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Bacterial Profile of Ground Beef Made from Carcass Tissue Experimentally Contaminated with Pathogenic and Spoilage Bacteria before Being Washed with Hot Water, Alkaline Solution, or Organic Acid and Then Stored at 4 or 12°C‡

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ABSTRACT

The long-term effectiveness of several beef-carcass surface-tissue (BCT) wash interventions on the microbiology of ground beef produced from this tissue was determined. BCT was inoculated with bovine feces containing one of two different levels (ca. 4 or 6 log CFU/ml) of *Escherichia coli* O157:H7, *Listeria innocua, Salmonella typhimurium,* and *Clostridium sporogenes.* The BCT was then subjected to one of several treatment washes: 2% (vol/vol) DL-lactic acid (LA), 2% (vol/vol) acetic acid (AA), 12% (wt/vol) trisodium phosphate (TSP), hot water (HW; 74 ± 2°C at the tissue surface), or water (WW; 32 ± 2°C at the tissue surface). A control group was left untreated. After treatments, BCT was held at 4°C for 24 h and then ground. The ground beef was packaged and incubated at 4°C for 21 days or 12°C for 3 days. AA-treated samples held at 12°C for 3 days yielded significantly lower aerobic plate counts than the control and also yielded the lowest levels of pseudomonads when compared to other sample groups. After being held at 4°C for 21 days or 12°C for 3 days, samples treated with antimicrobial compounds had lower or no detectable (<1 CFU/g) levels of *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes* than beef treated with a WW or the control. Ground beef produced from tissue treated with HW yielded lower populations of these bacteria when compared to WW or untreated control beef, but the populations were generally higher than those observed in any of the antimicrobial chemical-treated samples. These trends continued throughout all storage conditions over time. Results from this study indicate that the use of carcass interventions, especially antimicrobial compounds, presently available to the slaughter industry will lower bacterial counts in ground beef.

About 43% of beef consumed per capita in the U.S. is ground *(1),* and therefore pathogens present in this product can potentially affect large segments of the population. The growth potential and survival of various nonpathogenic and pathogenic bacteria found in ground beef have been thoroughly investigated over the years *(6, 8, 20, 21, 26, 30, 33).* The immediate effect various beef carcass intervention treatments (hot water, organic acid, and alkaline washes) have on the presence of bacteria has also been investigated *(4, 11, 12, 19, 27, 28, 34).*

Few studies have attempted to determine the fate of bacterial populations found in ground beef but originating from beef carcasses which have been subjected to various intervention processes before grinding *(24).* A recent microbial survey of midwestern red meat processing plants determined that ground beef contained an average of 4 to 6 log CFU of aerobic bacteria per g *(13).* These researchers state that the most important factor contributing to the source and level of microbial contamination for ground beef was the raw beef materials used for grinding. The purpose of this study was to document the long-term effect hot water, organic acid, and alkaline carcass wash treatments have on various pathogenic and nonpathogenic bacterial populations originating on carcasses and then being found to be present in the ground beef produced from these carcasses.

MATERIALS AND METHODS

Bacterial cultures and fecal inoculum. *Escherichia coli* O157:H7 CDC B6-914, *Listeria innocua* ATCC 33090, *Salmonella typhimurium* ATCC 14028, and *Clostridium sporogenes* ATCC 11437 were selected for resistance to antibiotics as described previously *(11).* To enhance the ability to selectively enumerate the marked bacteria in natural bovine feces, *E. coli* MARC1-S was enumerated on sorbitol McConkey agar (SMAC) (Difco Laboratories, Detroit, Mich.) containing 250 μg of streptomycin (Sigma Chemical Co., St. Louis, Mo.) per ml. Oxoid listeria selective agar (LSA) (Unipath, Ogdensburg, N.Y.) with 500 μg of streptomycin per ml was used to enumerate *L. innocua* MARC1-S. Clostridium botulinum isolation agar without egg yolk (CBI) with 50 μg of novobiocin (Sigma Chemical Co.) per ml was used to enumerate *C. sporogenes* MARC1-N. *S. typhimurium* MARC1-R was enumerated on Rambach agar (RA) (E. Merck, Gene-Trak Systems Corp., Hopkinton, Miss.) with 250 μg of nalidixic acid (Sigma Chemical Co.) per ml.

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‡ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

The inoculum used for each of six replications was prepared by collecting bovine feces immediately postdefecation from three heifers maintained on a hay-silage diet. Ten grams of each fecal sample was combined in a sterile Stomacher bag (Sterifil, Spiral Biotech, Bethesda, Md.) with 270 ml of sterile physiological saline and stomached for 1 min with a Model 400 Stomacher (Tekmar, Inc., Cincinnati, Ohio). An additional 1:10 dilution was made from this slurry.

Twenty milliliters of each culture, *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes,* grown quiescently overnight in tryptic soy broth plus yeast extract (TSBYE) or Schadler broth (Difco) with the appropriate antibiotic was transferred to separate 50-ml sterile conical centrifuge tubes, centrifuged at 1,690 × *g* for 15 min at 5°C. The resulting pellets of *E. coli* O157:H7 and *L. innocua* were resuspended in 20 ml of buffered peptone water (BPW; BBL, Cockeysville, Md.) and the pellets of *S. typhimurium* and *C. sporogenes* were each resuspended in 10 ml of BPW. Initial culture concentrations were determined from a McFarland standard curve obtained with a Spectronic 20 (Milton Roy Co., Rolling Meadows, Ill.) set at 650 nm. Serial dilutions, where appropriate, were made to a final concentration of ca. 10^5 CFU/ml (low-level inoculum) and 10^7 CFU/ml (high-level inoculum) for each bacterium. Separate low- (10^4 CFU/ml) and high- (10^6 CFU/ml) level final inocula were made in order to produce two separate sets of ground beef samples, one with high levels and one with low levels of bacterial contamination. This was accomplished by adding 1 ml of each culture of the appropriate dilution (low or high) to a 15-ml sterile conical centrifuge tube (4 ml), mixing by vortex, and then removing 2 ml of the resulting culture mix and adding it to 18 ml of the fecal slurry described above. No attempt was made to control the final levels of mesophilic aerobic bacteria (APC), lactic acid bacteria (LAB), or pseudomonads naturally occurring in the bovine feces.

