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Food Protection Trends, Vol. 23, No. 1, Pages 24-34

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Comparison of Intervention Technologies for Reducing *Escherichia coli* O157:H7 on Beef Cuts and Trimmings

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SUMMARY

This study evaluated the decontamination efficacy of water (W; 25° or 55°C), 2% acetic acid (AA), 0.001% acidified chlorine (AC), 2% lactic acid (LA; 55°C), 0.02% acidified sodium chlorite (ASC), 0.5% cetylpyridinium chloride (CPC), 1% lactoferricin B (LB), and 0.02% peroxyacetic acid (PAA) on *Escherichia coli* O157:H7 when applied to fresh beef carcass tissue (BCT) surfaces (40 cm^2) and lean tissue pieces (LTP; 300 g). Samples were inoculated with a five-strain composite of *E. coli* O157:H7 and then immersed in the treatment solutions for 30 s. Viable cell counts were enumerated by plating on sorbitol MacConkey (SMAC) agar. Overall, CPC was most effective ($P < 0.05$) and reduced bacterial populations by 4.8 log CFU/cm^2 and 2.1 log CFU/g on BCT and LTP, respectively. Of the treatments commonly used by industry, LA was the most effective ($P < 0.05$), as it reduced pathogen populations by 3.3 log CFU/cm^2 and 1.3 log CFU/g on BCT and LTP, respectively. Additionally, ASC, AA, PAA, LB, AC and W reduced pathogen populations when plated on SMAC by 1.9, 1.6, 1.4, 0.7, 0.4 and 1.2 log CFU/cm^2, when applied to BCT, while corresponding reductions following the above treatment applications to LTP were 1.8, 1.1, 1.0, 0.4, 0.5 and 0.3 log CFU/g, respectively. Results from this study indicated that LA and ASC were the most effective pathogen decontamination solutions currently approved for commercial use. Information regarding the antibacterial efficacy of decontamination solutions should prove beneficial to industry personnel as a means of improving microbiological quality as well as potentially improving the quality of non-intact beef tissue.

A peer-reviewed article.

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INTRODUCTION

Food safety concerns increased dramatically following the outbreak of illnesses caused by *Escherichia coli* O157:H7 in undercooked ground beef in 1993 (*4*). Awareness of the consequences of this meatborne pathogen has increased among those in the general public, making *E. coli* O157:H7 a household name in the 21st century. According to the Centers for Disease Control and Prevention (CDC), approximately 76 million cases of foodborne illness occur in the United States annually, with 14 million of those cases attributed to known pathogens (*27*). As a part of the Pathogen Reduction: Hazard Analysis Critical Control Point (HACCP) Systems Final Rule (*18*) by Food Safety Inspection Service, United States Department of Agriculture (FSIS-USDA) regulation, FSIS recommended that all beef, pork and lamb slaughter establishments apply at least one antimicrobial treatment to carcasses before chilling. Any antimicrobial compounds previously approved by the Food and Drug Administration (FDA) and FSIS could be used for carcass decontamination (*18*). Numerous carcass decontamination strategies have been researched to determine which are most effective against bacterial pathogens such as *E. coli* O157:H7 (*38*).

Van Donkersgoed et al. (*41*) reported that less than 10% of slaughter cattle carried *E. coli* O157:H7 in their feces or on their hide when entering the abattoir. Ransom et al. (*34*) found that 36.7% of lots of cattle contained positive hide samples and 13.3% had positive fecal samples. Elder et al. (*16*) showed that 43% of beef carcasses were positive for *E. coli* O157:H7 prior to evisceration but that, because of carcass decontamination strategies in place, only 18% and 2%, respectively, of carcasses sampled post-evisceration and post-processing were positive for *E. coli* O157:H7.

Carcass decontamination technologies previously studied include: (a) sanitizing solutions such as organic acids, hydrogen peroxide, trisodium phosphate, ozone, activated ozone and the like (*5, 7, 8, 10, 15, 17, 31, 33*), (b) spray-washing with water (*9, 12, 22*) (c) thermal (hot water) pasteurization (*3, 32*), (d) steam pasteurization (*29, 30*), (e) hot-water or steam vacuuming (*26*), and (f) conventional knife-trimming, with or without subsequent washing, to determine their efficacy in decontamination of carcasses, cuts and/or trimmings (*1, 2, 19, 20, 21, 23, 35, 37, 39, 40*). In general, washing and sanitizing agents have been effective in reducing bacterial counts by 1–3 logs and in decreasing occurrence of pathogens on beef carcasses and cuts (*37*).

