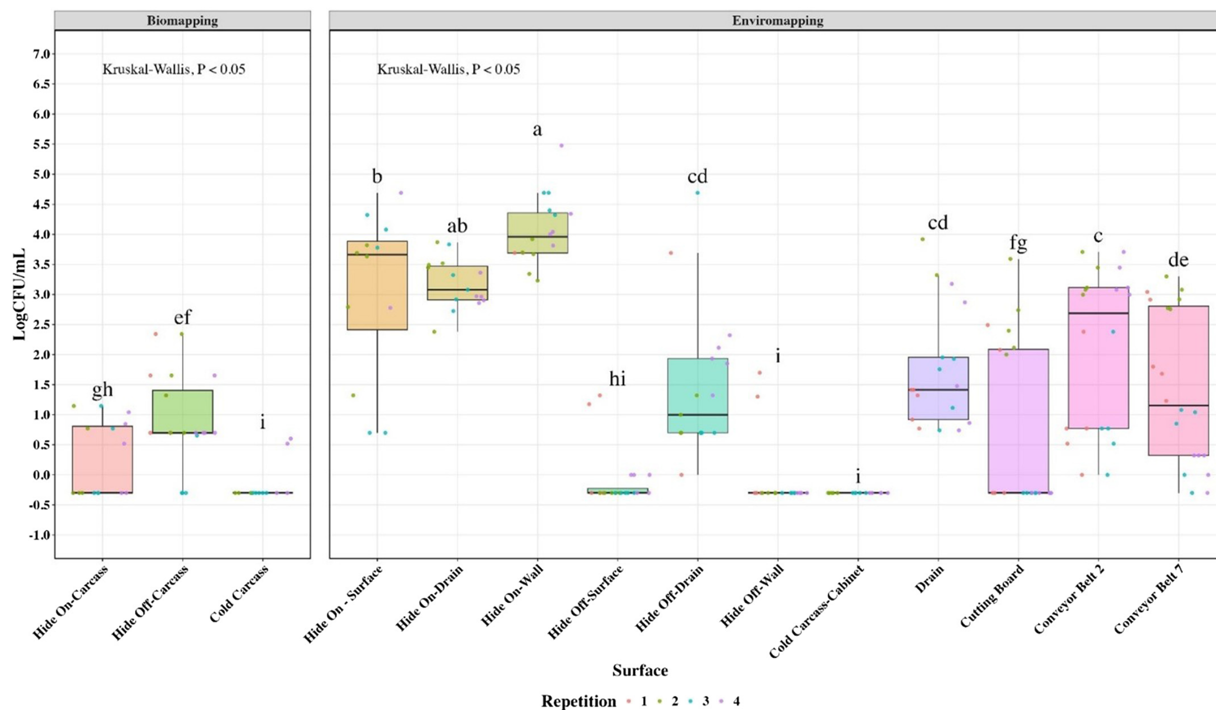

Figure 3. *E. coli* Biotype I counts (Log CFU/mL) at different surfaces and sampling locations throughout the processing environment. The figure is divided into biomapping and enviromapping sections. In each box plot, the horizontal line crossing the box represents the median, the bottom and top of the box are the lower and upper quartiles, and the vertical lines extend to show the data range. Individual points represent the actual data points, with colors indicating different repetitions (1–4). For each sampling location, boxes with different letters (a–i) are significantly different according to Kruskal-Wallis analysis ($P < 0.05$). The y-axis shows Log CFU/mL, while the x-axis displays various sampling locations including hide areas, processing surfaces, drains, and equipment.

The highest load was recorded in the hide-on drain at 3.18 ± 0.11, which was substantially reduced in the cold carcass cabinet. This is likely due to the application of lactic acid, which has been proven to be an effective single intervention against microbial indicators when applied through hand sprayers or cabinets (Carter et. al., 2021) These reductions were consistent across other indicators, demonstrating the effectiveness of the interventions employed.

