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Long-Term Effect of Alkaline, Organic Acid, or Hot Water Washes on the Microbial Profile of Refrigerated Beef Contaminated with Bacterial Pathogens after Washing†

WARREN J. DORSA,* CATHERINE N. CUTTER, AND GREGORY R. SIRAGUSA

United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933-0166, USA

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ABSTRACT

The effect of 2% (vol/vol) lactic acid, 2% (vol/vol) acetic acid, 12% (wt/vol) trisodium phosphate, water at 72°C and water at 32°C washes on bacterial populations introduced onto beef carcass surfaces after treatment was determined for up to 21 days at 4°C in storage in vacuum packaging. Beef carcass short plates were collected from cattle immediately after slaughter and subjected to the above treatments or left untreated (C). Short plates were then inoculated with low levels (ca. <2 $\log_{10}$) of *Listeria innocua, Salmonella typhimurium, Escherichia coli* O157:H7, and *Clostridium sporogenes* cells contained in a bovine fecal cocktail. In general, growth of these four bacteria and of aerobic bacteria, lactic acid bacteria, and pseudomonads was suppressed or not observed when lactic acid or acetic acid treatments were used. Bacteria introduced to trisodium phosphate-treated tissue underwent some growth suppression, but to a lesser extent than on acid-treated tissue, and in some cases grew as well as on untreated beef surfaces. Water washes at 72 or 32°C offered little growth suppression of pathogens during subsequent storage when these bacteria were introduced to beef tissue after treatment. The use of a final lactic or acetic acid wash during the processing of beef carcasses offers some residual efficacy in suppressing pathogen proliferation during refrigerated storage, should these bacteria be introduced immediately after carcass processing.

Thermal inactivation of *Escherichia coli, Listeria, Salmonella,* and *Clostridia* found in food and beef products has been well documented *(16, 17, 22, 23)*. Hot water and chemical antimicrobial carcass wash regimens have been thoroughly investigated *(3, 5, 6, 9, 12, 14, 19, 21, 27, 30, 31)*. The effects of thermal inactivation followed by storage at refrigeration temperatures have been investigated to a lesser extent *(13, 18)*. However, the suggestion by Jay *(20)* that the various carcass process methods proposed and used today are creating beef products that are primed for exponential growth of pathogenic bacteria introduced as postprocessing contaminants warrants investigation.

There are many opportunities for postslaughter cross-contamination to occur during the production of cut beef products *(7)*. Charlebois et al. *(8)* concluded that the cutting and boning operations are the most significant contributors to increased fecal coliform populations on boneless beef. Research that has considered the effects various beef carcass antimicrobial interventions have on bacteria introduced to the carcass surface after intervention has not, to our knowledge, been reported. Consequently, the present study was conducted to determine the effect hot water, alkaline, and organic acid wash intervention processes during slaughter have on postprocess contamination of pathogens on vacuum-packed beef subprimals stored under refrigeration for a 21-day period.

MATERIALS AND METHODS

Bacterial cultures. *Escherichia coli* O157:H7 CDC B6-914, *Listeria innocua* ATCC 33090, *Salmonella typhimurium* ATCC 14028, and *Clostridium sporogenes* ATCC 11437 were made resistant to antibiotics as described previously *(13)*. To enhance the ability to selectively enumerate the marked bacteria from natural bovine feces, *E. coli* MARC1-S was cultured on sorbitol McConkey agar (SMAC) (Difco Laboratories, Detroit, MI) plus 250 μg of streptomycin (Sigma Chemical, Co., St. Louis, MO) per ml. Oxoid listeria selective agar (LSA) (Unipath, Ogdensburg, NY) plus 500 μg of streptomycin per ml was used to culture *L. innocua* MARC1-S. *Clostridium botulinum* isolation agar without egg yolk (CBI) *(26)* plus 50 μg of novobicin (Sigma Chemical Co., St. Louis, MO) per ml was used to culture *C. sporogenes* MARC1-N. *S. typhimurium* MARC1-R was cultured on Rambach agar (RA) (E. Merck, Gene-Trak Systems Corp., Hopkinton, MS) plus 250 μg of nalidixic acid (Sigma) per ml.

Beef tissue preparation. Twelve beef short plates were collected as described by Dorsa et al. *(14)* from cows at a local processing facility immediately after slaughter (<12 min after exsanguination). Beef short plates are covered by the cutaneous trunci muscle and, therefore, consist of lean meat surface. As short plates were removed, they were placed individually into polypropylene bags, then held in an ice chest and transported within 1 h to the laboratory at Roman L. Hruska U.S. Department of Agriculture Meat Animal Research Center (Clay Center, NE). Prior to treat-

* Author for correspondence. Tel: (402)762-4228; Fax: (402)762-4149; E-mail: dorsa@marcvm.marc.usda.gov.