There are new additives/chemicals that may, singly or sequentially, be more effective against *E. coli* O157:H7 than are the microbiological interventions that have been previously proven effective or that might be useful as components of multiple hurdle decontamination systems for implementation by the beef packing/processing industry. Presently used by industry for decontamination of beef carcasses/cuts are thermal (hot-water) pasteurization, steam/hot-water vacuuming, steam pasteurization, and organic acid solution rinsing (*2*). Lactoferricin B, (recommended for preventing attachment and growth of pathogens on carcass surfaces) [*5, 28*], as well as peroxyacetic acid, acidified chlorine, acidified sodium chlorite, and cetylpyridinium chloride, are microbiological intervention technologies that have recently received attention for their antimicrobial properties (*6, 7, 10, 13, 17, 24, 25*).

The objective of this study was to compare the effectiveness of decontamination technologies presently in use with proposed chemicals not presently used as possible intervention strategies to determine their effectiveness in reducing *E. coli* O157:H7 counts on beef carcass adipose tissue and beef trimmings.

MATERIALS AND METHODS

Intervention treatment preparation

Chemical treatment solutions used in both phases of this experiment included: (1) 2% acetic acid (AA), vol/vol, prepared from glacial acetic acid, Mallinckrodt Baker Inc, Paris, KY; (2) 0.001% acidified chlorine (AC), vol/vol, prepared from 10% AC, Advanced Food Systems, Kamloops, B.C.; (3) 0.02% acidified sodium chlorite (ASC) vol/vol, prepared from a 7% sodium chlorite concentration, Birko Corporation, Denver, CO, and acidified with 2% lactic acid, Birko Corporation, Denver, CO; (4) 0.5% cetylpyridinium chloride (CPC), vol/vol, prepared from 40% CPC concentration, Cecure; Safe Foods Corporation, Little Rock, AR; (5) 2.0% lactic acid (LA), vol/vol, prepared from a 85% concentration, Birko Corporation, Denver, CO; (6) 1% lactoferricin B (LB), wt/vol, prepared from a 98% concentration, American Peptide Company, Sunnyvale, CA; (7) 0.02% peroxyacetic acid (PAA), vol/vol, prepared from a 5% peracetic acid solution, Birko Corporation, Denver, CO. All chemical solution treatments were prepared by thoroughly

mixing concentrated solutions with tap water to create the desired concentration levels.

Inoculum preparation

A composite culture of *E. coli* O157:H7 strains ATCC 43895, ATCC 43894, ATCC 43890, ATCC 43889 and EO139 was prepared for use in this study, as they were meat isolates that have been known to cause foodborne illness. These strains were available as frozen (-70°C) cultures in trypticase soy broth (BBL Becton Dickson Co., Sparks, MD) with 0.6% yeast extract (TSBYE) plus 20% glycerol (Mallinckrodt Baker Inc., Paris, KY). The cultures were activated by transferring 0.05 ml of stock culture in 10 ml of TSBYE and incubating (at 37°C) overnight. The strains were subcultured once (37°C, 24 h) by inoculating 100 µl of the activated culture in 10 ml TSBYE. The 10 ml overnight cultures were then mixed to form a 50 ml composite inoculum, which was serially diluted in 0.1% buffered peptone water (BPW; Difco Laboratories-Becton Dickinson Co, Sparks, MD) to obtain the required inoculum to recover either 3 to 4 $\log_{10}$ CFU/cm^2 (low inoculation level) or 5 to 6 $\log_{10}$ CFU/cm^2 (high inoculation level) of *E. coli* O157:H7. Each side of each piece of beef carcass adipose tissue (BCT) was inoculated with 500 µl of the culture composite and held at 4°C for 15 min to allow attachment.