Pathogen prevalence

The effectiveness of interventions in controlling pathogen prevalence was demonstrated across multiple strains. For *Salmonella* spp., there were no significant differences ($P > 0.05$) among pens in the lairage area because in this facility the pre-harvest bacteriophage intervention is not intended to reduce or control this pathogen. *Salmonella* spp. incidence was high, particularly in the outside pens (85%) and inside pens (100%) (Figure 4); however, after the first major intervention—hide removal—prevalence dropped to 5%. A study analyzing carcasses after hide removal and before antimicrobial interventions found a 47% prevalence of *Salmonella* spp. in carcasses and 1.6% in ground beef; DNA testing confirmed that the strains isolated from the carcasses matched those in the ground beef (Koohmaraie et al., 2012). The previously mentioned study reaffirms that processing facilities must ensure that proper dressing techniques are followed during hide removal to prevent cross-contamination between the hide and carcass, as well as among carcasses. Another study demonstrated that following hide removal, the implementation of antimicrobial interventions, such as steam vacuuming and the application of organic acid solution rinsing on carcasses, effectively reduced *Salmonella* spp. prevalence to 1.3% (Bacon et al., 2002). The application of antimicrobial treatments immediately after hide removal enhances the efficacy of pathogen control (Villarreal-Silva et al., 2016). In this facility, post-harvest interventions are effectively controlling *Salmonella* spp. prevalence throughout the process, with