Beef neck tissue preparation. Beef carcass necks were collected immediately after slaughter from a local cow and bull operation, individually placed in plastic bags, stored in an insulated carrier to prevent rapid cooling, and transported to the Roman L. Hruska U.S. Meat Animal Research Center (MARC). At MARC, each neck was placed on an aseptic plastic tray and three separate areas on each neck were marked with edible ink by using a sterile stainless-steel template and cotton swab. One area measured 10 by 10 cm (100 cm^2) and the other two measured 5 by 5 cm (25 cm^2). The two 25-cm^2 areas were used to determine the before- and after-treatment bacterial levels and the 100-cm^2 area was included in the ground beef as the contaminating source. The 100-cm^2 and 25-cm^2 surfaces were inoculated by pipeting 1 and 0.25 ml, respectively, of the appropriate inoculum (low or high level) onto the surface and using a sterile latex-gloved hand to spread it over the entire marked surface. This procedure resulted in an initial 2- to 3-log CFU/cm^2 (low level) and 4- to 5-log CFU/cm^2 (high level) inoculation of *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes* on the beef surface tissue. The inoculated beef was allowed to stand at room temperature for 15 min and then subjected to the intervention strategies described below. Control tissue received no treatments.

Wash cabinet and treatments. The wash cabinet used for this study was a stainless-steel insertable pod of the commercial carcass washer described by Dorsa et al. *(11).* Spray treatments were applied for 15 s at 80 lb/in^2 and 32 ± 2°C at the tissue surface except in the case of hot water, which was sprayed at 74 ± 2°C. All other physical parameters of the washer were set and monitored to parallel those used in previous research involving the commercial carcass washer *(10, 11).* Wash treatments applied to the beef necks were 2% (vol/vol) DL-lactic acid (LA) (Sigma Chemical Co., St. Louis, Mo.), 2% (vol/vol) glacial acetic acid (AA) (Fisher Scientific, St. Louis, Mo.), 12% (wt/vol) trisodium phosphate (TSP) (Rhone-Poulenc, Cranbury, N.J.), hot water (HW; 70 ± 2°C at the tissue surface), or water (WW; 32 ± 2°C at the tissue surface). The control group was left untreated. Each treatment was replicated six times.

Ground beef preparation. The inoculated and treated beef necks were then placed on aseptic plastic trays and placed in a segregated section of a 4°C walk-in cooler. After 24 h, 1,000 g of meat from the neck center was aseptically removed, including the marked 100-cm^2 inoculated surface, and ground through a 4.5-mm head on a Model MG12, 0.25-hp (186.5 = W) grinder (Davpol Enterprises, Inc., New York, N.Y.). Nonsterile fat was added as part of the total sample to yield ca. 90% lean ground beef. The resulting ground beef was placed into a sterile stomacher bag and thoroughly mixed by hand kneading for 2 min. Fat content of the ground beef was determined from a 56.7-g sample with a fat percentage analyzer model F-100 (Needham Mfg. Co. Inc., Needham Heights, Mass.). One-hundred-gram portions of the ground beef were placed into 5 by 18 cm 3.2-mil nylon-copolymer bags with an oxygen transmission rate of 52 cm^3/m^2 at 23°C dry (Holly Sales, Omaha, Nebr.) and heat sealed with a Hollymatic model LV 10 G (Hollymatic Corp., Countryside, Ill.). This bag material was selected as suggested by Hollymatic Corp. representatives who corroborated that it is standard packaging material used in the grinding industry to package ground beef for storage and shipment to retail outlets (personal communications). This point in the process is referred to as day 0 ground beef during the experiment. The individual ground beef samples were placed in cold storage to be sampled on subsequent days as described below.

Enumeration of bacteria from inoculated areas and ground beef. Immediately before treatments, one premarked inoculated 5 by 5 cm tissue section approximately 1 mm thick was excised from the beef neck and placed into a stomacher bag for analysis. The second 5 by 5 cm 1-mm-thick tissue section was taken from the beef neck for bacterial analysis after treatments and after 24 h at 4°C refrigeration storage. In both cases, following excision, the 25-cm^2 samples were placed into a stomacher bag with 25 ml of buffered peptone water (BPW) plus 0.1% (vol/vol) Tween 20. For ground beef analysis, an 85-g sample was taken immediately after grinding the beef neck meat (including the 100-cm^2 inoculated area) and placed into a stomacher bag with 85 ml of buffered peptone water (BPW) plus 0.1% Tween 20. The microbial profile of the ground beef was also determined at 7, 14, and 21 days after being incubated at 4°C and 1, 2, and 3 days after being incubated at 12°C as described above.