Beef carcass adipose tissue

Fresh (<6 h postmortem) BCT was obtained from a local abattoir and transported to the Center for Red Meat Safety at Colorado State University. The outer surface of the BCT was removed and the remaining tissue was portioned to obtain 100 pieces approximately 5 cm × 2.5 cm × 1 cm (total surface area of 40 cm^2) in size. This phase of the study was replicated twice with five samples per treatment per inoculation level in each replicate.

The uninoculated pieces were used as the negative control treatment, and the inoculated BCT were assigned to one of ten control or treatment groups: (1) uninoculated/untreated (negative control); (2) inoculated/untreated (positive control); (3) inoculated/AA (at 25°C); (4) inoculated/AC; (5) inoculated/ASC; (6) inoculated/CPC; (7) inoculated/LA (at 55°C); (8) inoculated/LB; (9) inoculated/PA; (10) inoculated/water (W; at 25°C). The inoculated BCT pieces (with exception of those in the two control groups) were dipped into 500 ml of either ambient temperature or heated (55°C) solutions for 30 s. Control and treated BCT samples were placed into plastic bags Whirlpak® (Nasco, Fort Atkinson, WI) with 0.1% BPW and then pummeled in a stomacher (IUL Instruments, Barcelona, Spain) for 2 min. Aliquots of 0.1 ml were plated on tryptic soy agar containing 0.6% added yeast extract (TSAYE, Difco) for the enumeration of all aerobic bacteria, including *E. coli* O157:H7, and on sorbitol MacConkey agar (SMAC, Difco) for enumeration of sorbitol-negative colonies, which included *E. coli* O157:H7. The TSAYE and SMAC plates were incubated at 37°C for 48 hours and then manually counted. The pH of each solution was measured (Accumet pH meter 50, Fisher Scientific, Houston, TX; glass probe, Denver Instruments, Fisher Scientific) before and after each sample dip in order to monitor the buffering capacity of the BCT in each solution.

Samples that yielded no growth on SMAC plates were also tested by enrichment. One ml of the original sample blend was transferred into 40 ml of both EC broth (Difco) and Brilliant Green Bile broth (Difco) and incubated at 37°C for 24 h, and then streaked on SMAC plates containing cefixime and tellurite supplement (0.5 mg/l; Dynal Inc., Lake Success, NY) (SMACct) for detection of *E. coli* O157:H7. In addition, 1 ml of enrichment broth from each sample was mixed with 20 µl of anti-O157 immunomagnetic beads (Dynal Inc.) on a rocker for 30 min at 25°C. The bead suspensions were washed 3 times in 1 ml of phosphate buffered saline (PBS; Difco) containing 0.05% Tween 20 (Fisher Scientific). After the final wash, the beads were resuspended in 100 µl of PBS/0.05% Tween 20 and 50 µl of the bead suspensions were directly spread-plated on SMACct and incubated overnight at 37°C, at which time sorbitol-negative colonies were manually counted.

Boneless beef tissue

Fresh boneless beef short plates (BSP) were obtained from a local abattoir approximately 36 hours after harvest and transported to the Center for Red Meat Safety at Colorado State University. BSP was portioned to obtain 132 pieces that were approximately 5 cm × 2.5 cm × 1 cm (total surface area of 40 cm^2). Pieces of BSP were inoculated to yield the low or high inoculation level of the *E. coli* O157:H7 composite culture as was described for the first phase. For each level of inoculation, 300g of uninoculated lean tissue pieces (LTP) plus 100 g of inoculated BSP were placed in oversized Whirlpak® (Nasco) bags

TABLE 1. Least-squares means (standard error) indicating survival and reduction of bacterial counts (log CFU/cm²) on beef carcass tissue inoculated with *Escherichia coli* O157:H7, by plating on sorbitol MacConkey agar (SMAC)