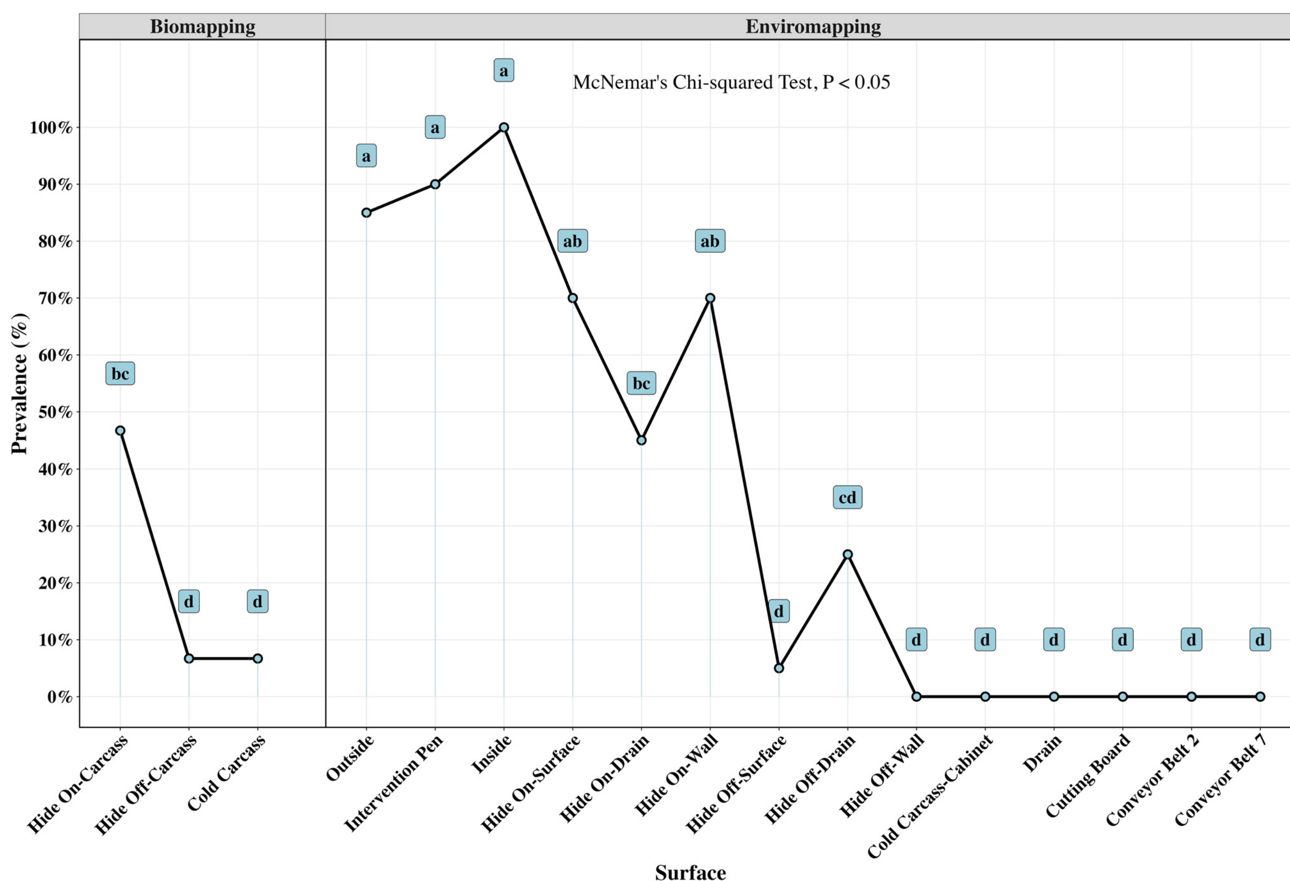

Figure 4. *Salmonella* prevalence (%) using BAX® System at different carcass regions and sampling locations throughout the beef processing line. The figure is divided into biomapping and enviromapping sections. The line graph represents the prevalence percentage, with points indicating specific measurements for each surface. Labels (a–d) above data points indicate statistically significant differences according to McNemar's Chi-squared test ($P < 0.05$). The y-axis shows prevalence percentage from 0% to 100%, while the x-axis displays various sampling locations including hide areas, processing surfaces, and equipment.

an initial average of 70% at the beginning of the process and an average of 0% on the environmental samples collected in the fabrication floor. The limit of detection for this assay was 10 CFU/mL.

The relationship between microbial indicators and pathogen prevalence provides valuable insights into the overall effectiveness of interventions. Figure 5 illustrates that *Salmonella* prevalence tended to increase when EB loads were high and decrease when EB counts were reduced. This suggests that managing EB levels could be an effective strategy in reducing *Salmonella* risk. According to Ruby and Ingham (2009), while EB has not been consistently correlated with *Salmonella* prevalence in previous studies, its absence has often been used as an indicator of *Salmonella* absence.

E. coli O157:H7 also showed a significant reduction throughout the process. Initially, *E. coli* O157:H7 had an 85% prevalence in the outside pens and 100% in the inside pens. The lowest prevalence in the lairage area was in the intervention pen, due to a bacteriophage treatment, reducing *E. coli* O157 prevalence to 30%. This is similar to a study by Flach (2023) in the same beef processing facility, which indicated that pens undergoing a bacteriophage intervention (Finalyse™) represent a low-risk area for contamination. The remaining prevalence could be attributed to the large amounts of organic matter in the lairage area, which could be a possible cause for the reduced efficacy of bacteriophages (Arthur et al., 2017). *E. coli* O157:H7 was effectively reduced throughout the process, with an initial prevalence of 60% in hide-on carcasses and none detected on the hide-off or chilled carcasses. The most notable reduction occurred after hide removal, in which prevalence dropped to approximately 7% in hide-off carcasses, followed by further reductions due to interventions like steam vacuum, lactic acid, and hot water (Figure 6). The reduction observed in this study post-harvest was lower compared to a study by Barkocy-Gallagher et al. (2003) on 3 beef processing facilities, in which *E. coli* O157:H7 was recovered from 60.6% of hide samples, 26.7% of pre-evisceration carcasses (before the pre-evisceration wash), and 1.2% of carcasses sampled at chilling. In another study by Rivera-Betancourt et al. (2004), *E. coli* O157:H7 was recovered from the surface of a preoperational conveyor belt in one of the plants sampled for this study. In comparison, the plant tested in this study demonstrated better control,