All meat samples were pummeled for 2 min with a Model 400 Stomacher (Tekmar, Inc., Cincinnati, OH). Serial dilutions were made from these samples, when required, in BPW. Samples were then spiral plated with a Model D spiral plater (Spiral Systems Instruments, Bethesda, Md.) in duplicate or spread plated (1 ml total volume, over 4 plates) on appropriate media. The number of CFU of each inoculated bacterium per square centimeter or gram was recorded with a digital counter or a CASBA IV computer-assisted colony image analyzer (Spiral Biotech, Inc., Bethesda, Md.) and converted to a logarithm.

The marked bacteria were enumerated on the media described above in the section concerning bacterial cultures and fecal inoculum. APC, LAB, and pseudomonads were enumerated on Trypticase soy agar (TSA) (BBL) at 37°C for 24 h, Bacto lactobacilli agar (MRS) (BBL) with 0.02% sodium azide in 5%

CO_2 at 30°C for 48 h, and pseudomonas isolation agar (PIA) (Difco) at 37°C for 24 h, respectively.

pH determination. The surface pH of the neck meat was determined using a flat-surface combination probe (Corning model 245, Corning, Inc., Corning, N.Y.) immediately after treatment and 24 h later (4°C incubation). A spear-tip probe (Corning, Inc.) was used to determine the pH of all ground beef samples prior to microbial analysis. The 15 g of ground beef not used for enumeration from each sample was used for pH determination.

Calculations and data analysis. The means of duplicate plate counts were converted to the logarithms of the number of CFU per square centimeter or gram where appropriate. To facilitate logarithmic analysis, any 0-count plate was assigned a value of 10 or 1 on the basis of the lowest limit of detection for the spiral plate or spread plate counting method, respectively. Least squares means (LSM) and population growth data were analyzed using the general linear model procedure (GLM) of SAS (SAS Institute, Cary, N.C.) with a probability of 0.05 used as the level of significance unless stated otherwise.

RESULTS

The immediate effect various wash treatments had on the beef surface pH is given in Table 1. The lowest and highest pH values observed on the organic acid- and alkaline-treated tissue, respectively, persisted for 24 h. Once the tissue was ground the average pH was 5.81 ± 0.19 regardless of the treatment received by the tissue prior to grinding. The pH for all ground meat samples dropped slightly over the 21-day study period to an average of 5.58 ± 0.25, within the range Gill and Newton *(17)* describe as "the ultimate pH of muscle tissue." The average percent fat content of ground beef from beef tissue treated with WW, HW, LA, AA, TSP, and the untreated control was 10.7, 10.6, 10.4, 10.6, 10.7, and 10.5, respectively. Excessive gas formation (blow-up) was observed at the 21-day sample period for three of six study replications (50%) in control packs of ground beef incubated at 4°C. There was also a single occurrence of blow-up in each of the W-, LA-, and TSP-treated samples at the 21-day sample period of 4°C storage. The control sample blow-ups occurred in samples inoculated at both high and low levels, while the W, LA, and TSP blow-ups occurred only in samples receiving high levels of inocula.

TABLE 1. *The average pH of beef tissue surfaces and resulting ground beef from samples that were inoculated at both high and low levels before treatment, stored at 4°C for 24 h, and then converted to ground beef*

Sample	Time of pH measurement after treatment[a]	pH at time after treatment:[b] None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP
Beef tissue surface	<0.5 hour	8.39	7.92	8.20	3.82	4.33	11.18
	24 h	7.39	8.09	7.46	3.84	4.91	9.14
Ground beef	Day 0	5.80	5.77	5.80	5.75	5.71	6.00

[a] Time <0.5 h, immediately after treatment; 24 h at 4°C; day 0, immediately after grinding.

[b] LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.

Regardless of the initial inoculation level, APC were reduced to significantly lower levels on beef surface tissue when hot water or any antimicrobial wash was used compared to APC on C and WW samples (Table 2). At the lower level of inoculation, there were no such significant differences in any samples of ground beef. This relationship was consistent throughout the study regardless of incubation temperature and time. However, at the higher initial inoculation level, beef subjected to HW and AA treatments exhibited significantly lower APC in the initial ground beef compared to the control. At the end of the 21-day 4°C storage period, there was no difference between any samples. AA-treated samples held at 12°C for 3 days exhibited significantly lower APC than the control.

After grinding, there were no significant differences in LAB and pseudomonad populations between any treated samples and the control (Tables 3 and 4). This relationship was maintained over all storage times, inoculation levels, and temperatures for LAB, although after samples had been held at 12°C for 3 days pseudomonads were generally present at notable to significantly lower levels in the organic acid-treated samples. AA-treated samples demonstrated the lowest levels of pseudomonads after 21 days of 4°C storage.

E. coli O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes* CFU were significantly reduced or brought to below detectable levels on the beef surface by HW and all antimicrobial washes (Tables 5 to 8). At high inoculation levels, this effect on *E. coli* O157:H7 populations was maintained when samples were converted to ground beef. At low inoculation levels of *E. coli* O157:H7, as much as a 1-log difference in the means between samples of treatments was observed, although these differences were not statistically significant. After being held at 12°C for 3 days, antimicrobial agent-treated samples had arithmetically lower levels of *E. coli* O157:H7 CFU, but only AA-treated samples were significantly lower. Ground beef made from tissue inoculated at higher initial levels and subjected to any antimicrobial treatment and then held at 12°C for 3 days yielded significantly lower *E. coli* O157:H7 counts than beef treated with a WW or the untreated control. A similar trend for *E. coli* O157:H7 was observed when samples were held at 4°C for 21 days.