Treatment Group	High Inoculation		Low Inoculation	
	Survival (log CFU/cm²)	Reduction (log CFU/cm²)	Survival (log CFU/cm²)	Reduction (log CFU/cm²)
Uninoculated/Untreated*	1.6 (0.16)	——	1.5 (0.09)	——
Inoculated/Untreated**	5.8 (0.05)[a]	——	4.1 (0.07)[a]	——
Inoculated/Water at 25°C	4.6 (0.16)[d]	1.2	3.5 (0.14)[b]	0.6
Inoculated/Acidified chlorine, 0.001% (AC)	5.4 (0.04)[b]	0.4	3.4 (0.05)[b]	0.8
Inoculated/Acetic acid, 2% (AA)	4.2 (0.12)[d]	1.6	2.0 (0.09)[d]	2.1
Inoculated/Lactic acid, 2% at 55°C (LA)	2.5 (0.16)[e]	3.3	1.1 (0.14)[e]	3.1
Inoculated/Lactoferricin B, 1% (LB)	5.1 (0.10)[c]	0.7	3.6 (0.06)[b]	0.6
Inoculated/Peroxyacetic acid, 0.02% (PAA)	4.4 (0.06)[d]	1.4	2.7 (0.11)[c]	1.4
Inoculated/Acidified sodium chlorite, 0.02% (ASC)	3.9 (0.16)[d]	1.9	2.1 (0.22)[d]	2.0
Inoculated/Cetylpyridinium chloride, 0.5% (CPC)	1.0 (0.23)[f]	4.8	0.5 (0.15)[f]	3.6

* Negative control

** Positive control

[a,b,c,d,e,f] Means within each column for survival bearing common superscript letter are not different ($P \geq 0.05$). Means in columns for reduction were not tested for statistical difference

n=10 samples per treatment and inoculation level

and shaken/rotated for one minute each to assure adequate mixing/cross contamination of inoculated and uninoculated pieces. To allow for attachment of the bacteria, the bags were stored at 4°C for 30 min. Uninoculated and inoculated pieces were assigned to each of 11 control or treatment groups: (1) uninoculated/untreated (negative control); (2) inoculated/untreated (positive control); (3) inoculated/A A(at 25°C); (4) inoculated/AC; (5) inoculated/ASC; (6) inoculated/CPC; (7) inoculated/LA (at 55°C); (8) inoculated/LB; (9) inoculated/PAA; (10) inoculated/W (at 25°C); (11) inoculated/W (at 55°C). For each control or treatment group, individual bags containing BSP and LTP were shaken/rotated (to assure adequate mixing of treatment with inoculated meat) for 30 s. Each bag of meat (400 g) was divided into three subsamples in order to be effectively homogenized in a sterile Waring Blender jar (Waring Product Division, New Hartford, CT). Aliquots of 1.0 ml were then removed from the subsamples for bacterial enumera-

tion, to determine the antimicrobial effectiveness of each antimicrobial intervention. This phase of the study was replicated twice with two samples per treatment and inoculation level in each replicate.

Statistical analysis

Five samples were evaluated per treatment subclass in the first phase of this experiment. In the second phase, two samples were subsampled three times and then evaluated per treatment subclass. Both phases of this experiment were replicated twice. Microbiological counts were converted to $\log_{10}$ CFU/cm^2 for the first phase and $\log_{10}$ CFU/g for the second phase before being analyzed. In the first phase of the experiment, the objective was to determine the effectiveness of treatment solutions on BCT. Analysis of fixed effects indicated that counts were dependent on type of media used (F-value = 77.79, $P < 0.0001$) and level of inoculum (F-value = 853.69, $P < 0.001$) determined using the general linear model procedure of SAS® Version 8.2 (*36*). The objective of the second phase was to determine the effectiveness of treatment solutions on LTP, and similarly, analysis of fixed effects indicated that counts were dependent on type of media (F-value = 38.90, $P < 0.0001$) and level of inoculum (F-value = 839.38, $P < 0.0001$). Thus in both phases these effects were dropped from the model and counts for each media are presented in different tables and separated by level of inoculum within tables. For each media and within level of inoculum, treatment least-squares means were segregated using a protected pairwise t-test of SAS® Version 8.2 (*36*) with significant differences considered at an alpha level = 0.05.