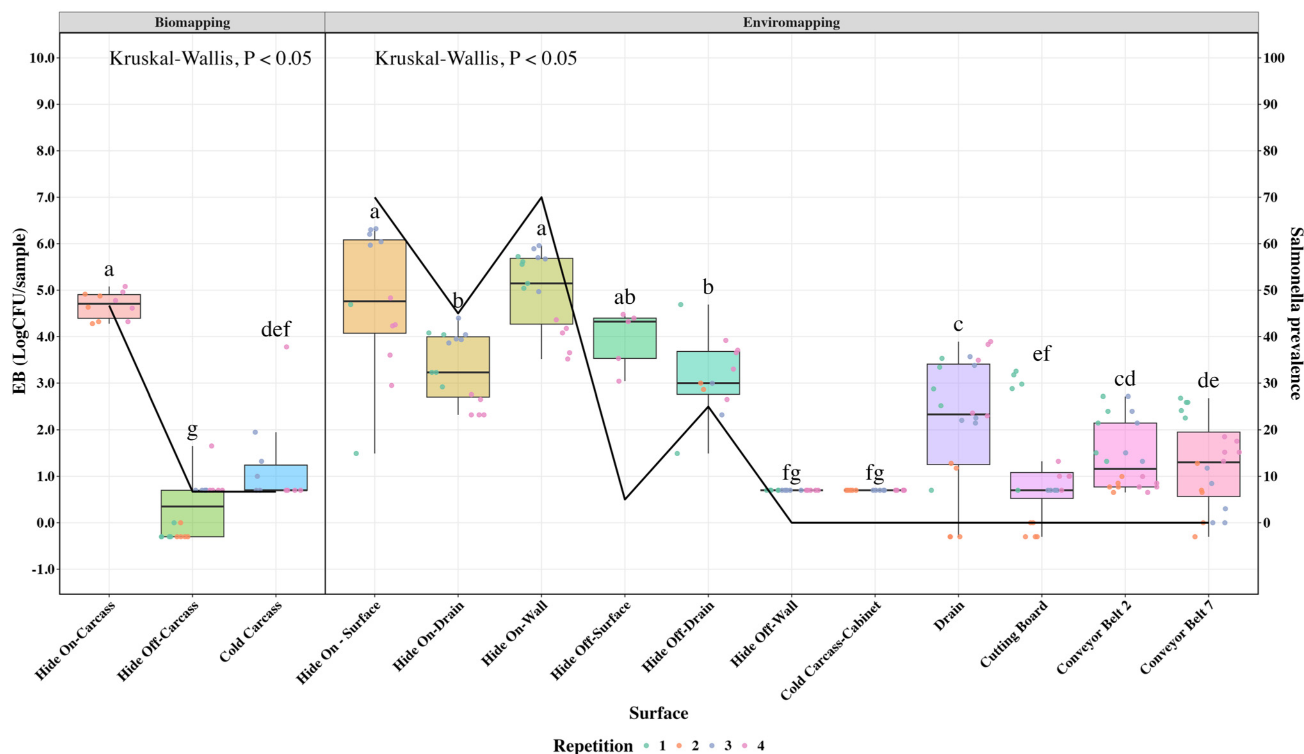

Figure 5. Box plots for Enterobacteriaceae (EB) counts Log CFU/sample (left y-axis) and *Salmonella* prevalence percentage (line, right y-axis) on samples collected from different surfaces throughout the beef processing line. Data are shown for 15 different sampling locations over 4 repetitions (indicated by color). The graph is divided into biomapping and enviromapping sections. Significant differences between sampling locations are shown by different letters (a–g) based on Kruskal-Wallis analysis ($P < 0.05$) for both EB counts and *Salmonella* prevalence. The box plots represent the distribution of EB counts, while the black line represents *Salmonella* prevalence.