† Mention of a trade name, proprietary product or specific equipment is 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.

ment, seven adjacent areas were marked on each short plate with a sterile stainless steel 5 by 5 cm template, a sterile cotton swab, and edible ink.

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. *(13)* (W. J. Cary Engineering, Inc., Springfield, MO).
Spray treatments were applied for 15 s at 80 lb/in^2 (5.5 bar) and 32 ± 2°C at the tissue surface, except in the case of hot water, which was applied at 72 ± 2°C. Initial trials with this system indicated 74°C at the nozzle would yield a maximum consistent temperature of 70°C at the tissue surface (data not presented). All other physical parameters of the washer were set and monitored to parallel those used in previous research involving the commercial carcass washer *(13)*. Wash treatments applied to the beef short plates were 2% (vol/vol) DL-lactic acid (LA) (Sigma), 2% (vol/vol) acetic acid, glacial (AA) (Fisher Scientific, St. Louis, MO), 12% (wt/vol) trisodium phosphate (TSP) (Rhone-Poulenc, Cranbury, NJ), hot water (HW) 70 ± 2°C at the tissue surface, or water (W) 32 ± 2°C at the tissue surface. A control group was left untreated.

Fecal inoculum. Before each replication, bovine feces were collected immediately after defecation from three heifers maintained on a hay-silage diet. Ten grams of each fecal sample were combined in a sterile stomacher bag (Sterifil, Spiral Biotech, Bethesda, MD) with 90 g of sterile physiological saline and stomached for 1 min with a Model 400 Stomacher (Tekmar, Inc., Cincinnati, OH). An additional 1:10 dilution was made from this slurry.

Ten-milliliter cultures of each bacterium (*E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes*), grown without shaking overnight in Trypticase soy broth (TSB; BBL Becton Dickinson, Cockeysville, MD) and the appropriate antibiotic, were transferred to separate 15-ml sterile conical centrifuge tubes, centrifuged at 1,690 × *g* for 15 min at 5°C, and resuspended in 20 ml of buffered peptone water (BPW) (BBL). Initial culture concentrations were determined from a McFarland standard using a Spectronic 20 spectrophotometer (Milton Roy Co., Rolling Meadows, IL) set at 650 nm and serial dilutions were made to a final concentration of 10^5 cells per ml for each bacterium. One-milliliter aliquots of each culture were then mixed together and 2 ml of the resulting culture mixture was added to 18 ml of the filtered fecal slurry described above for a final inoculum of 10^4 CFU/ml for all antibiotic-resistant bacteria.

Immediately after antimicrobial treatments and prior to inoculation, a single premarked 5 by 5 cm tissue section approximately 1 mm thick was excised and placed into a stomacher bag for analysis. Postintervention inoculation of the remaining five marked areas on each short plate was accomplished by pipetting 0.1 ml of the inoculum onto each marked area and spreading it with a sterile spatula. Immediately after inoculation, another premarked 25-cm^2 area of each beef short plate was sampled for analysis. The treated short plates were then placed on UV-sterilized plastic trays or tubs and covered with a polyurethane bag as described by Dorsa et al. *(14)*. At this point (<0.5 h after receiving treatments) the surface pH of the short plates was determined using a flat surface combination probe (Corning model 245, Corning, Inc., Corning, NY). The short plates were then placed into a walk-in cooler at 4°C for 48 h. After 48 h, the bag was removed, a third 25-cm^2 area excised for bacterial enumeration, and the surface pH of the short plate was measured. The remaining three marked areas were excised at this time, placed into stomacher bags that were placed into a suitably sized 3.2-mil nylon-copolymer bag (Holly Sales, Omaha, NE) with an oxygen transmission rate at 23°C dry of 52 cm^3/m^2, which was vacuum sealed with a Hollymatic model LV 10 G (Hollymatic Corp., Countryside, IL). These samples were stored at 4°C and removed for sampling at 7, 14, and 21 days. One additional tissue sample taken from each short plate was used throughout the study to obtain surface pH values on each of the sampling days. After the pH was taken from the time sample it was re-vacuum-packaged and stored at 4°C.