L. innocua was not detected in any of the samples of ground meat made from meat that had received a chemical antimicrobial treatment. Following storage at 12°C for 3 days, samples originating from low-level inoculation tissue receiving AA or TSP treatments still contained no detectable *L. innocua* CFU. The HW-, LA-, AA-, and TSP-treated samples had significantly lower levels of *L. innocua* than the control or W. This trend was observed in the cases of LA-, AA-, and TSP-treated samples that had received high initial inoculation levels and were held at 12°C for 3 days. *L. innocua* responded similarly in samples held at 4°C for 21 days.

S. typhimurium was either undetectable or detectable at low levels occasionally in samples of ground beef produced from tissue containing low initial inoculation levels and receiving HW or an antimicrobial treatment (Table 7).

TABLE 2. *The least squares means of mesophilic aerobic bacteria on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	Aerobic mesophils ($\log_{10}$ CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	4.0A[d]	4.2A	3.8A	3.9A	3.8A	4.0A	0.20
	B	4°C	3.7A	3.6A	2.2B	2.2B	2.5B	1.8B	0.23
Ground beef	0	—	3.5A	3.4A	3.1A	3.7A	3.4A	3.6A	0.42
	1	12°C	3.2A	3.3A	3.5A	3.3A	2.7A	3.7A	0.55
	2	12°C	4.3A	4.6A	4.5A	4.3A	4.3A	4.6A	0.62
	3	12°C	5.4A	5.8A	5.5A	5.1A	4.7A	5.7A	0.80
	7	4°C	5.1A	5.6A	5.3A	4.8A	4.7A	5.4A	0.99
	14	4°C	6.1A	6.1A	6.4A	5.5A	5.9A	6.0A	1.16
	21	4°C	6.2A	6.3A	6.6A	5.3A	5.9A	7.0A	1.04
	High inoculation level								
Beef tissue surface	A	—	5.8A	5.7A	5.7A	5.7A	5.7A	5.7A	0.09
	B	4°C	5.4A	5.3A	3.2B	2.9B	3.1B	2.5B	0.33
Ground beef	0	—	4.3A	3.9AB	3.2B	3.6AB	3.3B	3.6AB	0.36
	1	12°C	4.2A	4.3A	3.4A	3.4A	3.1A	3.7A	0.46
	2	12°C	5.5A	5.1A	4.8A	4.4A	4.1A	4.4A	0.61
	3	12°C	6.4A	5.9AB	6.1AB	5.2AB	4.6B	5.3AB	0.67
	7	4°C	5.7A	5.5A	5.4A	4.5A	5.0A	4.9A	0.86
	14	4°C	6.4A	6.1A	6.8A	5.6A	5.6A	5.9A	1.06
	21	4°C	6.8A	6.2A	6.9A	6.8A	5.4A	6.0A	0.95

[a] LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacteria counted from inoculated beef surface tissue before it was subjected to any treatment (A), after treatment and 24 h at 4°C (B), and from resulting ground beef on days 0, 1, 2, 3, 7, 14, and 21.
[c] Indicates initial before-treatment beef tissue surface inoculation levels of selected bacteria (i.e., *E. coli* O157:H7, *S. typhimurium, L. innocua,* and *C. sporogenes;* high level ca. 5 $\log_{10}$ CFU/cm^2 and low level ca. 2.5 $\log_{10}$ CFU/cm^2).
[d] Means within a row with no common following letter are significantly different ($P < 0.05$).

TABLE 3. *The least squares means of lactic acid bacteria on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	Lactic acid bacteria ($\log_{10}$ CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	2.0A[d]	1.9A	1.8A	1.8A	1.7A	1.8A	0.24
	B	4°C	1.4A	0.9A	0.3B	0.0B	0.1B	0.0B	0.22
Ground beef	0	—	1.4A	1.9A	1.5A	1.7A	1.4A	2.2A	0.51
	1	12°C	1.3A	1.5A	1.8A	2.2A	2.1A	2.4A	0.62
	2	12°C	3.5A	3.6A	4.4A	4.1A	4.5A	4.3A	0.84
	3	12°C	4.3A	4.2A	4.9A	4.4A	4.9A	5.0A	0.86
	7	4°C	4.3A	4.1A	4.2A	4.2A	4.4A	4.7A	1.05
	14	4°C	4.4A	4.3A	5.0A	4.7A	5.1A	4.4A	1.29
	21	4°C	4.6A	4.1A	4.6A	4.7A	5.1A	5.4A	1.34
	High inoculation level								
Beef tissue surface	A	—	2.4A	2.0AB	1.6B	2.2AB	1.8AB	1.8AB	0.22
	B	4°C	1.5A	0.9AB	0.2C	0.4C	0.0C	0.5BC	0.20
Ground beef	0	—	1.5A	1.4A	1.8A	1.4A	1.5A	1.6A	0.52
	1	12°C	1.3A	1.5A	2.0A	1.6A	1.8A	1.7A	0.69
	2	12°C	3.5A	3.1A	3.8A	3.6A	3.7A	3.6A	0.95
	3	12°C	4.3A	4.3A	4.9A	3.8A	4.1A	4.4A	0.92
	7	4°C	4.3A	4.2A	4.7A	4.5A	4.3A	4.6A	1.08
	14	4°C	4.4A	3.6A	5.6A	4.3A	4.7A	4.9A	1.24
	21	4°C	4.6A	3.7A	6.1A	4.2A	5.0A	5.4A	1.32

[a,b,c,d] See Table 2 footnotes.