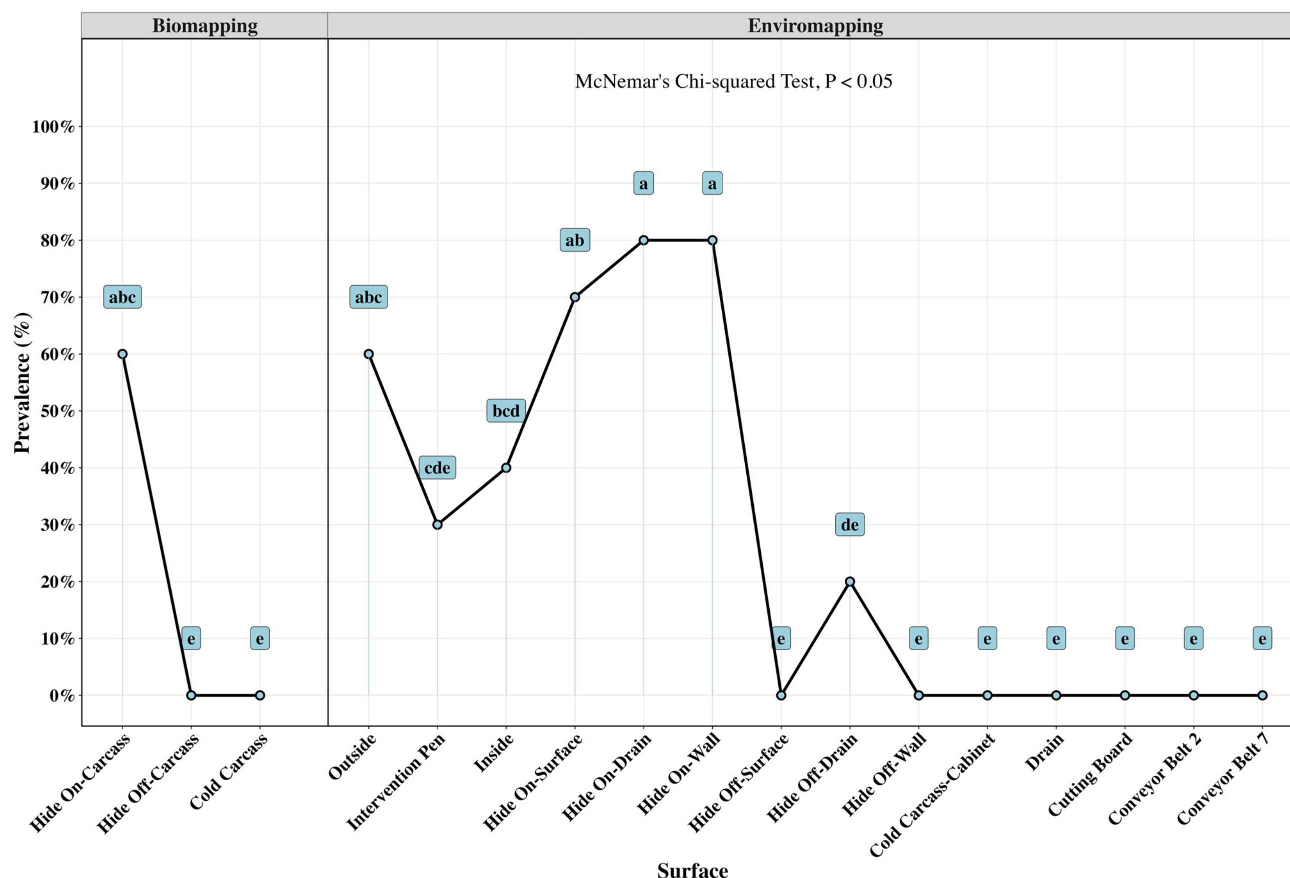

Figure 6. *E. coli* O157:H7 prevalence (%) using BAX® System at different carcass regions and sampling locations throughout the beef processing line. The figure is divided into biomapping and enviromapping sections. The line graph represents the prevalence percentage, with points indicating specific measurements for each surface. Labels (a–e) above data points indicate statistically significant differences according to McNemar's Chi-squared test ($P < 0.05$). The y-axis shows prevalence percentage from 0% to 100%, while the x-axis displays various sampling locations including hide areas, processing surfaces, and equipment.

with lower *E. coli* O157 counts on the fabrication floor, effectively reducing the prevalence to undetectable levels.

The prevalence of non-O157 STEC strains displayed significant reductions throughout the process. However, some strains behaved slightly differently compared to *E. coli* O157:H7, particularly after hide removal. Strains such as O26, O45, O103, and O121 initially showed prevalences of 95%, 93%, 73%, and 73% on hide-on samples, respectively, which were drastically reduced post-hide removal to 0%, 33%, 20%, and 0%. Results from a study performed during the summer at large processing facilities in the United States found that prior to evisceration, 58.3% of carcasses were positive for non-O157 STEC, and after antimicrobial interventions, the prevalence was reduced to 8.3% (Arthur et al., 2002). A study by Barkocy-Gallagher et al. (2003) found that 64.9% and 65.5% of pre-evisceration carcasses were positive for non-O157 STEC during the summer and fall, respectively. This study was performed during the summer and fall, and according to Arthur et al. (2002), studies completed during the summer tend to be associated with higher bovine STEC carriage, implicating that STEC carcass contamination could be lower in other seasons.

Figure 7 presents the biomapping and enviromapping results from the BAX® System Real-Time STEC Panel 1 kit (*E. coli* O26, O111, and O121). *E. coli* O111 had an initial average prevalence of 10% and was reduced to 0% when reaching the hide-off environmental samples. Interventions effectively controlled this strain throughout the process since it was the only strain that was not found on surfaces of the fabrication floor (cutting board and conveyor belts). Biomapping and enviromapping results demonstrated that *E. coli* O121 and *E. coli* O26 behaved similarly. Their prevalence on carcasses was effectively controlled after hide removal, and environmental samples collected post-hide removal were statistically different ($P < 0.05$) from the hide-on environmental samples; however, positive samples of O121 and O26 were still collected in the fabrication floor area. The prevalence of *E. coli* O121