Sample enumeration Following excision, the 25-cm^2 samples were placed into a stomacher bag with 25 ml of buffered peptone water (BPW) and 0.1% Tween 20. The 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 per cm^2 was calculated using a digital counter or a CASBA IV computer-assisted colony image analyzer (Spiral Biotech, Inc., Bethesda MD) and converted to logarithmic values.

Using the plating media described previously made isolation and enumeration possible for each antibiotic resistant bacterium from fresh unaltered feces inoculated with a mix containing all the marked bacteria *(13)*. Prior to inoculation with the marked bacteria, the feces used to produce the fecal inoculum was plated on each of the media to validate the absence of naturally resistant strains of the marked bacteria used in the current study. Total mesophilic aerobic bacteria (APC), lactic acid bacteria (LAB), and pseudomonads were enumerated on tryptic soy agar (TSA; Difco) at 37°C, Bacto lactobacilli agar (MRS) (BBL) in 5% CO_2 at 30°C, and pseudomonas isolation agar (PIA) (Difco) at 37°C, respectively.

Calculations and data analysis. The means of duplicate plate counts were converted to $\log_{10}$ CFU/cm^2. To facilitate log analysis, any 0-count plate was assigned a value of 10 or 1 based on 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 by the general linear model procedure (GLM) of SAS (SAS Institute, Cary, NC); a significance level of 0.05 was used unless otherwise stated.

RESULTS

Surface pH values of the samples immediately after organic acid treatments were lower than those of C (negative control) or W (water-washed at 32°C) samples by as much as 3.5 units (Table 1). Beef carcass tissue treated with TSP was as much as 3.6 pH units higher than C or W samples. After 2 days of cold storage, the pHs of all treated beef samples equilibrated toward a common value regardless of the wash treatment received. This trend continued throughout the study.

Beef tissue inoculated immediately after washes with HW, TSP, AA, or LA exhibited the lowest initial APCs of 2.3, 2.3, 2.7, and 2.3 $\log_{10}$ CFU/cm^2, respectively (Figure 1). The initial APCs after HW, TSP, and LA washes were significantly different from the APCs after W and C washes: 3.2 and 3.0 $\log_{10}$ CFU/cm^2, respectively. After 2 days of storage at 4°C all tissue treated with chemical antimicrobial agents (LA, AA, and TSP) exhibited no significant differences in APCs, but all three were significantly lower in APCs than the beef tissue treated by HW or W and the untreated tissue C. There was no significant difference in APCs observed between samples treated by HW, W, or C. By day 21 of storage at 4°C, LA- and AA-treated tissue

TABLE 1. *The average pH of beef tissue surfaces during 21 days of storage at 4°C*

	pH					
	treatment[a]					
Sample	Control	Water	Hot water	2.0% LA	2.0% AA	12% TSP
Wash solution[b]	NA	7.21	7.21	2.35	2.89	12.47
Beef on day: 0[c]	7.27	7.29	7.05	3.79	4.48	10.69
2	6.57	6.47	6.22	5.11	5.58	6.69
7	6.02	6.04	5.93	5.60	5.65	6.49
14	5.91	5.97	5.83	5.59	5.60	6.28
21	5.89	5.99	5.78	5.63	5.63	6.24

[a] TSP, trisodium phosphate; LA, lactic acid; AA, acetic acid.
[b] Wash solutions used for intervention treatments. NA, not applicable.
[c] pH for day 0 taken from beef surfaces shortly after wash treatments (<0.5 h).

exhibited significantly lower APCs than the tissues treated with TSP, HW, W, or C.

No beef tissue contained any detectable streptomycin-resistant strain of *E. coli* O157:H7 or *L. innocua,* nor any nalidixic acid-resistant strain of *S. typhimurium* or *C. sporogenes* prior to being inoculated.

Beef tissue inoculated immediately after being washed with LA exhibited the lowest initial *L. innocua* count, 0.2 $\log_{10}$ CFU/cm^2, while after washing with AA, TSP, HW, W, and C, initial counts of 0.6, 0.6, 1.0, 1.2, and 1.2 $\log_{10}$ CFU/cm^2, respectively, were not significantly different from one another (Figure 2). After 2 days of storage at 4°C all tissue treated with antimicrobial chemicals (LA, AA, and TSP) exhibited no significant difference in the presence of *L. innocua,* but the samples carried significantly lower levels than the beef tissue treated by HW, W, and C. There was no