TABLE 4. *The least squares means of pseudomonads on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	Pseudomonads (log_{10} CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	0.8A[d]	1.3A	0.7A	0.3A	1.5A	0.3A	0.40
	B	4°C	0.7AB	1.2A	0.0B	0.0B	0.3B	0.0B	0.28
Ground beef	0	—	1.0A	1.3A	0.9A	0.9A	0.6A	1.8A	0.57
	1	12°C	1.3A	1.0A	1.3A	0.8A	0.3A	2.2A	0.57
	2	12°C	2.4A	1.9A	2.8A	1.5A	0.7A	2.6A	0.66
	3	12°C	2.8A	3.1A	2.3AB	1.5AB	0.5B	2.8A	0.81
	7	4°C	2.5AB	1.8AB	2.2AB	1.7AB	0.4B	2.8A	0.80
	14	4°C	2.9A	3.2A	2.8A	1.0A	0.8A	2.8A	0.90
	21	4°C	2.8AB	3.1A	1.9AB	1.0AB	0.5B	3.1A	0.94
	High inoculation level								
Beef tissue surface	A	—	1.5A	1.0A	1.4A	1.3A	0.6A	0.6A	0.42
	B	4°C	1.3A	1.2A	0.3B	0.0B	0.2B	0.3B	0.30
Ground beef	0	—	1.1A	0.8A	0.9A	1.0A	0.9A	1.2A	0.49
	1	12°C	0.9A	0.8A	0.9A	1.1A	0.8A	1.3A	0.50
	2	12°C	1.6A	1.0A	1.9A	1.2A	0.6A	1.5A	0.63
	3	12°C	2.8A	2.6AB	2.4AB	1.9AB	0.9B	2.0AB	0.69
	7	4°C	1.4A	0.9A	1.5A	1.3A	0.7A	1.7A	0.67
	14	4°C	0.8A	1.0A	1.5A	1.8A	1.1A	2.5A	0.64
	21	4°C	1.3AB	1.2B	1.6AB	1.5AB	0.6B	3.2A	0.70

[a,b,c,d] See Table 2 footnotes.

TABLE 5. *The least squares means of* Escherichia coli *O157:H7 on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	*Escherichia coli* O157:H7 (log_{10} CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	1.8B[d]	2.2AB	2.1AB	2.5A	2.2AB	2.4A	0.20
	B	4°C	1.4A	0.8A	0.1B	0.0B	0.2B	0.0B	0.22
Ground beef	0	—	0.5A	1.0A	0.4A	0.0A	0.3A	0.5A	0.39
	1	12°C	0.4A	0.4A	0.0B	0.0B	0.0B	0.1B	0.11
	2	12°C	1.3A	1.1A	0.2B	0.5AB	0.0B	0.3B	0.32
	3	12°C	1.5A	0.8AB	0.7AB	0.4AB	0.0B	0.3AB	0.45
	7	4°C	0.4A	0.1AB	0.0B	0.2AB	0.0B	0.0B	0.13
	14	4°C	0.2A	0.0A	0.0A	0.3A	0.0A	0.0A	0.14
	21	4°C	0.1A	0.0A	0.0A	0.4A	0.0A	0.2A	0.16
	High inoculation level								
Beef tissue surface	A	—	5.2A	5.0A	5.0A	5.2A	5.1A	5.2A	0.13
	B	4°C	4.5A	4.2A	1.8B	1.4B	1.3BC	0.2C	0.38
Ground beef	0	—	3.0A	2.7A	0.6B	0.1B	0.2B	0.7B	0.29
	1	12°C	3.1A	2.7A	0.8BC	0.2C	0.6BC	1.1B	0.29
	2	12°C	3.3A	2.8B	1.5C	0.6C	1.2C	1.6C	0.37
	3	12°C	3.3A	3.2A	2.2AB	0.5C	0.9C	1.5BC	0.39
	7	4°C	2.9A	2.4A	1.0B	0.2C	0.3BC	0.5BC	0.28
	14	4°C	2.4A	1.2B	0.8B	0.1C	0.2C	0.2C	0.20
	21	4°C	1.8A	0.9B	0.4BC	0.0C	0.2C	0.0C	0.21

[a,b,c,d] See Table 2 footnotes.

TABLE 6. *The least squares means of* Listeria innocua *on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	*Listeria innocua* ($\log_{10}$ CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	2.2A[d]	2.1A	1.7A	2.1A	1.8A	1.7A	0.39
	B	4°C	1.7A	1.4A	0.0B	0.0B	0.4B	0.1B	0.26
Ground beef	0	—	0.7A	0.8A	0.1A	0.0A	0.0A	0.0A	0.29
	1	12°C	0.9A	0.7AB	0.2AB	0.0B	0.0B	0.1B	0.28
	2	12°C	1.6A	1.4A	0.2B	0.2B	0.0B	0.0B	0.37
	3	12°C	2.3A	2.2A	0.3B	0.2B	0.0B	0.0B	0.55
	7	4°C	1.5A	1.3AB	0.5AB	0.4AB	0.1AB	0.0B	0.51
	14	4°C	2.3A	2.3A	1.3AB	0.8AB	0.0B	0.0B	0.74
	21	4°C	2.6A	2.5A	1.5AB	0.6AB	0.1B	0.0B	0.76
	High inoculation level								
Beef tissue surface	A	—	4.8A	4.7A	4.4A	4.6A	4.7A	4.4A	0.29
	B	4°C	4.7A	4.2A	1.8B	0.9B	1.3B	1.0B	0.48
Ground beef	0	—	3.5A	3.1A	1.1B	0.3B	0.5B	0.8B	0.35
	1	12°C	3.4A	3.2A	1.4B	0.4B	0.6B	1.1B	0.39
	2	12°C	3.8A	3.4A	2.1B	0.8C	0.8C	1.4BC	0.42
	3	12°C	4.4A	4.0AB	2.9B	0.9C	0.6C	1.6C	0.42
	7	4°C	4.0A	3.4AB	2.4B	0.6C	0.5C	1.2BC	0.51
	14	4°C	4.7A	3.9A	3.5A	0.9B	0.8B	1.2B	0.62
	21	4°C	5.2A	3.9A	3.6A	1.0B	0.7B	1.5B	0.66

[a,b,c,d] See Table 2 footnotes.