RESULTS AND DISCUSSION

Beef carcass adipose tissue

The pH of each treatment solution was measured before and after treatment applications to ensure that the buffering capacity of the meat was not affecting the pH of the treatment solutions. No deviations greater than 0.5 were observed from the original pH to the post-treatment pH. The most effective chemical intervention used in this study was cetylpyridinium chloride (CPC), which is a quaternary ammonium compound that has been demonstrated to reduce bacterial counts on beef carcasses by up to 6.0 logs when sprayed at concentrations of 0.5% to 1.0% (*13*). In the present study, the application of CPC to BCT inoculated with high levels of *E. coli* O157:H7 resulted in a reduction ($P < 0.05$) of 4.8 log CFU/cm^2 as observed on both SMAC and TSAYE plates (Tables 1 and 2). The *E. coli* O157:H7 counts on the low-dose inoculated BCT were reduced by 3.6 log CFU/cm^2 on SMAC plates, to almost undetectable levels of 0.5 log CFU/cm^2 (Table 1). The reduction in bacterial counts was very extensive, as indicated by the need to enrich 85% of the CPC treated samples. However, all samples that were tested by enrichment were found to be positive for *E. coli* O157:H7. Levels of bacterial reduction achieved by application of CPC were comparable on both SMAC (Table 1) and TSAYE (Table 2) agar plates. It should be noted, however, that CPC is not currently permitted for direct application to fresh red meat products in the United States (*43*).

Lactic acid, one of the most widely studied of the organic acids currently used in the beef industry, has been applied both heated and at room temperature (*8, 9, 11, 12, 14, 31, 33*). The effects of the use of LA differ among studies but generally suggest the achievement of a 1.0 to 2.0 log CFU/cm^2 reduction. In this study, LA (2%; at 55°C) was the second most effective decontamination agent studied, as it reduced significantly ($P < 0.05$) the presence of *E. coli* O157:H7 and total bacterial populations on BCT (Tables 1 and 2). Lactic acid reduced *E. coli* O157:H7counts on SMAC on products inoculated with high inoculum, from the initial 5.8 log CFU/cm^2 to 2.5 log CFU/cm^2 (Table 1). Bacterial counts on BCT administered the low-dose inoculation were significantly ($P < 0.05$) reduced, by 2.6 log CFU/cm^2, on TSAYE plates (Table 2).

In the present study, AA achieved lower reductions in bacterial populations than did LA on both SMAC and TSAYE agar plates at both inoculation levels (Tables 1 and 2). More specifically, when 2% AA was applied at room temperature, a 1.6 log CFU/cm^2 reduction ($P < 0.05$) in bacterial counts was observed on the high-dose inoculated BCT (Table 1). Acetic acid was slightly more effective in reducing the pathogen load on the low-dose inoculated BCT, as shown by a 2.1 log CFU/cm^2 decrease in bacterial populations (Table 1). The use of either LA or AA would be feasible and effective for pathogen reduction of sufficient magnitude to aid in increasing the safety of beef.

Castillo et al. (*10*) demonstrated that acidified sodium chlorite (ASC), used in a washing system, reduced the presence of

TABLE 2. Least-squares means (standard error) indicating survival and reduction of bacterial counts (log CFU/cm^2) on beef carcass tissue inoculated with *Escherichia coli* O157:H7, by plating on tryptic soy agar with 0.6% yeast extract (TSAYE)

Treatment Group	High Inoculation		Low Inoculation	
	Survival (log CFU/cm^2)	Reduction (log CFU/cm^2)	Survival (log CFU/cm^2)	Reduction (log CFU/cm^2)
Uninoculated/Untreated*	1.5 (0.14)	——	1.5 (0.14)	——
Inoculated/Untreated**	6.4 (0.04)^a	——	4.3 (0.05)^a	——
Inoculated/Water at 25°C	4.8 (0.16)^c	1.6	3.9 (0.09)^b	0.4
Inoculated/Acidified chlorine, 0.001% (AC)	5.7 (0.03)^b	0.7	3.7 (0.15)^b	0.6
Inoculated/Acetic acid, 2% (AA)	4.9 (0.10)^c	1.4	2.5 (0.18)^d	1.8
Inoculated/Lactic acid, 2% at 55°C (LA)	3.7 (0.10)^e	2.7	1.7 (0.11)^e	2.6
Inoculated/Lactoferricin B, 1% (LB)	5.7 (0.06)^b	0.7	4.4 (0.12)^a	-0.1
Inoculated/Peroxyacetic acid, 0.02% (PAA)	4.8 (0.10)^c	1.5	3.2 (0.07)^c	1.1
Inoculated/Acidified sodium chlorite, 0.02% (ASC)	4.3 (0.16)^d	2.1	3.0 (0.19)^c	1.3
Inoculated/Cetylpyridinium chloride, 0.5% (CPC)	1.5 (0.28)^f	4.8	0.8 (0.24)^f	3.5