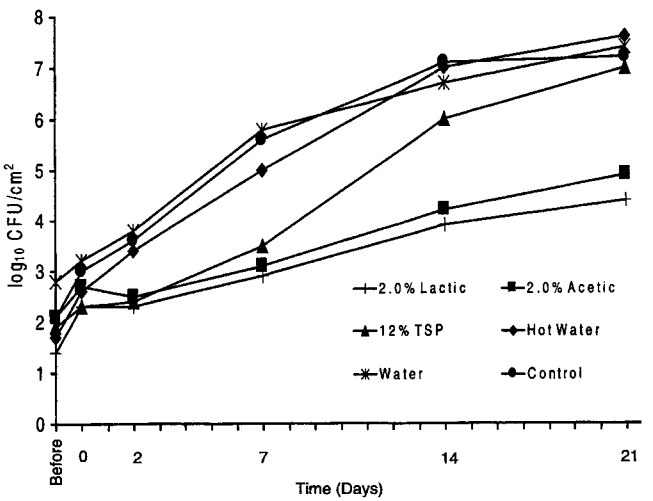

FIGURE 1. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and during subsequent outgrowth of mesophilic aerobic bacterial populations (least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

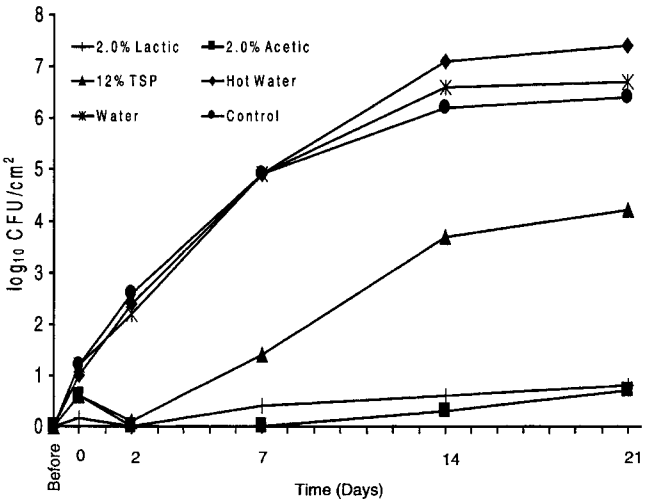

FIGURE 2. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and during subsequent survival and/or growth of* Listeria innocua *(least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

significant difference observed between samples washed with HW, W, or C. This relationship remained true throughout the study period with the exception of TSP-treated beef tissue, on which *L. innocua* exhibited growth significantly greater than LA- or AA-treated beef tissue by 14 days. However, this growth remained significantly less than that exhibited by samples washed with HW, W, and C, while the growth on the latter three remained statistically similar throughout the 21 days of storage at 4°C.

Beef tissue inoculated immediately after being washed with LA or TSP exhibited the lowest initial *E. coli* O157:H7 count, 0.2 $\log_{10}$ CFU/cm^2, and undetectable, respectively (Figure 3). Samples washed with AA, HW, W, and C resulted in initial counts of *E. coli* O157:H7 of 1.1, 1.3, 1.1, and 1.1 $\log_{10}$ CFU/cm^2, respectively, which were not significantly different from one another. After 2 days of storage at 4°C, all tissue washed with antimicrobial chemicals (LA, AA, and TSP) exhibited no significant differences in *E. coli* O157:H7 levels, but significantly lower levels than beef tissue treated by HW, W, and C. This relationship remained true throughout the study period; however, by 21 days the untreated control (C) beef tissue had significantly higher *E. coli* O157:H7 populations than any of the treated beef tissues.

S. typhimurium was not detectable on beef tissue inoculated immediately after being washed with LA or TSP (Figure 4). Samples washed with AA, HW, W, and C had initial mean counts of 0.4, 0.8, 0.8, and 0.8 $\log_{10}$ CFU/cm^2, respectively; these were significantly greater than 0.0 $\log_{10}$ CFU/cm^2 and not significantly different as a group. Between 2 and 7 days of storage at 4°C, all tissue treated with antimicrobial chemicals (LA, AA, and TSP) exhibited no significant difference in population levels of *S. typhimurium,* but significantly lower levels than beef tissue treated by HW, W, and C. However, by 14 days, reductions in the average