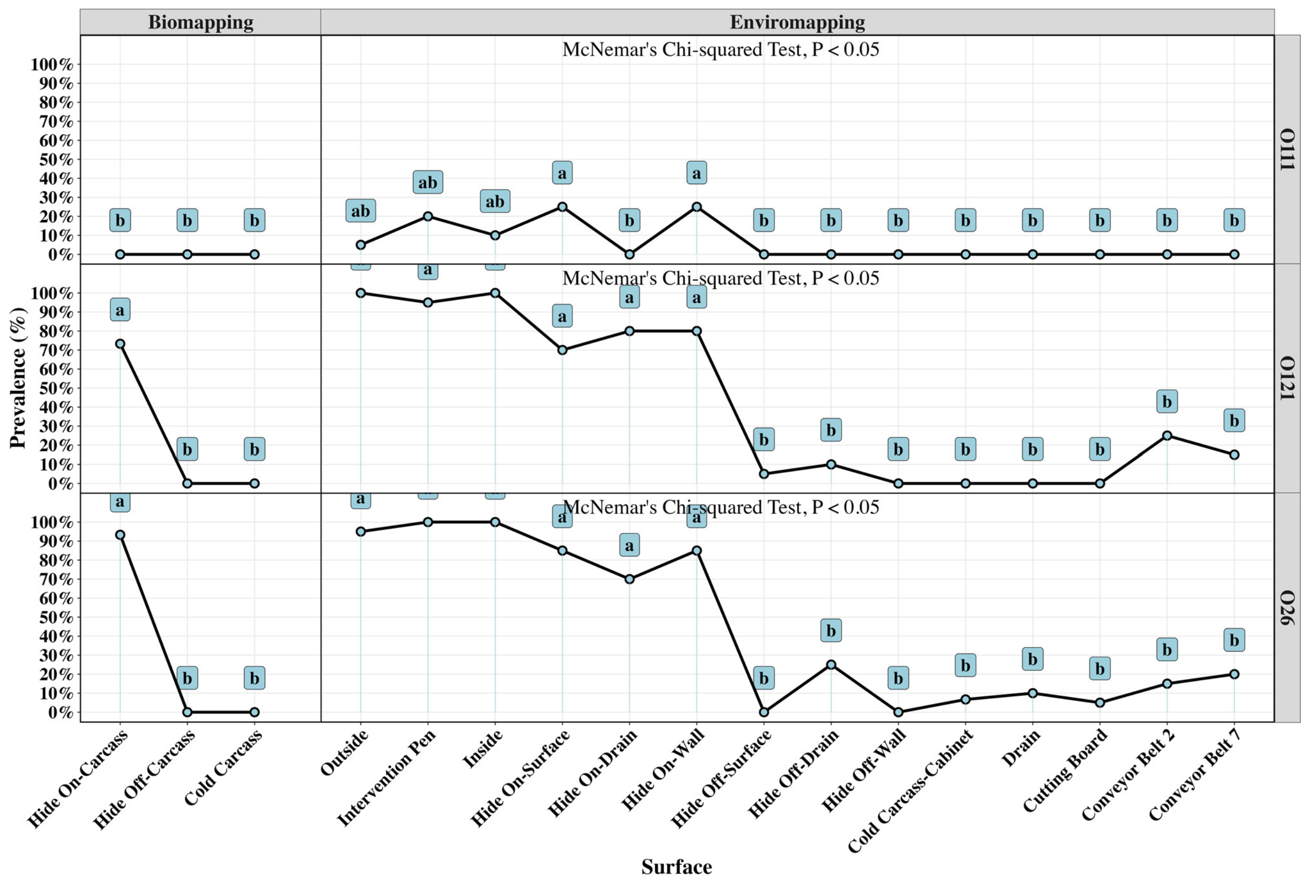

Figure 7. *E. coli* Panel 1 (O111, O121, O26) prevalence (%) using BAX® System at different carcass regions and sampling locations throughout the beef processing line. The figure is divided into biomapping and enviromapping sections. The line graph represents the prevalence percentage, with points indicating specific measurements for each surface. Labels (a–e) above data points indicate statistically significant differences according to McNemar's Chi-squared test ($P < 0.05$). The y-axis shows prevalence percentage from 0% to 100%, while the x-axis displays various sampling locations including hide areas, processing surfaces, and equipment.

on conveyor belts 2 and 7 was 25% and 15%, respectively, while the prevalence of *E. coli* O26 was 15% and 20% on conveyor belts 2 and 7, respectively. Although interventions controlled O121 and O26, it is possible that these non-O157 STEC could accumulate over time on surfaces, increasing the risk of cross-contamination.