TABLE 7. *The least squares means of* Salmonella typhimurium *on inoculated beef tissue surfaces and in resulting ground beef*

Sample	Sample time[b]	Storage temp.	*Salmonella typhimurium* ($\log_{10}$ CFU/cm^2 or g) LSM after treatment[a]: None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	3.4A[d]	3.4A	3.4A	3.6A	3.5A	3.7A	0.18
	B	4°C	2.9A	2.0A	0.4B	0.1B	0.7B	0.0B	0.29
Ground beef	0	—	0.6A	0.8A	0.1A	0.0A	0.1A	0.0A	0.23
	1	12°C	1.6A	1.6A	0.8AB	0.4B	0.2B	0.1B	0.32
	2	12°C	2.0AB	2.3A	1.3AC	0.8BC	0.3C	0.4C	0.45
	3	12°C	3.4A	3.5A	2.2AB	1.2BC	0.5C	0.6C	0.57
	7	4°C	0.8A	0.7AB	0.2AB	0.3AB	0.0B	0.0B	0.25
	14	4°C	0.7A	0.7A	0.2A	0.4A	0.0A	0.1A	0.31
	21	4°C	0.3A	0.2A	0.4A	0.5A	0.0A	0.0A	0.28
	High inoculation level								
Beef tissue surface	A	—	5.4A	5.3A	5.3A	5.3A	5.2A	5.4A	0.12
	B	4°C	4.6A	4.3A	2.4B	1.6B	1.8B	0.5C	0.35
Ground beef	0	—	3.3A	2.7A	1.3B	0.1C	0.5C	0.2C	0.23
	1	12°C	3.5A	3.1A	1.9B	0.5C	0.8BC	0.9BC	0.38
	2	12°C	4.4A	4.0A	2.9B	1.5C	1.4C	1.8C	0.34
	3	12°C	5.5A	5.0AB	4.2B	2.5C	1.5C	2.0C	0.44
	7	4°C	3.3A	2.9A	1.8B	0.1C	0.6C	0.2C	0.37
	14	4°C	2.8A	2.4A	1.5B	0.1C	0.6C	0.1C	0.31
	21	4°C	3.0A	2.0AB	1.6BC	0.2D	0.5CD	0.1D	0.47

[a,b,c,d] See Table 2 footnotes.

TABLE 8. *The least squares means of* Clostridium sporogenes *on inoculated beef tissue surfaces and in resulting ground beef*

			Clostridium sporogenes ($\log_{10}$ CFU/cm^2 or g)						
			LSM after treatment[a]:						
Sample	Sample time[b]	Storage temp.	None (control)	Water	Hot water	2.0% LA	2.0% AA	12% TSP	Pooled SEM for row
	Low inoculation level[c]								
Beef tissue surface	A	—	2.2A[d]	2.5A	2.1A	2.7A	2.7A	2.5A	0.33
	B	4°C	0.8A	0.3B	0.0B	0.0B	0.0B	0.0B	0.33
Ground beef	0	—	0.3A	0.8A	0.1A	0.0A	0.0A	0.2A	0.19
	1	12°C	0.1A	0.2A	0.0A	0.0A	0.0A	0.3A	0.13
	2	12°C	0.2A	0.2A	0.0A	0.0A	0.0A	0.3A	0.14
	3	12°C	0.2A	0.1AB	0.0B	0.0B	0.0B	0.0B	0.03
	7	4°C	0.0A	0.0A	0.0A	0.0A	0.0A	0.0A	0.00
	14	4°C	0.0A	0.0A	0.0A	0.0A	0.0A	0.0A	0.00
	21	4°C	0.2A	0.0A	0.0A	0.0A	0.0A	0.0A	0.08
	High inoculation level								
Beef tissue surface	A	—	4.4A	4.4A	3.6A	4.1A	4.3A	4.1A	0.33
	B	4°C	2.0A	1.8A	0.5B	0.8AB	0.3B	0.0B	0.33
Ground beef	0	—	1.7A	1.8A	0.5B	0.1B	0.0B	0.3B	0.33
	1	12°C	1.6A	1.8A	0.4B	0.3B	0.0B	0.5B	0.37
	2	12°C	1.7A	1.5A	0.1B	0.2B	0.0B	0.3B	0.31
	3	12°C	1.5A	1.4A	0.1B	0.4B	0.0B	0.0B	0.31
	7	4°C	0.6A	0.2AB	0.0B	0.0B	0.0B	0.0B	0.17
	14	4°C	0.4A	0.0A	0.0A	0.0A	0.0A	0.0A	0.16
	21	4°C	0.1A	0.0A	0.0A	0.0A	0.0A	0.0A	0.02

[a,b,c,d] See Table 2 footnotes.

Throughout the 21-day storage period at 4°C, the control and WW-treated samples consistently yielded higher, but not statistically higher, levels of *S. typhimurium.* Samples receiving antimicrobial treatments and held at 12°C exhibited significantly lower *S. typhimuirum* populations by 3 days of storage when compared to control samples.