* Negative control

** Positive control

^{a,b,c,d,e,f} Means within each column for survival bearing common superscript letter are not different ($P \geq 0.05$). Means in columns for reduction were not tested for statistical difference

n=10 samples per treatment and inoculation level

E. coli O157:H7 by 3.8 to 4.5 $\log_{10}$ CFU/cm^2 while use of water alone, without subsequent application of ASC, resulted in a 2.3 log CFU/cm^2 reduction. It could be speculated that the force at which the ASC was applied (1,320 Kpa) in the Castillo et al. (*10*) study aided in the reduction of pathogens on the surface of beef carcass tissue. In the present study, ASC reduced ($P < 0.05$) the pathogen counts (SMAC) on high-inoculum BCT by 1.9 log CFU/cm^2 and on low-inoculum BCT by 2.0 log CFU/cm^2. In addition, total counts recovered on the TSAYE plates (Table 2) showed ASC reduced the counts by 2.1 and 1.3 log CFU/cm^2 on high-inoculum and low-inoculum BCT, respectively.

In previous research, Farrell et al. (*17*) evaluated peroxyacetic acid as a sanitizer for meat contact surfaces. Peroxyacetic acid was effective in reducing the bacterial load, but total elimination of *E. coli* O157:H7 was not achieved. Use of PAA significantly ($P < 0.05$) reduced (1.4 log CFU/cm^2) pathogen counts on both high and low inoculated BCT, while a slightly higher reduction of the total bacterial populations (TSAYE) was observed on the high-inoculated product (Tables 1 and 2). In this experiment, PAA was just as effective at the high-inoculated level as AA ($P > 0.05$).

An inexpensive and simple contamination reduction strategy may be washing with water, which has been studied by many researchers (*39*). The effectiveness of water as a decontamination technology is determined by the temperature, pressure and time

TABLE 3. Least-squares means (standard error) indicating survival and reduction of bacterial counts (log CFU/g) on homogenized boneless beef short plates and lean tissue pieces inoculated with *Escherichia coli* O157:H7, by plating on sorbitol MacConkey agar (SMAC)

Treatment Group	High Inoculation		Low Inoculation	
	Survival (log CFU/g)	Reduction (log CFU/g)	Survival (log CFU/g)	Reduction (log CFU/g)
Uninoculated/Untreated*	2.3 (0.12)	——	2.3 (0.12)	——
Inoculated/Untreated**	5.8 (0.04)[a]	——	4.2 (0.29)[a]	——
Inoculated/Water at 25°C	5.4 (0.18)[a]	0.3	4.1 (0.12)[a]	0.2
Inoculated/Acidified chlorine, 0.001% (AC)	5.3 (0.10)[a]	0.5	4.0 (0.32)[a]	0.3
Inoculated/Acetic acid, 2% (AA)	4.7 (0.10)[b]	1.1	2.9 (0.09)[bc]	1.4
Inoculated/Lactic acid, 2% at 55°C (LA)	4.5 (0.19)[b]	1.3	2.5 (0.23)[c]	1.7
Inoculated/Lactoferricin B, 1% (LB)	5.4 (0.06)[a]	0.4	4.1 (0.10)[a]	0.1
Inoculated/Peroxyacetic acid, 0.02% (PAA)	4.8 (0.06)[ab]	1.0	3.1 (0.05)[b]	1.2
Inoculated/Acidified sodium chlorite, 0.02% (ASC)	4.0 (0.19)[c]	1.8	2.8 (0.24)[bc]	1.5
Inoculated/Cetylpyridinium chloride, 0.5% (CPC)	3.7 (0.18)[c]	2.1	2.3 (0.12)[c]	1.9
Inoculated/Water at 55°C	4.7 (0.09)[b]	1.1	3.4 (0.12)[b]	0.8

* Negative control

** Positive control

[a,b,c,] Means within each column for survival bearing common superscript letter are not different ($P \geq 0.05$). Means in columns for reduction were not tested for statistical difference

n=4 samples per treatment and inoculation level with 3 subsamples per sample

at which it is applied; therefore, increasing the temperature, pressure and time should enhance the effectiveness of contamination reduction (*22*). In the present study, water was applied at room temperature (25°C); its use reduced ($P < 0.05$) the presence of the pathogen (SMAC) by 1.2 and 0.6 log CFU/cm^2 on high-inoculum and low-inoculum BCT, respectively, and similar results were observed on TSAYE plates (Tables 1 and 2). Because of its current availability, water will likely continue to be the most widely used intervention in beef slaughtering facilities.