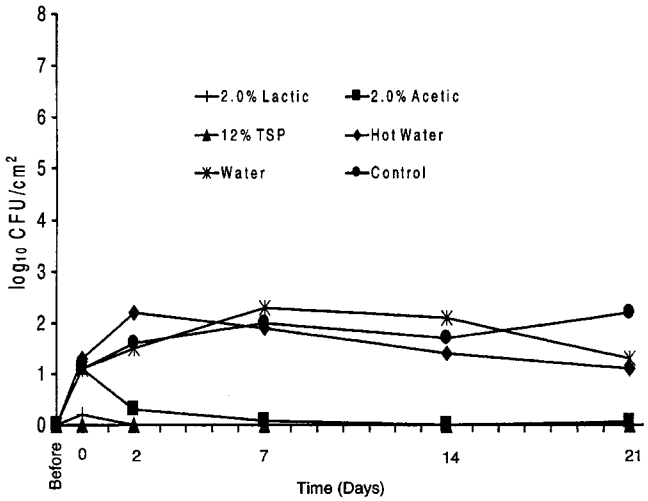

FIGURE 3. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and during subsequent survival and/or outgrowth of* Escherichia coli *O157:H7 (least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

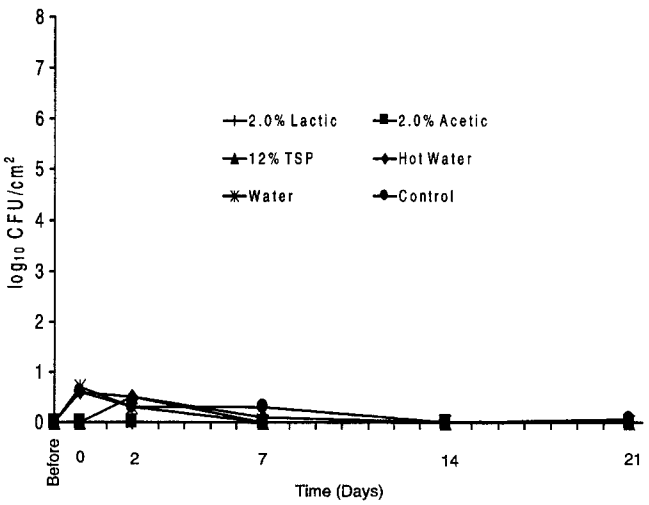

FIGURE 5. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and during subsequent survival of* Clostridium sporogenes *(least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

populations present on HW, W, and C beef tissue resulted in no significant difference in *S. typhimurium* populations on any of the beef tissue samples and this relationship remained true at 21 days.

C. sporogenes cells were not detectable on beef tissue inoculated immediately after being washed with LA, AA, or TSP (Figure 5). Washes HW, W, and C resulted in initial counts on the samples of 0.6, 0.7, and 0.6 $\log_{10}$ CFU/cm^2, respectively, which were significantly larger than 0.0 $\log_{10}$ CFU/cm^2, but not significantly different from one another. By day 2 of storage at 4°C, *C. sporogenes* populations on beef tissue samples washed with HW no longer exhibited

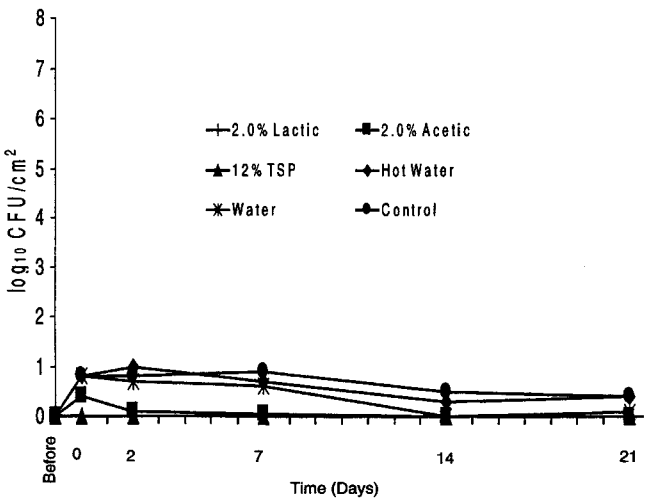

FIGURE 4. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and during subsequent survival and/or growth of* Salmonella typhimurium *(least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

counts significantly different from 0.0 $\log_{10}$ CFU/cm^2. *C. sporogenes* counts on W-treated tissue were undetectable by 7 days, and by 14 days no treated beef tissue samples exhibited a detectable presence of *C. sporogenes*. At 21 days, *C. sporogenes* was detectable on some C beef tissue samples; however, the mean values were not significantly different from 0.0.