Ground beef produced from tissue inoculated at high initial levels and treated with any antimicrobial compound contained significantly lower populations of *S. typhimurium.* While ground beef produced from tissue treated with HW yielded significantly lower populations of *S. typhimurium* than WW or untreated control, the populations were significantly higher than those observed in any of the antimicrobial chemical-treated samples. This trend continued throughout storage periods of 3 days at 12°C and 21 days at 4°C. The exception to this trend was observed in samples receiving HW and AA. These samples were not significantly different from one another after 21 days of 4°C storage.

At low initial inoculation levels vegetative cells of *C. sporogenes* were reduced to below detectable levels by all antimicrobial agents tested, remaining that way throughout the study for both 12 and 4°C, 3- and 21-day storage periods, respectively (Table 8). Generally, *C. sporogenes* initially detectable in ground beef resulting from antimicrobial chemical-treated beef tissue inoculated with high levels of bacteria was not detectable after 3 (12°C) or 21 (4°C) days of storage. However, LA-treated samples held at 12°C continued to yield low detectable levels of *C. sporogenes* after 3 days.

DISCUSSION

Blow-ups of ground beef packs due to excessive gas production from bacteria are a sporadic but detrimental occurrence in the beef industry. During this study blow-ups were observed to be a sporadic event occurring only when initial inoculation levels were high for one replication each of W-, LA-, and TSP-treated meat, and only after 21 days of refrigerated storage. In contrast, this phenomenon occurred in 50% of the untreated control packs at both high and low inoculation levels. Various carcass treatments do not seem to eliminate this sporadic event but do appear to reduce the occurrence when compared to untreated samples. This experiment was not designed to determine the effects of carcass wash treatments on blow-up occurrence and therefore no conclusions can be drawn; however, these observations would support a need for additional research.

All chemical antimicrobial and HW treatments tested in this study demonstrated significant initial reductions on the beef carcass surface tissue. After grinding, however, there was no significant difference between any treated samples and the untreated control. While it appears the APC counts increase after the beef is ground, it is impossible to infer any conclusions from this data, since the pregrind samples are a surface count given in CFU/cm^2 and the ground beef counts are of the total meat sample in CFU/g. However, variation within meat models passing through a process has been described previously *(25)*. The investigators determined that as boned carcass beef was minced and chopped the mean log

of the number of bacterial counts increased, probably due to more thorough mixing.

After incubation of ground beef samples at 4 or 12°C, there generally were no statistical differences observed in APC. It is important, however, to consider that the APC levels in ground beef from necks initially treated with AA and LA tended to be lower after 3 or 21 days of storage at 12 or 4°C than APCs from the control or WW-treated ground beef. This apparent delay in effectiveness of organic acid treatments is in agreement with observations of other researchers who have studied the effects of lactic or acetic acid applications on various packaged beef products *(29, 32)*. Prasai et al. *(29)* observed a significant difference ($P < 0.05$) of aerobic plate counts (log CFU/cm^2) on vacuum-packaged beef subprimals which received 1.5% (vol/vol) lactic acid spray treatments and underwent 28 days of −1.1 or 2°C storage, when compared to untreated controls. They concluded that "Although organic acid decontamination may not dramatically reduce bacterial contamination from meat surfaces, it can improve the microbial quality of meat." It appears this statement is also true for ground beef produced from beef carcasses receiving either lactic or acetic acid wash interventions.

All chemical antimicrobial and HW treatments demonstrated significant initial reductions of LAB on the beef carcass surface tissue when compared to the control samples. As observed for APC postgrind, there was no significant difference in LAB populations between any treated samples and the untreated control. Additionally, LAB counts increased after the beef was ground in a manner similar to that observed for APC.

After incubation at 12 or 4°C, for 3 or 21 days, respectively, there were no statistical differences observed between treated samples and the control. In fact, the LAB levels within all treatments increased over time. The overall levels of LAB after the refrigerated incubation periods resemble those observed by other researchers after vacuum packaging and −1.5°C storage for 24 days of ground beef *(14)*. However, within the same samples, *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes* populations were decreased to levels below detection in many cases. Taken together, this information would indicate that the resulting environmental changes brought on by LA, AA, TSP, or HW did not negatively impact the general population of LAB, but effected a decrease in the populations of specific pathogens. This effect was maintained over 21 days of 4°C storage.

Changes in the ecology of the product resulting from a uniform and general antimicrobial treatment initially lowered the populations of LAB, but were still not conducive to the unchecked outgrowth of the inoculated strains of specific pathogens. Our model is representative of the situation of fecally contaminated carcasses that are subjected to antimicrobial treatments; it is not a model of carcasses that are sterile or free of any microorganisms, a situation that does not exist in the industry today. LAB have been shown to be a dominant microflora in ground beef displayed for retail sale *(15)*. The present study demonstrated that initially reducing their levels by antimicrobial agent carcass washes did not result in population increases of *E. coli* O157:H7 through lack of competitive inhibition as suggested by Jay *(22)*. Consequently, the belief that an explanation for various haemorrhagic colitis outbreaks resulting from raw ground beef is that the meat is "so clean that the low numbers of pathogens had no antagonists" *(22)* is not supported.