There is no evidence to date of the effectiveness of acidified chlorine (AC), which is a new intervention proposed for use in Canada to enhance the microbiological status of meat. When used as recommended by Advanced Food Systems, Kamloops, B. C., at 10 ppm (0.001%), its effectiveness in reducing total bacteria (TSAYE) and pathogen (SMAC) populations was slight (Tables 1 and 2). Acidified chlorine reduced pathogen counts (SMAC) by 0.7 and 0.6 CFU/cm^2 for high and low levels of inoculation, respectively (Table 1). Although there is no scientific evidence, increasing the concentration of the solution may enhance the effectiveness of this intervention.

Lactoferricin B, a peptide in lactoferrin, has been shown to have antimicrobial activity (*5*). In previous research (*42*), it was demonstrated that use of 50 and 100 μg/ml of LB reduced *E. coli* O157:H7 by 0.7 and 2.0 log CFU/ml, respectively. In contrast, it has been suggested (*5*) that the effective dose of lactoferricin B for a 3.0 log CFU/cm^2 reduction in *E. coli* IID:861 was 10 μg/ml. As applied in the present study, LB (10 μg/ml; 1.0%) reduced the to-

TABLE 4. Least-squares means (standard error) indicating survival and reduction of bacterial counts (log CFU/g) on homogenized boneless beef short plates and lean tissue pieces inoculated with *Escherichia coli* O157:H7, by plating on tryptic soy agar with 0.6% yeast extract (TSAYE)

Treatment Group	High Inoculation		Low Inoculation	
	Survival (log CFU/g)	Reduction (log CFU/g)	Survival (log CFU/g)	Reduction (log CFU/g)
Uninoculated/Untreated*	2.8 (0.05)	——	2.8 (0.05)	——
Inoculated/Untreated**	5.9 (0.06)[a]	——	4.3 (0.26)[a]	——
Inoculated/Water at 25°C	5.8 (0.09)[a]	0.1	4.3 (0.07)[a]	0.0
Inoculated/Acidified chlorine, 0.001% (AC)	5.7 (0.01)[a]	0.2	4.0 (0.29)[a]	0.3
Inoculated/Acetic acid, 2% (AA)	5.3 (0.18)[b]	0.6	3.2 (0.29)[b]	1.1
Inoculated/Lactic acid, 2% at 55°C (LA)	4.7 (0.06)[c]	1.1	2.8 (0.13)[c]	1.5
Inoculated/Lactoferricin B, 1% (LB)	5.4 (0.04)[ab]	0.5	4.5 (0.20)[a]	-0.3
Inoculated/Peroxyacetic acid, 0.02% (PAA)	4.9 (0.04)[c]	1.0	3.3 (0.11)[b]	1.0
Inoculated/Acidified sodium chlorite, 0.02% (ASC)	4.9 (0.07)[c]	1.0	3.2 (0.05)[b]	1.1
Inoculated/Cetylpyridinium chloride, 0.5% (CPC)	3.9 (0.17)[d]	2.0	2.6 (0.12)[c]	1.7
Inoculated/Water at 55°C	5.3 (0.18)[b]	0.6	3.7 (0.05)[ab]	0.6

* Negative control

** Positive control

[a,b,c,d] Means within each column for survival bearing common superscript letter are not different ($P \geq 0.05$). Means in columns for reduction were not tested for statistical difference

n=4 samples per treatment and inoculation level with 3 subsamples per sample

tal bacterial populations (TSAYE) on high-inoculum product by 0.7 log CFU/cm^2 and did not reduce ($P \geq 0.05$) the level of bacteria on the low-inoculum product (Table 2). Reductions of 0.7 and 0.6 log CFU/cm^2 in pathogen counts on SMAC (Table 1) at high and low inoculation levels, respectively, were observed when LB was used. Naidu (*28*) suggests that lactoferricin B is effective in preventing pathogens from attaching on the surface of carcasses; further studies should be conducted to test this suggestion.