Twenty-five percent of the beef short plates used contained detectable low levels of LAB prior to being inoculated and 75% had no detectable LAB present. After inoculation 100% of the short plates contained detectable levels of LAB. While LA-washed beef tissue exhibited the lowest mean levels of LAB immediately after inoculation (0.5 $\log_{10}$ CFU/cm^2), there was no significant difference between any of the mean levels of LAB on treated and untreated tissue. LAB remained undetectable or not significantly different from 0.0 $\log_{10}$ CFU/cm^2 on LA-washed beef tissue for the duration of the study (Figure 6). This was also true for AA-washed beef tissue until 14 days, when LAB levels became significantly greater than 0.0 $\log_{10}$ CFU/cm^2, but remained significantly lower than levels on tissue washed with TSP, HW, W, or C tissue. At 21 days there was no significant difference between the LAB counts of beef tissue samples treated with TSP, HW, W, or untreated C, and these samples exhibited significantly greater LAB counts than those from AA- or LA-washed beef tissue. While the counts observed on AA-washed beef tissue were greater than those on LA-washed beef tissue, there was no significant difference at 21 days.

Pseudomonads were not detectable on beef tissue used for LA, TSP, and HW washes (Figure 7). Pseudomonads were detectable at low levels on beef tissue used for AA washes and C (0.4 and 0.5 $\log_{10}$ CFU/cm^2, respectively) prior to treatment and inoculation, and present at higher levels on tissue used for W treatments (2.1 $\log_{10}$ CFU/cm^2).

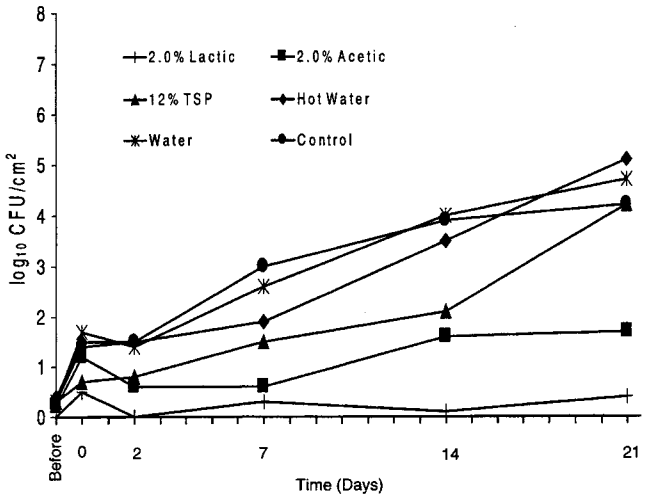

FIGURE 6. *Effects of antimicrobial and water wash interventions on beef surfaces on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and subsequent survival and/or growth of lactic acid bacteria (least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

The higher starting point of the pseudomonad population on W-washed beef tissue was reflected throughout the study. Consequently, the 21-day mean value of 4.1 $\log_{10}$ CFU/cm^2 observed from beef tissue subjected to W treatments was significantly higher than those observed for untreated C or HW-washed beef tissue, 3.0 and 2.5 $\log_{10}$ CFU/cm^2, respectively. However, the total differences from pretreatment and inoculation to 21 days in HW, W, and C washed beef tissue, of 2.5, 2.0, and 2.5 $\log_{10}$ CFU/cm^2, respectively, indicates no difference in the rate of increase. The HW- and W-washed, and C beef tissue samples all yielded significantly greater pseudomonad counts by 21 days than LA-, AA-, or TSP-treated beef tissue. Pseudomonads were not detectable at 21 days on beef tissue treated with LA and AA.

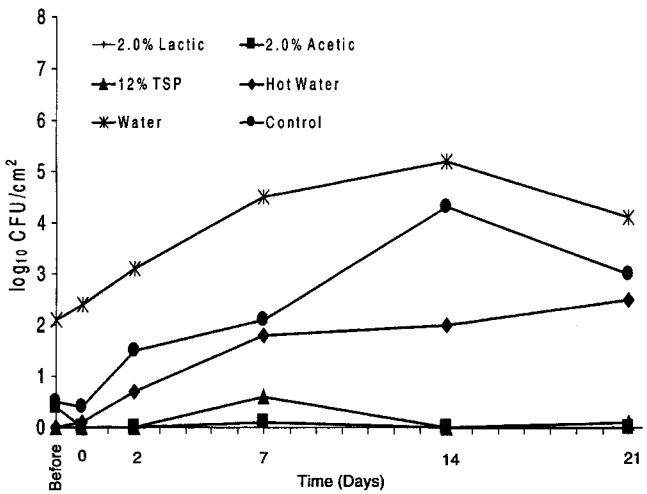

FIGURE 7. *Effects of antimicrobial and water wash interventions on the initial numbers immediately after treatments before inoculation (Before), after inoculation (Time 0), and subsequent survival and/or growth of pseudomonads (least squares means $\log_{10}$ CFU/cm^2; n = 6) during beef storage at 4°C aerobically for 2 days followed by vacuum packaging and storage for an additional 19 days.*