Pseudomonads have been shown to establish themselves on raw beef as early in the slaughter process as hide pulling *(18)* and are the dominate aerobic flora of meat *(16)*. *Pseudomonas fluorescens* or *Pseudomonas fragi* inoculated at levels of 4 log CFU/cm^2 onto cut beef surfaces containing no background microflora have been observed to grow to levels exceeding 9 log CFU/cm^2 in as little as 8 days when beef was incubated at 4°C *(9)*. In the present study pseudomonads on beef carcass surface tissue responded to treatments and subsequent incubation in ground beef in a manner similar to APC and LAB. Blickstad et al. *(7)* determined that spoilage bacteria are only present in low numbers before the cold storage of meat. In the present study low initial numbers demonstrated minimal growth in ground beef, regardless of the treatment received. AA treatments yielded the best overall growth suppression of pseudomonads out of the current treatments. By days 3 and 21 of 12 and 4°C incubation, respectively, this suppression was significant when compared to the control samples. Previous studies have demonstrated that *Pseudomonas* spp. populations on beef pieces are initially reduced by 35.7% more when dipped in 1.2% acetic acid than when dipped in water *(5)*. It appears that organic acid carcass washes applied prior to carcasses entering refrigerated storage will suppress the outgrowth of pseudomonads in the resulting ground beef. This suppression could extend the time before the onset of spoilage resulting from their presence in ground beef.

The procedures of the present study yielded ground beef containing a background APC flora of ca. 3 log CFU/g regardless of the initial treatment. However, *E. coli* O157:H7 originally present on the neck surface tissue was in many cases undetectable in samples receiving a treatment, ground, then stored for 3 days at 12°C or 21 days at 4°C. This effect was most noticeable for AA-treated samples at 3 days (4°C), when samples contained no detectable cells. In all cases, ground samples made from tissue receiving a chemical antimicrobial treatment or HW were lower in *E. coli* O157:H7 counts than the untreated controls at the end of the study periods. This effect was most profound when high-level inocula were used to yield an initial ground beef inoculum of ca. 3 log CFU of *E. coli* O157:H7 per g in untreated samples.

Palumbo et al. *(26)* observed that *E. coli* O157:H7 did not grow in fresh ground beef held at 5, 8, 12, and 15°C, and in some cases fell below detectable levels, but that viable counts remained constant. Observations from the present study are congruent with this observation in the case of ground beef made from beef treated by WW and HW or in untreated controls, i.e., samples most relatable to their work. However, in AA- and TSP-treated samples, *E. coli* O157:H7 levels declined over time and in some cases became undetectable regardless of initial inoculation level and incubation temperature. This data might suggest that if *E.*

coli O157:H7 cells are in some way injured while on the carcass surface prior to being incorporated into ground beef, and if the antimicrobial agent used is residual, an added degree of long-term safety during cold storage could be achieved.

The inability of *Listeria* spp. to grow in refrigerated ground beef has been previously documented *(20, 23, 30)*. Barbosa et al. *(3)* recently documented slow growth of *L. monocytogenes* in vacuum-packed refrigerated ground beef. During the present study both situations were observed, depending upon treatment and initial inoculation level. Low initial inoculations of *L. innocua* reduced during carcass interventions utilizing antimicrobial chemicals exhibited little to no growth during all storage periods, and in some cases were undetectable. In all other situations during the present study *L. innocua* exhibited some growth, although it was minimal. The ability of various carcass intervention procedures to significantly reduce the levels of *L. innocua* and the inability of the bacterium to grow well in the resulting refrigerated ground beef could be utilized to produce safer food products. This is exemplified by significantly lower numbers observed at the end of all storage periods of ground beef from tissue receiving an antimicrobial treatment, regardless of initial inoculation levels, when compared to WW and controls.

The behavior of *S. typhimurium* in response to various treatments was similar to that of *E. coli* O157:H7 and *L. innocua*. At low initial inoculation levels, *S. typhimurium* was in some cases below detectable levels immediately after grinding, and then increased to detectable levels after 3 days of 12°C incubation. By the end of all storage periods of samples receiving an antimicrobial treatment regardless of initial inoculation level, *S. typhimurium* levels were significantly lower than the controls. The exception to this was observed in samples receiving low-level initial inoculation and 4°C incubation where there were no significant differences observed between any treated samples and controls. This lack of difference can be attributed to the inability of *S. typhimurium* to grow even in the control samples at this temperature in ground beef. It is worth noting that AA- and TSP-treated samples yielded no detectable levels at the 21-day storage period when ground beef was held at 4°C. This study indicates that the benefits of any carcass intervention used to reduce initial levels of *S. typhimurium* will be maintained in the resulting ground beef even if the product is subjected to mild levels of temperature abuse.

Vegetative cell levels of *C. sporogenes* decreased to below or near-below detectable levels in the ground beef made from tissue subjected to HW, LA, AA, or TSP treatments. At the end of all storage periods, with few exceptions, the bacterium was not detectable. When the bacterium was present in the control and WW samples, it was at reduced levels. This record parallels the decline of inoculated *C. sporogenes* observed by other researchers in ground poultry and beef held in air at temperatures ranging from 2 to 13°C *(2, 31)*.

The use of carcass interventions can be helpful in producing microbially safer ground beef. In particular, it appears the residual effects of organic acids will offer the best overall protection. It is likely the use of a multi-hurdle approach, incorporating various combinations of beef carcass WW, HW, and organic acid applications will offer the highest level of microbial control for the resulting ground beef. The interventions employed in this study reduce bacteria indiscriminately and do not appear to produce a shift in the bacterial flora resulting from fecal contamination capable of promoting accelerated growth of particular pathogens. In fact, when antimicrobial chemicals were used, beef pathogens were reduced, while APC, LAB, and pseudomonads increased during storage. The present study indicates that the application of interventions normally utilized in the beef industry to date will not produce a selectively aseptic carcass capable of promoting the growth of some pathogens typically associated with raw beef.

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