Results from the biomapping and enviromapping using the BAX® System Real-Time STEC Panel 2 kit (*E. coli* O45, O103, and O145) are presented in Figure 8. Serogroups O103 and O45 were highly prevalent in both enviromapping and biomapping results. The prevalence for hide-on carcasses was statistically different ($P < 0.05$) from hide-off carcasses for both strains, but the prevalence was not reduced to 0%. The prevalence of O103 was significantly different for hide-off surface and hide-off wall, but there were no statistical differences between outside pen and hide-off drain ($P > 0.05$) and between conveyor belt 2 (55% prevalence) and lairage area environmental samples. For O45, there were no statistical differences ($P > 0.05$) between conveyor belts 2 and 7 and the inside pen located in the lairage area. O103 and O45 serotypes never reached a 0% prevalence, compared to other strains. For *E. coli* O145, biomapping results indicated that the prevalence from hide-on carcasses was 20%, and it was completely controlled on hide-off carcasses and cold carcass samples. The highest prevalence was found in the environmental samples collected from the inside pen (60%), and despite finding statistical differences from most hide-off environmental samples ($P < 0.05$), there were no statistical differences between the inside pen and conveyor belt 7.

These results provide important insights to make targeted improvements in sanitary dressing practices within the plant. Specifically, the data on the prevalence and persistence of certain *E. coli* strains, such as O103, O45, and O121, highlight areas where current interventions may need to be enhanced or modified to