DISCUSSION

Spray applications of both AA and LA reduced the pH of the samples by as much as 3.5 units. Woolthuis and Smulders *(33)* observed similar reductions of 3.3 units when calf carcasses were treated with 1.25% lactic acid sprays. Hardin et al. *(19)* sprayed 2% lactic acid onto beef tissue and observed a reduction range of 3.7 to 4.7 units depending on which region of the carcass was used. Woolthuis and Smulders *(33)* reported an increase of ca. 2 pH units after overnight storage of calf carcasses and found that these surface pH values were still significantly ($P < 0.001$) below the controls. These results are similar to those observed during cold storage of the samples in the present study.

The application of LA to beef carcass surface tissue significantly suppressed the growth of aerobic bacteria and lactic acid bacteria placed on the beef surface after treatment. Any growth of pseudomonads, present at very low levels initially, was completely suppressed. Quattara et al. *(25)* demonstrated in vitro that organic acids in general appear to be inhibitory to meat spoilage bacteria. *E. coli* O157:H7, *S. typhimurium,* and *C. sporogenes* were no longer detectable after 14 days of storage at 4°C. *L. innocua* was only detectable at very low levels, and in many individual replications its presence was undetectable. Lactic acid appeared to be bactericidal during the first 48 h after bacteria were introduced to the beef surface. An immediate and short-term bactericidal effect of lactic acid has been observed by many other researchers on meat and in broth during refrigerated storage *(1, 2, 29, 32)*. However, studies have typically been conducted by exposing meat pieces already contaminated with bacteria to the lactic acid treatment. El-Khateib et al. *(15)* observed immediate reductions of 0.79 $\log_{10}$ CFU per cube for *L. monocytogenes* with a maximum difference between populations on treated and untreated meat of 1.7 $\log_{10}$ CFU after 48 h of 4°C storage. Woolthuis and Smulders *(33)* observed substantial immediate reductions of aerobic bacteria on calf carcasses treated with 1.25% lactic acid. Dickson and Anderson *(10)* state in a review of decontamination methods for animal carcasses that organic acids have been reported to have an immediate effect on the microflora of meat when applied during carcass processing. The present study demonstrated this bactericidal effect will occur on beef surfaces if bacteria are introduced onto the surface even after a lactic acid treatment.

Smulders and Woolthuis *(28)* also determined that a 1.25% lactic acid spray applied to calf carcasses immediately after processing had some residual bactericidal effect on the naturally occurring aerobic bacteria. They reported that aerobic populations were significantly lower on lactic acid-treated carcasses than on untreated carcasses after storage for 14 days at 3 ± 1°C. The present study demonstrates the short-term bactericidal effect observed by Smulders and Woolthuis *(28)* is also observable for various specific bacteria populations introduced onto beef carcasses immediately after a LA treatment. Additionally, it appears the effects of a LA treatment on certain specific and general

bacteria populations introduced onto a carcass immediately after treatment persists if carcasses are held in cold storage for 48 h, fabricated, and the fabricated meat then stored at 4°C for up to 21 days. However, the persistent inhibitory effect observed might not be consistently inhibitory at all. We speculate the observed inhibitions of various bacteria are likely the combined effects of low pH, organic acids, and refrigeration temperatures that have inhibited the outgrowth of the inoculated bacteria. Whether initial numbers were reduced and outgrowth slowed or whether initial numbers were not reduced but impaired, and hence outgrowth slowed, was not determined.

Upon inoculating the treated tissue (beef carcass surface tissue covered by an intact superficial fascia) three events could have occurred: cell death, sublethal cellular injury, or no antimicrobial effect. In the event of injury, the remaining viable cells would probably not grow as rapidly as the normal growth rate under the conditions tested (4°C, vacuum packaging) or would not be detected in the selective media used. If some portion of the initial population was killed, there would be fewer cells left to begin outgrowth and hence the population levels would remain low or lag over a longer time period than those subjected to the control and water wash treatments. If the antimicrobial effects of the organic acids were the sole reason for the observed prolonged inhibition, then this effect would dissipate as the pH of the tissue approached its final value (pH 5.6), since the proportion of undissociated lactic or acetic acid would also decrease.