Boneless beef trimmings

Pieces of BSP and LTP were used to simulate the decontamination of beef trimmings. Trends in reducing *E. coli* O157:H7 counts due to intervention chemicals used on BSP and LTP were generally similar to the counts recovered from BCT, except that fewer chemical interventions significantly ($P < 0.05$) reduced the level of total bacterial and pathogen contamination (Tables 3 and 4). Cetylpyridinium chloride was the most effective at reducing the microbiological load on BSP and LTP at both inoculation levels.

It was observed that, with use of SMAC plates, CPC reduced ($P < 0.05$) pathogen populations, from an initial count of 5.8 log CFU/g to a final count of 3.7 log CFU/g and from an initial count of 4.2 log CFU/g to a final count of 2.3 log CFU/g at high and low inoculation levels, respectively (Table 3). On TSAYE plates, CPC reduced bacterial populations by comparable levels (Table 4).

When bacterial populations were enumerated with TSAYE agar plates, it was found that

heated (55°C) LA (2%) resulted in a 1.1 log CFU/g reduction of total bacterial populations on BSP and LTP at the high inoculation level and a 1.5 log CFU/g reduction of bacteria at the low inoculation level (Table 4). An even greater reduction in *E. coli* O157:H7 counts on BSP and LTP was observed after treatment with LA (2% at 55°C) when counts were enumerated on SMAC agar plates (Table 3). Although the reduction in total bacterial counts (TSAYE) from the original inoculation level was significant ($P < 0.05$), the use of AA was only modestly effective in the decontamination of BSP and LTP, as evidenced by reductions of only 0.6 log CFU/g and 1.1 log CFU/g at the high-dose and low-dose inoculation levels, respectively (Table 4). However, AA was slightly more effective in reducing *E. coli* O157:H7 numbers, as shown by a reduction of 1.1 log CFU/g and 1.4 log CFU/g for the high and low inoculation levels, respectively (Table 3).

Acidified sodium chlorite was also effective at reducing ($P < 0.05$) total bacterial populations (TSAYE) and pathogen populations (SMAC) on BSP and LTP (Tables 3 and 4). The ASC solution treatment was effective in reducing *E. coli* O157:H7 on BSP and LTP based on enumeration from SMAC plates, showing 1.8 and 1.5 log CFU/g reductions at high and low inoculation levels, respectively (Table 3). However, ASC was less effective in reducing total aerobes (TSAYE) on BSP and LTP, as shown by a 1.0 and 1.1 log CFU/g reduction for high and low inoculation levels, respectively (Table 4).

Peroxyacetic acid was shown to reduce bacterial populations on BSP and LTP by 1.0 and 1.2 log CFU/g as determined on SMAC plates (Table 3), and by 1.0 and 1.0 log CFU/g as determined on TSAYE plates (Table 4) at high and low inoculation levels, respectively. When water was applied to BSP and LTP at 55°C, a 1.1 and 0.8 log CFU/g reduction in bacterial counts was observed on SMAC (Table 3) plates at high and low inoculation levels, respectively. On TSAYE plates the reduction in pathogen populations associated with the use of water (55°C) as a decontamination agent was less than 1.0 log CFU/g at either inoculation level (Table 4). The use of acidified chlorine, lactoferricin B or water (at 25°C) did not reduce ($P \geq 0.05$) bacterial populations at either level of *E. coli* O157:H7 inoculation on either SMAC or TSAYE plates when used as a chemical sanitizer on BSP or LTP (Tables 3 and 4).

The results of this study indicated that LA, which is commonly used in microbiological intervention strategies, was the most effective antimicrobial agent currently approved for use in reducing total bacterial populations on beef carcass tissue and beef trimmings. Similar reductions were observed on total bacterial and pathogen populations on BSP and LTP treated with federally approved AA, ASC, and PAA compounds. The most effective microbiological intervention used in both phases of this study was CPC, which, however, is currently not approved as a chemical intervention on beef carcasses or on food contact surfaces (*43*).

ACKNOWLEDGMENTS

Funding for this study was provided by the National Cattlemen's Beef Association and the Colorado State University Agricultural Experiment Station.

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