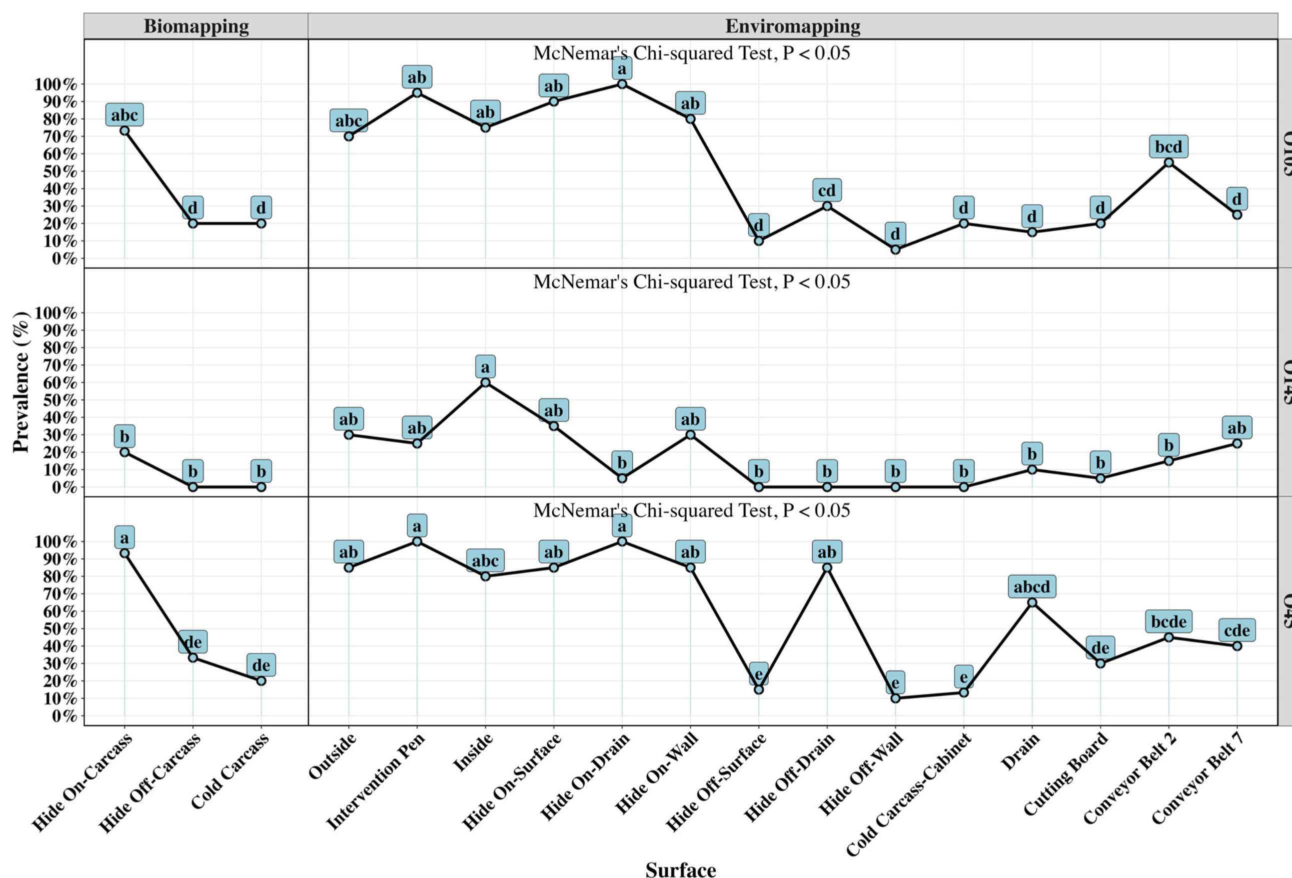

Figure 8. *E. coli* Panel 2 (O103, O145, O45) prevalence (%) using BAX® System at different carcass regions and sampling locations throughout the beef processing line. The figure is divided into biomapping and enviromapping sections. The line graph represents the prevalence percentage, with points indicating specific measurements for each surface. Labels (a–e) above data points indicate statistically significant differences according to McNemar's Chi-squared test ($P < 0.05$). The y-axis shows prevalence percentage from 0% to 100%, while the x-axis displays various sampling locations including hide areas, processing surfaces, and equipment.

further reduce the risk of cross-contamination. The findings of this study suggest that particular attention should be given to improving the sanitation of conveyor belts and other surfaces on the fabrication floor to prevent the accumulation of non-O157 STEC over time. Implementing these changes based on biomapping and enviromapping data could significantly reinforce the effectiveness of microbial control strategies throughout the processing stages.

Prevalence results were similar to a study by Kanankege et al. (2017) on a large-scale slaughter facility located in the U.S., in which the most prevalent strains were O103 (57%) and O45 (54%) (same highly prevalent serotypes in this study), followed by O121 (50%), O145 (33.33%), O26 (28.57%), and O111 (28.57%). On the other hand, results from a study performed on abattoirs from Alberta, Canada revealed that the pre-evisceration prevalence of non-O157 STEC on carcasses is 3.2%, and for pre-chilled carcasses is 3.9% (Essendoubi et al., 2019). Another study from a meat processing plant, located in Brazil, directly evaluated final meat products for *E. coli* O26, O111, O121, O45, O103, and O145 and did not find any positive sample (evidenced by the absence of the genes encoding the Shiga toxin *stx1* and *stx2*), which indicates that these pathogens are effectively controlled throughout the facility (Gonçalves et al., 2022). Further investigation into the prevalence of non-O157 STEC in U.S. beef processing facilities is needed to assess the efficacy of current intervention strategies and improve pathogen control.

The use of real-time PCR has also been encouraged for the detection of non-O157 STEC due to its higher sensitivity (Kanankege et al., 2017). Factors such as cross-contamination or buildup over time, which could be mitigated by more frequent cleaning, may influence conveyor belt prevalence. This underscores the need for continuous sanitation efforts to prevent cross-contamination. The importance of these interventions is further highlighted by USDA-FSIS regulations, which mandate testing for non-O157 in non-intact raw beef due to their classification as adulterants (USDA-FSIS, 2011).

Conclusions

Biomapping allows manufacturers to visualize data, evaluate the effectiveness of the process, and make decisions about whether a process is contributing to the overall reduction of indicators (Vargas et al., 2022). One of the advantages of using this tool is that decisions can be made such as removing interventions or adding new ones to prevent and reduce loads of indicators or pathogens. As with biomapping, environmental mapping is a useful tool to provide important information regarding the process and prevent cross-contamination (Sanchez, 2023).

Using these tools can help prevent cross-contamination and identify microbial harborage in different stages of the process. Biomapping provides useful information regarding the process: In terms of this facility, a reduction can be observed in different stages of the process, meaning that interventions are reducing the incoming load of microbial indicators and pathogens in the latest stages.

Enviromapping provides information regarding the environment of a food manufacturing facility helping to avoid cross-contamination and evaluate food safety programs within a processing facility. As part of the study, a reduction of microbial indicators and pathogens was observed as samples were collected in the latest stages of the process; however, for O103, O145, and O45, there was an increase in the prevalence of conveyor belts and cutting boards. This could represent that it is necessary to deep clean conveyor belts and cutting boards when time for shift change; however, this could be very unpractical for the facility due to the time required to disable and wash conveyor belts. Additional studies are needed to determine how the 6 STEC O groups can be controlled in beef fabrication environments.

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