There was no significant difference between the effectiveness of AA and LA treatments in the present study; however, in most cases carcass tissue treated with lactic acid exhibited better growth suppression over time. This observation is not surprising since it has previously been determined in vitro and on beef surfaces that lactic acid caused greater degrees of injury and was more effective against *L. monocytogenes* and *E. coli* O157:H7 than acetic acid at similar concentrations *(3, 19, 24)*. Avens et al. *(4)* did not find a significant difference in total aerobic bacteria, lactic acid bacteria, total coliforms, and fecal streptococcus present on beef carcasses when these carcasses received either a spray application of 1% acetic acid (at 49°C) or none. They concluded dilute acetic acid sprays were ineffective on the day of slaughter prior to carcass chilling when low bacterial counts (<4 $\log_{10}$ CFU/cm^2) were present on the carcass surfaces. If only the immediate effect is considered, this conclusion would be supported by results from the present study, since there was no significant difference determined between AA- samples and W-treated samples initially. However, as with LA, it appears that the effectiveness of an AA treatment on certain specific and general bacteria populations introduced onto a carcass immediately after treatment persists if carcasses are held in cold storage for 48 h, fabricated, and the fabricated meat is then stored at 4°C for up to 21 days. A previous study *(12)* conducted on beef carcass surface tissue containing high inoculation levels (ca. 6 $\log_{10}$ CFU/cm^2) also determined that acetic acid washes provide no additional immediate difference when compared to water washes. As with the present study, after a period of cold storage AA-treated beef tissue had significantly lower populations of aerobic bacteria, *E. coli* O157:H7, *L. innocua,* lactic acid bacteria, and pseudomonads than did C-, W-, or HW-treated beef carcass surface tissue. As with LA, it is likely these persistent effects are a result of either an initial complete elimination of certain bacteria or a consequence of cell injury and subsequent refrigeration storage.

TSP seemed to have a minimal effect on the initial growth of aerobic bacteria and lactic acid bacteria; however, by 21 days there was no significant difference in populations between TSP-treated tissue and C-, W-, or HW-treated surfaces. Dorsa et al. *(12)* observed somewhat similar growth patterns, but growth suppression was observed for both bacterial groups when beef tissue was inoculated at high levels prior to receiving wash treatments. By 21 days there was no significant difference for aerobic bacteria populations between TSP-treated beef carcass surface tissue and C or W samples. In the present study, no growth was observed of *E. coli* O157:H7, *S. typhimurium,* and *C. sporogenes* on the beef tissue after receiving TSP treatments, and growth of *L. innocua* was suppressed when compared to samples treated by C, W, or HW. While there was no significant difference between the growth of *S. typhimurium* on the C-, W-, or HW-, and TSP-washed samples, it is worth noting that *S. typhimurium* was detectable on samples treated by C, W, and HW, but not on TSP-treated samples. Bactericidal effects of TSP toward other *Salmonella* spp. have also been demonstrated previously on beef tissue and using a different food model *(11, 34)*. *E. coli* O157:H7 was also not detectable after 21 days on TSP-treated samples and this result was significant when compared to samples treated by C, W, or HW. *L. innocua* was able to grow on TSP-treated tissue, however, to a significantly lesser extent than on C-, W-, or HW-treated tissue. Somers et al. *(30)* and Dorsa et al. *(12)* demonstrated that *L. monocytogenes* and *L. innocua* are more resistant to the effects of TSP than other bacteria on beef surfaces and, therefore, growth of *L. innocua* was not unexpected.

Water washes at a variety of temperatures have been shown to be effective for immediate reductions of bacterial populations present on beef carcass surfaces before washing *(5, 9, 14, 19)*. Thus, hot water washes are valuable bacterial hurdles when included in carcass processing. Dorsa et al. *(13)* observed that when *E. coli* O157:H7 and *C. sporogenes* are initially present at high levels, a hot water wash or other forms of moist heat can effectively reduce their presence on beef surfaces, and this initial benefit is not lost over time during refrigerated storage. It was also noted during this study that mesophilic aerobic bacteria, lactic acid bacteria, and *L. innocua* recovered and grew on moist heat-treated tissue to levels equal to those on untreated tissue during long-term refrigerated storage. During the present study all bacteria introduced onto the beef carcass surface after receiving HW or W treatments grew as well as those introduced onto untreated controls. While moist heat treatments are effective for reducing bacteria populations present on beef carcass tissue before these treatments, they offer no advantage for carcasses subsequently contaminated during refrigerated storage and fabrication.

This study considered only the introduction of specific bacteria immediately after treatment. Carcass surfaces are potentially exposed to contamination by pathogens for as long as 72 h after whole carcass wash treatments. Thus, additional studies conducted to consider the long-term effects of any whole carcass treatment on carcasses contaminated during this extended time frame should be considered.

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